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Friday
September 27, 1985

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Animal and Plant Health Inspection Service

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DEPARTMENT OF AGRICULTURE

Agricultural Marketing Service

7 CFR Parts 916 and 917

Nectarines, Pears, Plums and Peaches Grown in California; Amendment of Container and Pack Requirements

AGENCY: Agricultural Marketing Service, USDA.

ACTION: Final rule.

SUMMARY: This final rule amends the container and pack regulations for nectarines, peaches, and plums grown in California. The principal changes add labeling requirements for consumer packages and a new container, the No. 22G standard lug box used in shipping these fruits. This action would be in accordance with the Agricultural Marketing Agreement Act of 1937, as amended, and Marketing Orders 916 and 917. These actions were unanimously recommended by the commodity committees established under these orders.

EFFECTIVE DATE: September 27, 1985.

FOR FURTHER INFORMATION CONTACT: William J. Doyle, Chief, Fruit Branch, F&V, AMS, USDA, Washington, DC 20250, telephone (202) 447–5975.

SUPPLEMENTARY INFORMATION: This action has been reviewed under Secretary's Memorandum 1512–1 and Executive Order 12291 and has been designated a "non-major" rule. William T. Manley, Deputy Administrator, Agricultural Marketing Service, has determined that this action will not have a significant economic impact on a substantial number of small entities.

This final rule is issued under the marketing agreements, as amended, and Marketing Orders 916 and 917, as amended (7 CFR Parts 916 and 917), regulating the handling of nectarines, pears, plums, and peaches grown in California. The agreements and orders are effective under the Agricultural Marketing Agreement Act of 1937, as amended (7 U.S.C. 601–674). Shipments of these California fruits are regulated by container and pack under Nectarine Regulation 8 (7 CFR Part 916), Peach Regulation 8 (7 CFR Part 917), and Plum Regulation 17 (7 CFR Part 917). Because these regulations do not change substantially from season to season, they were issued on a continuing basis subject to amendment, modification or suspension as may be recommended by the applicable committee and approved by the Secretary. This final rule is based upon recommendations and information submitted by the committees and other available information.

This action establishes marking requirements for master containers of consumer packages of nectarines, peaches and plums and individual consumer packages of such fruit. They are designed to prevent misrepresentation of the pack, size and net weight of the specific fruits and promote buyer confidence. Since consumer packages are removed from master containers, they should also be marked to inform the buyer of the contents of the package. This action specifies marking requirements for a new container (No. 22G standard lug box) for plums and nectarines and experimental containers for peaches to assure that fruit packed in these containers is appropriately represented to the trade.

This final rule exempts master containers of consumer packages of nectarines and peaches and individual consumer packages of such fruit from the requirements of standard pack except that nectarines shall be fairly uniform in size. A similar size uniformity requirement is currently in effect for peaches and plums. Other standard pack requirements are generally applicable to fruit packed in boxes and not consumer packages, e.g. polybags. This action requires that master containers of consumer packages of nectarines, peaches and plums be marked with the number of individual consumer packages and the net weight of each individual consumer package within the master container, minimum size of the fruit (e.g. 80 size), and name and address of the shipper. Individual consumer packages of the specified fruits are to be marked with the net weight and name and address of the shipper. Currently only the regulation for plums requires that master containers of consumer packages and individual consumer packages be marked with the net weight.

This final rule also requires the new container, the No. 22G standard lug box, of nectarines and plums and experimental containers of peaches packed in molded forms to be marked with the number of fruit in each container such as "80 count", "88 count", etc. and to be marked with the size corresponding to the number of such fruit when packed in molded forms in the No. 22D standard lug box. The action requires each No. 22G standard lug box of loose-filled nectarines to be marked with the words "25 pounds net weight". This is the same requirement in effect for loose-filled nectarines in the No. 22D standard lug box.

A proposed rule was published on July 30, 1985 (50 FR 30855) with a 15-day comment period. No comments were received. It is hereby found that this action will tend to effectuate the declared policy of the act.

Accordingly, the Secretary finds that upon good cause shown this final rule will be effective less than 30 days after publication in the **Federal Register** (5 U.S.C. 553), because: (1) Shipments of the current crop of nectarines, peaches, and plums grown in California are underway; (2) the amendments to the container and pack regulations for the specified commodities was recommended by the commodity committees following discussion at a public meeting at which there were no opposing views; (3) a proposed rule was issued on July 30, 1985 with a 15-day comment period and no opposing views were received; and (4) handlers of these commodities have been apprised of these requirements.

List of Subjects

7 CFR Part 916

Marketing agreements and orders, Nectarines, California.

7 CFR Part 917

Marketing agreements and orders, Pears, Plums, Peaches, California.

1. The authority citations for 7 CFR Parts 916 and 917 continue to read as follows:

Authority: Secs. 1–19, 48 Stat. 31, as amended; 7 U.S.C. 601–674.

PART 916—NECTARINES GROWN IN CALIFORNIA

2. Section 916.350 is revised to read as follows:

§ 916.350 Nectarine Regulation 8.

(a) No handler shall ship any package or container of any variety of nectarines except in accordance with the following terms and conditions:

(1) Such nectarines, when packed in any closed package or container, except master containers of consumer packages and individual consumer packages therein, shall conform to the requirements of standard pack: *Provided,* That nectarines in any container shall be fairly uniform in size.

(2) Each package or container of nectarines shall bear, on one outside end in plain sight and in plain letters, the name "nectarines" and except for consumer packages in master containers the name of the variety, if known or, when the variety is not known, the words "unknown variety."

(3) Each package or container of nectarines shall bear, on one outside end in plain sight and in plain letters, the following count and/or size description of the nectarines as applicable:

(i) The size of nectarines packed in molded forms (tray-packs) in No. 22D standard lug boxes, No. 22G standard lug boxes, cartons, No. 12B fruit (peach) boxes or flats and the size of wrapped nectarines packed in rows in No. 12B fruit (peach) boxes shall be indicated in accordance with the number of nectarines in each container, such as "80 count," "88 count," etc.

(ii) The size of nectarines in molded forms (tray-packs) in No. 22G standard lug boxes shall be indicated according to the number of such nectarines when packed in molded forms in the No. 22D standard lug box in accordance with the requirements of standard pack, such as "80 size," "88 size," etc, along with count requirements in paragraph (a)(3)(i) of this section.

(iii) The size of nectarines loose-filled or tight-filled in any container shall be indicated according to the number of such nectarines when packed in molded forms in the No. 22D standard lug box in accordance with the requirements of standard pack, such as "80 size," "88 size," etc.

(4) Each No. 22D standard lug box and No. 22G standard lug box of loose-filled nectarines shall bear on one outside end, in plain sight and in plain letters, the words "25 pounds net weight."

(5) Each No. 22E standard lug box of loose-filled nectarines shall bear on one outside end, in plain sight and in plain letters, the words "35 pounds net weight."

(6) Each bulk bin container of loose-filled nectarines shall contain not less than 400 pounds net weight, and bear on one outside panel, in plain sight and in plain letters, the following information:

(i) The name and address (including zip code) of the shipper.

(ii) The net weight.

(7) Each master container when filled with nectarines packed in consumer packages shall bear on one outside end in plain sight and in plain letters the following information:

(i) The number of individual consumer packages, the net weight of each consumer package, and the size description of the contents.

(ii) The name and address (including zip code) of the shipper.

(8) Each individual consumer package shall bear the name and address of the shipper and the net weight.

(b) As used herein, "standard pack" and "fairly uniform in size" shall have the same meanings as set forth in U.S. Standards for Grades of Nectarines (§§ 51.3145 to 51.3160) and all other terms shall have the same meaning as when used in the amended marketing agreement and order. "No. 12B standard fruit box" measures 2⅜ to 7⅛ x 11½ x 16⅛ inches, "No. 22D standard lug box" measures 2⅞ to 7⅛ x 13½ x 16⅛ inches, "No. 22E standard lug box" measures 8¾ x 13½ x 16⅛ inches, "No 22G standard lug box" measures 7⅜ to 7½ x 13¼ x 15⅞ inches. All dimensions are given in depth (inside dimension) by width by length (outside dimension).

PART 917—FRESH PEARS, PLUMS, AND PEACHES GROWN IN CALIFORNIA

3. Section 917.442 is revised to read as follows:

§ 917.442 Peach Regulation 8.

(a) No handler shall ship any package or container of any variety of peaches except in accordance with the following terms and conditions:

(1) Such peaches, when packed in any closed package or container, except master containers of consumer packages and individual consumer packages therein, shall conform to the requirements of standard pack.

(2) Each package or container of peaches shall bear, on one outside end in plain sight and in plain letters the name "peaches" and except for consumer packages in master containers the name of the variety, if known or, when the variety is not known, the words "unknown variety."

(3) Each package or container of peaches shall bear, on one outside end, in plain sight and in plain letters, the following count and/or size description of the peaches as applicable:

(i) The size of peaches packed in molded forms (tray-packs) in No. 22D standard lug boxes, experimental containers, cartons, No. 12B fruit (peach) boxes, or flats and the size of wrapped peaches packed in rows in No. 12B fruit (peach) boxes shall be indicated in accordance with the number of peaches in the container, such as "80 count," "88 count," etc.

(ii) The size of peaches in molded forms in experimental containers shall be indicated according to the number of such peaches when packed in molded forms in the No. 22D standard lug box in accordance with the requirements of standard pack, such as "80 size," "88 size," etc, along with count requirements in paragraph (a)(3)(i).

(iii) The size of peaches loose-filled or tight-filled in any container shall be indicted according to the number of such peaches when packed in molded forms in No. 22D standard lug boxes, in accordance with the requirements of standard pack, such as "80 size," "88 size," etc.

(4) The variation in diameter between the smallest and largest peach in any individual container shall not exceed one-forth (¼) inch for size 80 and smaller and three-eights (⅜) inch for peaches larger than size 80: *Provided,* That not more than five (5) percent, by count, of the peaches in any individual container may fail to meet the diameter requirements of this paragraph.

(5) Each No. 22D standard lug box of loose-filled peaches shall bear on one outside end, in plain sight and in plain letters, the words "25 pounds net weight."

(6) Each No. 22E standard lug box of loose-filled peaches shall bear on one outside end, in plain sight and in plain letters, the words "35 pounds net weight."

(7) Each bulk bin container of loose-filled peaches shall contain not less than 400 pounds net weight, and bear on one outside panel, in plain sight and in plain letters, the following information:

(i) The name and address (including zip code) of the shipper.

(ii) The net weight.

(8) Each master container when filled with peaches packed in consumer packages shall bear on one outside end in plain sight and in plain letters the following information:

(i) The number of individual consumer packages, the net weight of each consumer package, and the size description of the contents.

(ii) The name and address (including zip code) of the shipper.

(9) Each individual consumer package shall bear the name and address of the shipper and the net weight.

(b) As used herein, "standard pack" shall have the same meaning as set forth in U.S. Standards for Grades of Peaches (§§ 51.1210 to 51.1223) and all other terms shall have the same meaning as when used in the amended marketing agreement and order. "No. 12B standard fruit box" measures 2⅜ to 7⅛ x 11½ x 16⅛ inches, "No 22D standard lug box" measures 2⅞ to 7⅛ x 13 ½ x16⅛ inches. "No. 22E standard lug box" measures 8¾ x 13½ x 16⅛ inches. All dimensions are given in depth (inside dimensions) by width by length (outside dimensions).

4. Section 917.454 is amended by revising paragraphs (a) and (d) to read as follows:

§ 917.454 Plum Regulation 17.

(a) No handler shall ship any package or container of any variety of plums except in accordance with the following terms and conditions:

(1) Such plums, when shipped in closed in packages or containers, except master containers of consumer packages and individual consumer packages therein, shall conform to the requirements of standard pack:

(2) The diameters of the smallest and largest plums in any individual package or container shall not vary more than one-fourth (¼) inch: *Provided,* That a total of not more than five (5) percent, by count, of the plums in any package or container may fail to meet this requirement.

(3) Each package or container of plums shall bear on one outside end, in plain sight and in plain letters, the name "plums" and except for consumer packages in master containers the name of variety if known or, when the variety is not known, the words "unknown variety".

(4) Each package or container of plums shall bear on one outside end, in plain sight and in plain letters, the size description of the contents which description shall conform to the following as applicable:

(i) The size of plums in four-basket crates shall be indicated in accordance with the arrangement of the plums in the top layer of the baskets, such as "4x4 size," "4x5 size," etc.

(ii) The size of plums loose-filled or tight-filled in standard lug boxes, cartons, or other packages or containers shall be indicated in accordance with the equivalent size designation for such plums when packed in four-basket crates, such as "4x4 size," etc.

(iii) The size of plums packed in molded forms (tray-packs) in cartons or lugs and of wrapped plums packed in No. 12B fruit (peach) boxes shall be indicated in accordance with the number of plums in the container, such as "88 count," "108 count," etc.

(iv) The size of plums packed in molded forms (tray-packs) in No. 22G standard lug boxes shall be indicated according to the number of such plums when packed in molded forms in the No. 22D standard lug box in accordance with the requirements of standard pack, such as "80 size," "88 size," etc., along with count requirements in paragraph (a)(4)(iii) of this section.

(5) Each package or container of loose-filled or tight-filled plums other than bulk bin containers, master containers of consumer packages, and individual consumer packages in master containers shall bear on one outside end, in plain sight and in plain letters, the words "28 pounds net weight."

(6) Each bulk bin container of loose-filled plums shall contain not less than 400 pounds net weight, and bear on one outside panel, in plain sight and in plain letters, the following information:

(i) The name and address (including zip code) of the shipper.

(ii) The net weight.

(7) Each master container when filled with plums packed in consumer packages shall bear on one outside end in plain sight and in plain letters the following information:

(i) The number of individual consumer packages, the net weight of each consumer package, and the size description of the contents.

(ii) The name and address (including zip code) of the shipper.

(8) Each individual consumer package shall bear the name and address of the shipper and the net weight.

(b) * * *

(c) * * *

(d) When used herein "standard pack" and "diameter" shall have the same meanings as set forth in the U.S. Standards for Grades of Fresh Plums and Prunes (§§ 51.1520 to 51.1538) and all other terms shall have the same meaning as when used in the amended marketing agreement and order. "No. 12B standard fruit box" measures 2⅜ to 7⅛ x 11½ x 16⅛ inches, "No. 22D standard lug box" measures 2⅞ to 7⅛ x 13½ x 16⅛ inches and "No. 22G standard lug box" measures 7⅜ to 7½ x 13¼ x 15⅞ inches. All dimensions are given in depth (inside dimensions) by width by length (outside dimensions).

Dated: September 24, 1985.

Thomas R. Clark,

Deputy Director, Fruit and Vegetable Division, Agricultural Marketing Service.

[FR Doc. 85–23183 Filed 9–26–85; 8:45 am]

BILLING CODE 3410-02-M

DEPARTMENT OF JUSTICE

Immigration and Naturalization Service

8 CFR Part 238

Contracts With Transportation Lines; Addition of Five Star Airlines

AGENCY: Immigration and Naturalization Service, Justice.

ACTION: Final rule.

SUMMARY: This rule adds Five Star Airlines to the list of carriers which have entered into agreements with the Service to guarantee the passage through the United States in immediate and continuous transit of aliens destined to foreign countries.

EFFECTIVE DATE: September 18, 1985.

FOR FURTHER INFORMATION CONTACT: Loretta J. Shogren, Director, Policy Directives and Instructions, Immigration and Naturalization Service, 425 I Street, NW., Washington, DC 20536, Telephone: (202) 633-3048.

SUPPLEMENTARY INFORMATION: The Commissioner of Immigration and Naturalization entered into an agreement with Five Star Airlines on September 18, 1985 to guarantee passage through the United States in immediate and continuous transit of aliens destined to foreign countries.

The agreement provides for the waiver of certain documentary requirements and facilitates the air travel of passengers on international flights while passing through the United States.

Compliance with 5 U.S.C. 553 as to notice of proposed rulemaking and delayed effective date is unnecessary because the amendment merely makes an editorial change to the listing of transportation lines.

In accordance with 5 U.S.C. 605(b), the Commissioner of Immigration and Naturalization certifies that the rule will not have a significant impact on a substantial number of small entities.

This order constitutes a notice to the public under 5 U.S.C. 552 and is not a rule within the definition of section 1(a) of E.O. 12291.

List of Subjects in 8 CFR Part 238

Airlines, Aliens, Government contracts, Travel, Travel restriction.

Accordingly, Chapter I of Title 8 of the Code of Federal Regulations is amended as follows:

PART 238—CONTRACTS WITH TRANSPORTATION LINES

1. The authority citation for Part 238 continues to read as follows:

Authority: Secs. 103 and 238 of the Immigration and Nationality Act, as amended (8 U.S.C. 1103 and 1228).

§ 238.3 [Amended]

In § 238.3 Aliens in immediate and continuous transit, the listing of transportation lines in paragraph (b) *Signatory lines* is amended by: Adding in alphabetical sequence, Five Star Airlines.

Dated: September 20, 1985.

Marvin J. Gibson,

Acting Associate Commissioner, Examinations, Immigration and Naturalization Service.

[FR Doc. 85-23105 Filed 9-26-85; 8:45 am]

BILLING CODE 4410-10-M

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

9 CFR Part 78

[Docket No. 85-086]

Brucellosis in Cattle; State and Area Classifications

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Affirmation of interim rule.

SUMMARY: This document affirms the interim rule which amended the regulations governing the interstate movement of cattle because of brucellosis by including Medina County, Texas, in the portion of Texas designated as Class B rather than in the portion of the State designated as Class C. This rule is necessary because it has been determined that Medina County together with the previously designated Class B Area of Texas meets the standards for Class B. The effect of the rule is to relieve certain restrictions on the interstate movement of cattle from Medina County, Texas.

EFFECTIVE DATE: September 27, 1985.

FOR FURTHER INFORMATION CONTACT: Dr. G.H. Frye, Cattle Diseases Staff, VS, APHIS, USDA, Room 814, Federal Building, 6505 Belcrest Road, Hyattsville, MD 20782, 301-436-8711.

SUPPLEMENTARY INFORMATION:

Background

A document published in the **Federal Register** on June 4, 1985 (50 FR 23393-23394), amended the brucellosis regulations in 9 CFR Part 78 by including Medina County, Texas, in the Area of Texas designated as Class B. Prior to the effective date of the interim rule, Medina County, Texas, was included in the Area of Texas designated as Class C. The amendment, which was made effective June 4, 1985, relieves certain restrictions on the interstate movement of cattle from Medina County, Texas.

Comments were solicited for 60 days after publication of the amendment. No comments were received. The factual situation which was set forth in the document of June 4, 1985, still provides a basis for the amendment.

Executive Order and Regulatory Flexibility Act

This rule is issued in conformance with Executive Order 12291 and has been determined to be not a major rule. Based on information compiled by the Department, it has been determined that this rule will not have a significant effect on the economy; will not cause a major increase in costs or prices for consumers, individual industries, Federal, State, or local government agencies, or geographic regions; and will not have any significant adverse effects on competition, employment, investment, productivity, innovation, or on the ability of United States-based enterprises to compete with foreign-based enterprises in domestic or export markets.

For this action, the Office of Management and Budget has waived its review process required by Executive Order 12291.

Changing the status of a portion of the State of Texas reduces testing and other requirements on the interstate movement of certain cattle. Cattle moved interstate are moved for slaughter, for use as breeding stock, or for feeding. Testing requirements for cattle moved interstate for immediate slaughter, or to quarantined feedlots are not affected by the change in status. Also, cattle from Certified Brucellosis-Free Herds moving interstate are not affected by the change in status. It has been determined that the change in brucellosis status made by this rule will not affect marketing patterns and will not have a significant economic impact on those persons affected by this document.

Under these circumstances, the Administrator of the Animal and Plant Health Inspection Service has determined that this action will not have a significant economic impact on a substantial number of small entities.

Executive Order 12372

This program/activity is listed in the Catalog of Federal Domestic Assistance under No. 10.025 and is subject to the provisions of Executive Order 12372 which requires intergovernmental consultation with State and local officials. (See 7 CFR 3015, Subpart V, 48 FR 29112, June 24, 1983; 49 FR 22675, May 31, 1984; 50 FR 14088, April 10, 1985).

List of Subjects in 9 CFR Part 78

Animal Diseases, Cattle, Hogs, Quarantine, Transportation, Brucellosis.

PART 78—BRUCELLOSIS

Accordingly, the interim rule amending 9 CFR Part 78 which was published at 50 FR 23393-23394 on June 4, 1985, is adopted as a final rule.

Authority: 21 U.S.C. 111-114a-1, 114g, 115, 117, 120, 121, 123-126, 134b, 134f; 7 CFR 2.17, 2.51, and 371.2(d).

Done at Washington, D.C., this 20th day of September 1985.

B.G. Johnson,

Acting Deputy Administrator, Veterinary Services.

[FR Doc. 85-23029 Filed 9-26-85; 8:45 am]

BILLING CODE 3410-34-M

NUCLEAR REGULATORY COMMISSION

10 CFR Part 11

Criteria and Procedures for Determining Eligibility for Access to or Control Over Special Nuclear Material

AGENCY: Nuclear Regulatory Commission.

ACTION: Final rule.

SUMMARY: The Nuclear Regulatory Commission is amending the requirements for applications for initial special nuclear material "U" and "R" access authorizations and for the renewal of "U" access authorizations by allowing utilization of information on file with the Federal government on those individuals who possess current active clearances based upon equivalent investigations. The amendments will eliminate unnecessary duplication of administrative and investigative costs to licensees for affected individuals as well as reduce certain NRC administrative costs.

EFFECTIVE DATE: October 28, 1985.

FOR FURTHER INFORMATION CONTACT: Dr. W.B. Brown, Office of Nuclear

Material Safety and Safeguards, U.S. Nuclear Regulatory Commission, Washington, DC 20555, telephone: (301) 427–4185.

SUPPLEMENTARY INFORMATION:

Background

On March 17, 1977 (42 FR 14880), the NRC published proposed amendments to its regulations which would establish an access authorization program for individuals who have unescorted access to or control over special nuclear material. Written comments were invited and received. On December 28, 1977 (42 FR 64703), the Commission issued a notice of public hearings on the proposed regulations and subsequently established a Hearing Board to gather additional testimony. A final rule, based upon recommendations of the Hearing Board regarding only fuel cycle facilities and transportation, was published in the **Federal Register** amending 10 CFR Parts 11, 50, and 70 on November 21, 1980 (45 FR 76968).

One of the reasons for undertaking the NRC access authorization program for individuals having access to or control over special nuclear material was to maintain comparability with a similar program of clearances at Department of Energy (DOE) facilities and at mixed DOE/NRC facilities. Subsequently, the population of individuals affected has become one in which DOE/NRC mixed facility employees are by far the dominant segment. Accordingly, the incentive to establish and maintain a comparable and independent NRC program has been replaced by an incentive to use as much of the existing Federal system as possible. The revisions to 10 CFR Part 11 accomplish this end.

Conversion of Clearances

Under the revised Part 11 requirements the NRC would accept an existing personnel access clearance from any Federal agency, including NRC, DOE or DOD, if the investigation upon which the clearance is based is equivalent to the investigation required for the special nuclear material access authorization. This avoids having to conduct an investigation on an individual for whom an adequate investigation already exists.

Acceptance of Non-NRC Application Forms

Acceptance of an application for access authorizations on other than NRC forms allows the NRC to use existing information on individuals with current Federal clearances. It also allows an individual applying for renewal of a DOE "Q" personnel clearance to use some of the DOE documents in making application for the NRC "U" material access authorization. This reduces the administrative burden upon applicants.

Use of Department of Energy Reinvestigation Program

Application for renewal of an NRC "U" material access authorization may follow the schedule of the DOE Reinvestigation Program and utilize signed and dated copies of Part I of the DOE Personnel Security Questionnaire if an individual is subject to both DOE "Q" clearance and NRC "U" access authorization requirements.

Minor Administrative Changes

A number of administrative changes which are not related to reducing duplication are included in this rule revision. The schedule date for revision of fees has been changed from December to July; the fee for a full field investigation has been changed from $1550.00 to $1580.00 to reflect the current cost; and a new section has been added regarding withdrawal or cancellation of applications.

Omission of a Proposed Rule

These changes in the procedures in 10 CFR Part 11 were initiated in response to a request by a licensee for elimination of needless duplication between the NRC special nuclear material access authorization program and the Department of Energy personnel clearance program. The changes have been discussed in detail with all affected licensees. All agree that the changes are desirable. The changes do not alter the list of jobs identified in 10 CFR Part 11 as requiring material access authorization; therefore, individual applicants for access authorizations are either not affected or are benefited by the elimination of duplication. No other persons are affected by the changes. For these reasons good cause exists for omitting notice and public rulemaking as unnecessary. Accordingly, these changes in 10 CFR Part 11 are issued as a final rule effective in 30 days.

Environmental Impact: Categorical Exclusion

The NRC has determined that this final rule is the type of action described in categorical exclusion 10 CFR 51.22(c)(1). Therefore, neither an environmental impact statement nor an environmental assessment has been prepared for this final rule.

Paperwork Reduction Act Statement

This final rule amends information collection requirements that are subject to the Paperwork Reduction Act of 1980 (44 U.S.C. 3501 et seq.). These requirements were approved by the Office of Management and Budget approval number 3150–0062.

Regulatory Analysis

The Commission has prepared a regulatory analysis on this final regulation. The analysis examines the costs and benefits of the alternatives considered by the Commission. The analysis is available for inspection in the NRC Public Document Room, 1717 H Street NW., Washington, DC. Single copies of the analysis may be obtained from Dr. W.B. Brown, Office of Nuclear Material Safety and Safeguards, U.S. Nuclear Regulatory Commission, Washington, DC 20555, telephone: (301) 427–4185.

List of Subjects in 10 CFR Part 11

Hazardous materials transportation, Nuclear materials, Reporting and recordkeeping requirements, Security measures, Special nuclear material.

PART 11—CRITERIA AND PROCEDURES FOR DETERMINING ELIGIBILITY FOR ACCESS TO OR CONTROL OVER SPECIAL NUCLEAR MATERIAL

For the reasons set out in the preamble and under the authority of the Atomic Energy Act of 1954, as amended, the Energy Reorganization Act of 1974, as amended, and 5 U.S.C. 553, the NRC is adopting the following amendments to 10 CFR Part 11.

1. The authority citation for Part 11 is revised to read as follows:

Authority: Sec. 161, including 161i, 68 Stat. 948, as amended (42 U.S.C. 2201); sec. 201, 88 Stat. 1242, as amended (42 U.S.C. 5841). Section 11.15(e) also issued under sec. 501, 65 Stat. 290 (31 U.S.C. 9701).

2. In § 11.7, Paragraph (c) is revised to read as follows:

§ 11.7 Definitions.

* * * * *

(c) "NRC–'U' special nuclear material access authorization" means an administrative determination based upon a national agency check and a full-field background investigation, normally conducted by the Office of Personnel Management, that an individual in the course of employment is eligible to work at a job falling within the criteria of § 11.11(a)(1) or § 11.13.

* * * * *

3. In § 11.11, the introductory text of paragraph (a) and the entire text of paragraph (b) are revised to read as follows:

§ 11.11 General requirements.

(a) Each licensee who uses, processes, stores, transports, or delivers to a carrier for transport, formula quantities of special nuclear material (as defined in Part 73 of this chapter) subject to the physical protection requirements of §§ 73.20, 73.25, 73.26, 73.45, and 73.46, and each person subject to the general licensing requirements of § 70.20a, shall identify at its facility or plant (excluding all non-power reactor facilities and storage of fuel incident thereto and facilities and plants in which the licensee possesses or uses only irradiated special nuclear material subject to the exemption of § 73.6(b) of Part 73), describe, and if not already provided, provide to the Commission, by December 26, 1985 by amendment to its security plan:

* * * * *

(b) After 365 days following Commission approval of the amended security plan submitted in accordance with paragraph (a) of this section, no individual may be permitted to work at any job determined by the Commission to fall within the criterion of paragraph (a)(1) of this section without an NRC–U special nuclear material access authorization, and no individual may be permitted unescorted access to any protected area at any site subject to this Part without either an NRC–U or NRC–R special nuclear material access authorization. The exceptions to the requirement for an NRC–U and NRC–R special nuclear material access authorization are as follows:

(1) Exceptions to the requirement for an NRC–U special nuclear material access authorization for an individual to work at a job within the criteria of paragraph (a)(1) are provided for (i) any individual employed in such a job on October 28, 1985, who is not yet in receipt of an NRC–U special nuclear material access authorization from the Commission, provided that a complete application has been submitted to and is pending before the NRC for processing for that employee in accordance with § 11.15 (a) and (b); or (ii) any individual in possession of an NRC–L or R access authorization or an equivalent active Federal security clearance but not yet in receipt of the NRC–U special nuclear material access authorization, provided that a complete application has been submitted to and is pending before the NRC for processing for that employee in accordance with § 11.15 (a) or (b), or both.

(2) Exceptions to the requirement for an NRC–R special nuclear material access authorization for an individual to have unescorted access to a protected area are provided for (i) any individual employed in such a job on October 28, 1985 who is not yet in receipt of an NRC–R special nuclear material access authorization from the Commission, provided that a complete application has been submitted to and is pending before the NRC for processing for that employee in accordance with § 11.15 (a) and (b); or (ii) any individual in possession of an NRC–L access authorization or an equivalent active Federal security clearance, provided that a complete application has been submitted to the NRC for processing for that employee in accordance with § 11.15 (a) or (b), or both.

§ 11.13 [Revised]

4. Section 11.13, is revised to read as follows:

§ 11.13 Special requirements for transportation.

(a) All individuals who, after 365 days following approval of the amended security plan submitted in accordance with § 11.11(a), transport, arrange for transport, drive motor vehicles in road shipments of special nuclear material, pilot aircraft in air shipments of special nuclear material, act as monitors at transfer points, or escort road, rail, sea, or air shipments of special nuclear material subject to the appropriate physical protection requirements of §§ 73.20, 73.25, 73.26, or 73.27 of this chapter, or who are authorized to alter the scheduling and routing of such transport shall have NRC–U special nuclear material access authorization. Exceptions are provided for (1) any individual who is employed in such a job on October 28, 1985 and who is not yet in receipt of an NRC–U special nuclear material access authorization from the Commission, provided that a complete application has been submitted to and is pending before the NRC for processing for that employee in accordance with § 11.15 (a) and (b) or (2) any individual in possession of an NRC–L or R access authorization or equivalent active Federal security clearance but not yet in receipt of the NRC–U special nuclear material access authorization, provided that a complete application has been submitted to and is pending before the NRC for processing for that employee in accordance with § 11.15 (a) or (b), or both.

(b) Licensees who, after 365 days following Commission approval of the amended security plan submitted in accordance with § 11.11(a), transport or deliver to a carrier for transport special nuclear material subject to the physical protection requirements of §§ 73.20, 73.25, 73.26, or 73.27 of this chapter shall confirm and record prior to shipment the name and special nuclear material access authorization number of all individuals identified in paragraph (a) of this section assigned to the shipment. However, the licensee need not confirm and record the special nuclear material access authorization number in the case of any individual for whom an application has been submitted and is pending before the NRC in accordance with paragraph (a) of this section.

5. Section 11.15 is revised to read as follows:

§ 11.15 Application for special nuclear material access authorization.

(a)(1) Application for special nuclear material access authorization, renewal, or change in level shall be filed by the licensee on behalf of the applicant with the Director, Division of Security, U.S. Nuclear Regulatory Commission, Washington, DC 20555. Applications for affected individuals employed on October 28, 1985, shall be submitted within 60 days of notification of Commission approval of the amended security plan.

(2) Licensees who wish to secure NRC–U or NRC–R special nuclear material access authorizations for individuals in possession of an active NRC Q or L access authorization or other security clearance granted by another Federal agency based on an equivalent investigation shall submit a "Security Acknowledgement" (NRC Form 176) and a "Request for Access Authorization" (NRC Form 237). NRC will process these requests by verifying the data on an NRC cleared individual, or by contacting the Federal agency which granted the clearance, requesting certification of the security clearance, and determining the investigative basis and level of the clearance. Licensees may directly request the Federal agency which administered the security clearance, if other than NRC, to certify to the NRC that it has on file an active security clearance for an individual and to specify the investigative basis and level of the clearance.

(b) Applications for special nuclear material access authorization for individuals, other than those qualifying under the provisions of § 11.15(a)(2), must be made on forms supplied by the Commission, including:

(1) Personnel Security Questionnaire (PSQ) (NRC Form 1, Parts I and II);

(2) National Agency Check-Data for Nonsensitive or Noncritical-Position (SF–85A) for R cases only (This form must be typed.);

(3) Two completed standard fingerprint cards (FD–258);

(4) Security Acknowledgement (NRC Form 176);

(5) Authority to Release Information (NRC Form 259);

(6) Related forms where specified in accompanying instructions (NRC–254); and

(7) A statement by the employer, prospective employer, or contractor identifying the job to be assigned to or assumed by the individual and the level of authorization needed, justified by appropriate reference to the licensee's security plan.

(c)(1) Except as provided in paragraph (c)(2) of this section, NRC–U and R special nuclear material access authorizations shall expire 5 years from the date of issuance. If continued NRC–U and R special nuclear material access authorization is required, an application for renewal must be submitted at least 120 days prior to its expiration date. Failure to make a timely application will result in expiration of special nuclear material access authorization. Special nuclear material access authorization for which a timely application for renewal has been made may be continued beyond the expiration date pending final action on the application. An application for renewal will include:

(i) A statement by the licensee that at the time of application for renewal the individual's assigned or assumed job requires an NRC–U special nuclear material access authorization, justified by appropriate reference to the licensee's security plan;

(ii) Personnel Security Questionnaire (NRC Form 1, Parts I and II);

(iii) National Agency Check-Data for Nonsensitive or Noncritical-sensitive position (SF–85A) (This form must be typed.);

(iv) Two completed standard fingerprint cards (FD Form 258);

(v) Authority to Release Information (NRC Form 259); and

(vi) Other related forms where specified in accompanying NRC instructions (NRC Form 254).

(2) An exception to the NRC–U special nuclear material access authorization expiration date and the time for submission of NRC–U special nuclear material access authorization renewal applications is provided for those individuals who have a current and active DOE–Q access authorization and who are subject to DOE Selective Reinvestigation Program requirements. For these individuals, the time for submission of NRC–U special nuclear material access authorization renewal applications may coincide with the time for submission to DOE of Part I of a DOE Personnel Security Questionnaire pursuant to DOE Selective Reinvestigation Program requirements. The licensee may submit to NRC, concurrent with its submission to DOE, a copy of Part I of the individual's DOE Personnel Security Questionnaire which is dated and bears an original signature together with Part II of an NRC Personnel Security Questionnaire and the forms and information required by paragraphs (c)(1) (i), (iii), (iv), (v) and (vi) of this section, as the supporting documentation for an NRC–U special nuclear material access authorization renewal application. Any NRC–U special nuclear material access authorization issued in response to a renewal application submitted pursuant to this paragraph will not expire until the date set by DOE for the next reinvestigation of the individual pursuant to DOE's Selective Reinvestigation Program (generally every five years). At that time (and at the time of each subsequent reinvestigation of the individual), the licensee may again submit, concurrent with its submission to DOE, a copy of Part I of the individual's DOE Personnel Security Questionnaire which is dated and bears an original signature together with Part II of an NRC Personnel Security Questionnaire and the forms and information required by paragraphs (c)(1) (i), (iii), (iv), (v), and (vi) of this section as the supporting documentation for the renewal application. Failure to file such a renewal application concurrent with the time for submission of an individual's Part I of a DOE Personnel Security Questionnaire to DOE pursuant to DOE Selective Reinvestigation Program requirements will result in the expiration of the individual's NRC special nuclear material access authorization. NRC–U special nuclear material access authorizations for which timely applications for renewal have been made may be continued beyond the expiration date, pending final action on the application.

(3) Notwithstanding the above, in no instance shall the period of time for the initial and each subsequent NRC–U renewal application to NRC exceed 7 years. Any individual who is subject to the DOE Selective Reinvestigation Program requirements but, for administrative or other reasons, who does not submit reinvestigation forms to DOE within 7 years of the previous submission, shall submit a renewal application to NRC using the forms prescribed in paragraph (c)(1) above before the expiration of the 7 year period. Failure to request an NRC–U renewal for any individual within the 7 year period will result in termination of the individual's NRC–U access authorization.

(d) If at any time, due to new assignment or assumption of duties, a change in special nuclear material access authorization level from NRC "R" to "U" is required, the individual shall apply for a change of level of special nuclear material access authorization. The application must include a description of the new duties to be assigned or assumed, justified by appropriate reference to the licensee's security plan.

(e)(1) Each application for special nuclear material access authorization, renewal or change in level must be accompanied by the licensee's remittance payable to the U.S. Nuclear Regulatory Commission according to the following schedule:

i.	NRC–U requiring full field investigation.	$1,580
ii.	NRC–U based on certification of comparable full field background investigation.	[1] 0
iii.	NRC–U or R renewal	[1] 15
iv.	NRC–R	[1] 15
v.	NRC–R based on certification of comparable investigation.	[2] 0

[1] If a full field investigation is deemed necessary, a subsequent fee of $1580 will be assessed.

[2] If a National Agency Check investigation is deemed necessary, a subsequent fee of $15.00 will be assessed; however, if a full field investigation is deemed necessary, a subsequent fee of $1580 will be assessed.

(2) Material access authorization fees will be published in July of each year and will be applicable to each access authorization request received during the following calendar year. Applications from individuals having current Federal access authorizations may be processed expeditiously at no cost, since the Commission may accept the certification of access authorizations and investigative data from other Federal government agencies which grant personnel access authorization.

(f)(1) Any Federal employee, employee of a contractor of a Federal agency, licensee, or other person visiting an affected facility for the purpose of conducting official business, who possesses an active NRC or DOE–Q access authorization or an equivalent Federal security clearance granted by another Federal agency ("Top Secret") based on a comparable full field background investigation may be permitted in accordance with § 11.11 the same level of unescorted access that an NRC–U special nuclear material access authorization would afford. (2) Any Federal employee, employee of a contractor of a Federal agency, licensee, or other person visiting an affected facility for the purpose of conducting official business, who possesses an active NRC or DOE-L access authorization or an equivalent security clearance granted by another Federal agency ("Secret") based on a background investigation or national agency check may be permitted in accordance with § 11.11 the same level of unescorted access that an NRC-R special nuclear material access authorization would afford.

6. A new § 11.16 is added to read as follows:

§ 11.16 Cancellation of request for special nuclear material access authorization.

When a request for an individual's access authorization is withdrawn or cancelled, the licensee shall immediately notify the Chief, Facilities and Personnel Security Branch, NRC Division of Security, by telephone, so that the full investigation or National Agency Check may be discontinued. The caller shall provide the full name and date of birth of the individual, the date of request, and the type of access authorization originally requested ("U" or "R"). The licensee shall promptly submit written confirmation of the telephone notification to the Facilities and Personnel Security Branch, NRC Division of Security. A portion of the fee for the "U" special nuclear material access authorization may be refunded depending upon the status of the full field investigation at the time of withdrawal or cancellation.

Dated at Bethesda, Maryland, this 13th day of September 1985.

For the Nuclear Regulatory Commission.

William J. Dircks,

Executive Director for Operations.

[FR Doc. 85-23166 Filed 9-26-85; 8:45 am]

BILLING CODE 7590-01-M

DEPARTMENT OF COMMERCE

International Trade Administration

15 CFR Part 399

[Docket No. 50944-5144]

Exports to COCOM Countries

Correction

In FR Doc. 85-22650 beginning on page 38511 in the issue of Monday, September 23, 1985, make the following correction:

On page 38513, third column, paragraph 20., third line from the bottom, "(vi)" should read "(iv)".

BILLING CODE 1505-01-M

COMMODITY FUTURES TRADING COMMISSION

17 CFR Part 3

Review of National Futures Association Registration Proceedings

AGENCY: Commodity Futures Trading Commission.

ACTION: Final rules.

SUMMARY: The Commodity Futures Trading Commission ("Commission") has adopted rules pursuant to which the Commission, in accordance with the provisions of section 17(o)(2) of the Commodity Exchange Act ("Act"), 7 U.S.C. 21(o)(2) (1982), will review registration proceedings conducted by the National Futures Association ("NFA") to deny, condition, suspend, restrict or revoke registration. The Commission has authorized NFA to conduct such proceedings effective September 30, 1985.

EFFECTIVE DATE: September 30, 1985.

FOR FURTHER INFORMATION CONTACT: Linda Kurjan, Special Counsel, or Philip V. McGuire, Attorney, Division of Trading and Markets, Commodity Futures Trading Commission, 2033 K Street, NW., Washington, DC 20581. Telephone: (202) 254-8955.

SUPPLEMENTARY INFORMATION: On August 14, 1985, the Commission published in the **Federal Register** proposed rules pursuant to which the Commission, in accordance with the provisions of section 17(o)(2) of the Act, 7 U.S.C. 21(o)(2) (1982), will review registration proceedings conducted by NFA to deny, condition, suspend, restrict or revoke registration. 50 FR 32737. By order dated August 22, 1985, the Commission has authorized NFA to conduct such proceedings, effective September 30, 1985, with respect to futures commission merchants, introducing brokers, commodity pool operators, commodity trading advisors and their respective associated persons. 50 FR 34885.[1]

The Commission received no comments on its proposed rules. After careful consideration of its own, the Commission has determined to adopt them essentially as proposed. However, the second sentence of proposed rule 3.84(a), relating to the contents of an applicant's or registrant's brief in support of a petition for review, has been revised for the sole purpose of clarity. As proposed, this sentence read:

> The brief shall include a statement of the reasons why it is claimed that the order of the National Futures Association comports with the standards for disposition upon review set forth in § 3.87 of this subpart and the specific facts which support those reasons.

As revised, this sentence reads as follows:

> The brief shall include a statement of the reasons why it is claimed that the order of the National Futures Association should not be affirmed in accordance with the provisions of § 3.87 of this subpart and the specific facts which support those reasons.

This provision now more closely parallels § 3.87(b), which provides that the order of NFA shall be affirmed unless the Commission makes one or more of the findings set forth in that paragraph.

The Regulatory Flexibility Act

The Commission has previously determined that neither futures commission merchants nor registered commodity pool operators should be considered small entities for purposes of the Regulatory Flexibility Act ("RFA"), 5 U.S.C. 601 *et seq.*[2] Accordingly, the requirements of the RFA do not apply to those entities. With respect to the remaining entities, introducing brokers, commodity trading advisors and associated persons, the Commission noted when it proposed these rules that it believed they would not impose any additional burdens upon such parties, since all registrants are already subject to similar requirements under Part 3 of the Commission's regulations, and the proposed rules simply provide a procedure to petition for review of an order concerning the registration of such parties. Therefore, pursuant to section 3(a) of the RFA, 5 U.S.C. 605(b), the Chairman certifies that these regulations will not have a significant economic impact on a substantial number of small entities.

Effective Date

Section 4(c) of the Administrative Procedure Act, 5 U.S.C. 553(d), provides that rules promulgated by an agency generally may not be made effective less than thirty days after publication in the **Federal Register** except, *inter alia*, "for good cause." The Commission finds that good cause exists to make these rules effective September 30, 1985. As previously noted, the Commission has authorized NFA to conduct proceedings to deny, condition, suspend, restrict or revoke registration as of that date, and the Commission believes it is

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[1] The rules in accordance with which NFA will conduct such proceedings are set forth as an appendix to the Commission's order. 50 FR at 34887.

[2] *See* 47 FR 18618 (April 30, 1982).

appropriate that these rules take effect at the same time.

Lit of Subjects in 17 CFR Part 3

Registration requirements, Conditional registration, Temporary licenses, Statutory disqualifications, Authority delegations, Fingerprinting, Associated persons, Floor brokers, Introducing brokers, Commodity trading advisors, Commodity pool operators, Futures commission merchants, Leverage transaction merchants, Petitions for review.

PART 3—REGISTRATION

1. The authority citation for Part 3 continues to read as follows:

Authority: Sections 2(a)(1), 4, 4b, 4c, 4d, 4e, 4f, 4g, 4h, 4i, 4k, 4m, 4n, 4o, 4p, 6, 8, 8a, 14, 15, 17 and 19 of the Commodity Exchange Act, 7 U.S.C. 2 and 4, 6, 6b, 6c, 6d, 6e, 6f, 6g, 6h, 6i, 6k, 6m, 6n, 6o, 6p, 8, 9, 9a and 13b, 12, 12a, 18, 19, 21 and 23 (1982).

2. Subpart F is added to 17 CFR Part 3 to read as follows:

Subpart F—Review of National Futures Association Registration Proceedings

Sec.

3.80 Scope of rules
3.81 Service.
3.82 Notice and effective date of order affecting registration.
3.83 Petition for review.
3.84 Briefs.
3.85 Filing of the record.
3.86 Grant or denial of petition for review.
3.87 Decision of the Commission.
3.88 Default.
3.89 Applicability of the Rules of Practice.
3.90 Reservation of authority.
3.91 Participation of Commission staff.

Subpart F—Review of National Futures Association Registration Proceedings

§ 3.80 Scope of rules.

This subpart governs review by the Commission, under section 17(o) of the Act, of any proceeding conducted by the National Futures Association, pursuant to delegated authority, to deny, condition, suspend, restrict or revoke the registration of any applicant or registrant.

§ 3.81 Service.

(a) For the purposes of this subpart, service upon an applicant or registrant and the National Futures Association shall be effected in accordance with the provisions of § 3.50(a). Service upon the National Futures Association shall be at its principal office, 200 West Madison Street, Chicago, IL 60606.

(b) Any document which is required to be served upon the Commission under this subpart shall be served upon the Hearing Clerk and shall be effected in accordance with the provisions of § 3.50(c).

(c) A copy of any document which is required to be served upon or filed with the Commission under this subpart shall be filed concurrently with the Division of Trading and Markets (Attn: Chief Counsel) and the Division of Enforcement (Attn: Registration Appeals) at the Commission's Washington, DC Office, 2033 K Street, NW., Washington, DC 20581.

§ 3.82 Notice and effective date of order affecting registration.

(a) Any order issued by the National Futures Association upon completion of a proceeding to deny, condition, suspend, restrict or revoke registration, which is the final decision of the National Futures Association, shall be filed with the Division of Trading and Markets (Attn: Chief Counsel) and the Division of Enforcement (Attn: Registration Appeals) at the same time it is served upon the applicant or registrant.

(b) Such order shall take effect fifteen days after the date it is served upon the applicant or registrant unless the applicant or registrant files with the Commission a petition for review thereof in accordance with the provisions of this subpart: *Provided, however,* That if such order grants registration to, or conditions the registration of, an applicant, it shall not take effect until thirty days after the date it is served upon the applicant, unless (1) the Director of the Division of Trading and Markets, or the Director's designee, with the concurrence of the Director of the Division of Enforcement and the General Counsel, or their designees, by authority delegated hereby, directs that such registration may take effect at an earlier date, (2) the Commission, on its own motion, institutes review of the proceeding in accordance with the provisions of § 3.90 of this subpart, or (3) the Division of Trading and Markets or the Division of Enforcement requests the Commission to institute review of the proceeding in accordance with the provisions of § 3.91.

(c) If an applicant or registrant files a timely petition for review in accordance with the provisions of § 3.83, the effective date of the order of the National Futures Association shall be stayed pending a final determination and order by the Commission with respect to such petition.

§ 3.83 Petition for review.

(a) *Time to file.* Within fifteen days of service of a final order of the National Futures Association to deny, condition, suspend, restrict or revoke registration, the applicant or registrant may serve upon the Commission a petition for review thereof, together with proof of service upon the National Futures Association.

(b) *Contents.* The petition for review shall include:

(1) The name of the applicant or registrant;

(2) The statutory disqualification to which the applicant or registrant has been found to be subject;

(3) A concise statement of the facts underlying the statutory disqualification; and

(4) A copy of the order of the National Futures Association and the relief sought therefrom.

§ 3.84 Briefs.

(a) Within thirty days after service of a petition for review, the applicant or registrant shall file a brief in support thereof, together with proof of service upon the National Futures Association. The brief shall include a statement of the reasons why it is claimed that the order of the National Futures Association should not be affirmed in accordance with the provisions of § 3.87 of this subpart and the specific facts which support those reasons.

(b) Within thirty days after service of the brief in support of the petition for review, the National Futures Association may file an answering brief, together with proof of service upon the applicant or registrant. If no answering brief is filed, the final order of the National Futures Association shall be deemed the answer to the petition.

(c) No further briefs shall be permitted.

§ 3.85 Filing of the record.

Within thirty days of service of the petition for review, the National Futures Association shall file with the Commission the record of the proceeding which shall include: a certified copy of any order issued by a court of competent jurisdiction, federal or state governmental agency, or self-regulatory organization or such other document which sets forth the statutory disqualification to which the applicant or registrant has been found to be subject; the notice issued by the National Futures Association of its intent to deny, condition, suspend, restrict or revoke the registration of the applicant or registrant; the answer and all documentary evidence submitted therewith by the applicant or registrant; the reply filed by the National Futures Association; any interim order issued by the National Futures Association; the transcript of any oral hearing which may have been held, including any documentary evidence submitted in connection therewith; and the final order of the National Futures Association. Upon request, the National Futures Association shall serve a copy of such record upon the applicant or registrant at the same time it is filed with the Commission, provided the applicant or registrant agrees to pay the National Futures Association reasonable fees for such copy.

§ 3.86 Grant or denial of petition for review.

(a) *Determination.* The determination to review any proceeding conducted by the National Futures Association to deny, condition, suspend, restrict or revoke registration is a matter committed to the Commission's discretion.

(b) *Denial of petition.* Should the Commission decline to grant review, the Commission shall issue an order to that effect which shall be deemed a final order of the Commission five days after service upon the applicant or registrant. A copy of such order shall also be served upon the National Futures Association at the same time.

(c) *Granting of petition.* Based upon the petition, the briefs and the record submitted pursuant to § 3.85, the Commission may grant review of the order of the National Futures Association.

§ 3.87 Decision of the Commission.

(a) Upon review, the Commission may affirm, modify, set aside or remand for further proceedings, in whole or in part, the order of the National Futures Association.

(b) The Commission shall affirm the order of the National Futures Association, unless the Commission finds that:

(1) The proceeding was not conducted in accordance with the rules of the National Futures Association;

(2) The National Futures Association did not observe fundamental fairness in the conduct of the proceeding;

(3) The order issued by the National Futures Association was not in accordance with the policies of the Commission with respect to the statutory disqualification provisions of the Act; or

(4) The weight of the evidence in the record does not support a finding that the applicant or registrant is subject to a statutory disqualification under section 8a(2), 8a(3) or 8a(4) of the Act.

§ 3.88 Default.

In the event that the applicant or registrant or the National Futures Association fails to file any document required under this subpart, the Commission, in its discretion, may dismiss the petition or, once instituted, the proceeding or, based on the record before it, may affirm, modify, set aside or remand for further proceedings the order of the National Futures Association. An order issued pursuant to this section shall be deemed a final order of the Commission five days after service upon the applicant or registrant. A copy of such order shall also be served upon the National Futures Association at the same time.

§ 3.89 Applicability of the Rules of Practice.

With the exception of §§ 10.4–10.6, 10.10, 10.11, and 10.12(a)(2)–10.12(g) of this chapter, and except as otherwise may be provided by the Commission by order, the Commission's Rules of Practice shall not apply to a proceeding under this subpart. For purposes of this subpart, functions assigned by the Rules of Practice to an "Administrative Law Judge" shall be performed by the Commission.

§ 3.90 Reservation of authority.

(a) Nothing in this subpart shall prevent the Commission from initiating a proceeding in an appropriate case to deny, condition, suspend, restrict or revoke registration under the provisions of section 6(b) of the Act or subpart C of this part.

(b) Nothing in this subpart affects the authority of the Commission under section 17(o)(3) of the Act to review the granting of a registration application by the National Futures Association.

(c) On its own motion, the Commission may institute review of any proceeding conducted by the National Futures Association to deny, condition, suspend, restrict or revoke the registration of any applicant or registrant.

(d) If the Commission institutes review under paragraph (b) or (c) of this section of a proceeding conducted by the National Futures Association, it shall thereafter issue an order establishing a schedule for the filing of briefs and requiring the submission of the record of the proceeding, or designated portions of the record, and such documents applicable to the particular proceeding as may aid the Commission in the review of such proceeding and of the decision rendered therein. If the order issued by the National Futures Association has not become effective, such order shall be stayed pending a final determination and order by the Commission with respect to such petition.

§ 3.91 Participation by Commission staff.

(a) If an applicant or registrant files a petition for review in accordance with § 3.83 the Division of Trading and Markets or the Division of Enforcement may file with the Commission, together with proof of service upon the applicant or registrant and upon the National Futures Association, a memorandum stating its views with respect to the matters addressed in such petition or in the brief in support thereof. Such memorandum, which shall be made a part of the record, must be filed within the time provided for the National Futures Association to file an answering brief in accordance with the provisions of § 3.84(b).

(b) At any time after an order issued by the National Futures Association is served upon the Commission in accordance with the provisions of § 3.82(a), the Division of Trading and Markets or the Division of Enforcement may file with the Commission, together with proof of service upon the applicant or registrant and upon the National Futures Association, a memorandum requesting the Commission, on its own motion, to institute review of the granting of a registration application by the National Futures Association or of any proceeding conducted by the National Futures Association to deny, condition, suspend, restrict or revoke registration. The memorandum shall set forth with particularity the reasons why the Commission should institute such review. Upon filing of the memorandum, and unless otherwise ordered by the Commission, if the order issued by the National Futures Association has not become effective, such order shall be stayed an additional fifteen days in order to afford the Commission the opportunity to consider the request.

Issued in Washington, D.C. on September 24, 1985.

Lynn K. Gilbert,

Deputy Secretary of the Commission.

[FR Doc. 85–23086 Filed 9–26–85; 8:45 am]

BILLING CODE 6351-01-M

DEPARTMENT OF HOUSING AND URBAN DEVELOPMENT

Office of the Secretary

24 CFR Parts 44, 111, 203, 207, 236, 290, 511, 570, 850, 880, 881, 882, 883, 884, 886, 941, 968, and 990

[Docket No. R-85-1255; FR-2075]

Implementation of the Single Audit Act of 1984

AGENCY: Office of the Secretary, HUD.

ACTION: Interim rule.

SUMMARY: This interim rule implements, with reference to HUD program authorities, the requirements of the Single Audit Act of 1984 and OMB Circular A-128.

DATES: Effective Date: November 1, 1985.

Comment Due Date: November 26, 1985.

ADDRESS: Interested persons are invited to submit comments regarding this rule to the Office of the General Counsel, Rules Docket Clerk, Room 10276, Department of Housing and Urban Development, 451 Seventh Street SW., Washington, DC 20410. Communications should refer to the above docket number and title. A copy of each communication submitted will be available for public inspection during regular business hours at the above address.

FOR FURTHER INFORMATION CONTACT: Steven A. Switzer, Assistant Inspector General for Audit, Office of Inspector General, Department of Housing and Urban Development, Room 8284, 451 Seventh Street SW., Washington, DC 20410. Telephone (202) 755-6364. (This is not a toll-free number.)

SUPPLEMENTARY INFORMATION:

I. Background

In 1979, the General Accounting Office (GAO) and the Joint Financial Management Improvement Project (JFMIP) published studies citing certain ineffectual practices by State and local governments in auditing Federal financial assistance. A principal problem identified in these studies was the practice of State and local governments in auditing on a grant-by-grant basis, rather than organization-wide. On October 22, 1979, the Office of Management and Budget (OMB) responded to these concerns by issuing Attachment P to Circular A-102, "Uniform requirements for grants to State and local governments."

Attachment P set forth uniform guidelines for organization-wide audits of State and local governmental and Indian tribal recipients of Federal assistance. It explicitly provided that auditing procedures for State and local governments must be on an organization-wide basis, rather than grant-by-grant. This "single audit" approach was to be monitored by one "cognizant agency" assigned for a particular recipient organization by OMB. According to Attachment P, the cognizant agency must assess and coordinate the review of the auditing of its designated recipient organizations. Where significant inadequacies in an audit are disclosed, the recipient organization was to be advised and the auditor was to be requested to take corrective action. Other affected audit agencies were to be informed of irregularities uncovered.

Attachment P also included:

1. A requirement that a representative sample of auditing charges to Federal grants be tested, reflecting all Federal awards of financial assistance received and all cost categories that materially affected the award;
2. A requirement that audits must be conducted at least every two years; and
3. General requirements governing the scope of audits, content of the audit report, and auditor's comments on compliance and internal controls.

In the **Federal Register** of December 30, 1983 (48 FR 57483), HUD promulgated a rule at 24 CFR Part 44 implementing Attachment P across a broad range of the Department's programs. (Given the purpose of the December 1983 rule to incorporate Attachment P by reference, it contained only a minimal statement of regulatory provisions.)

II. The Single Audit Act of 1984

The Single Audit Act of 1984, 31 U.S.C. 7501-7507, establishes a more comprehensive framework for single audit requirements than did Attachment P. The enactment of the Single Audit Act reflected findings from a GAO report, "Study of Progress Made in Implementing the Single Audit Concept" (March 1984), indicating that the principal problems inhibiting implementation of Attachment P were: (1) Uncertainty among State and local officials concerning the scope and purpose of the single audit, (2) diffusion of responsibility among Federal cognizant agencies for certain audit functions, and (3) conflicts between the provisions of Attachment P and existing statutory grant audit requirements.

Key provisions of the Single Audit Act are directed toward establishing clearer guidelines for audit functions by State and local governments. The audit requirements in the Act apply to fiscal years of States and local governments that begin after December 31, 1984. According to OMB Circular A-128, the audit provisions of Attachment P to Circular A-102 must continue to be observed for fiscal years that begin before December 31, 1984. Among the provisions in the Single Audit Act that differ from requirements of Attachment P are:

1. *Definition of "Financial Assistance".*

The Act defines "financial assistance" to include a broad range of HUD's program activities: "grants, contracts, loans, loan guarantees, property, cooperative agreements, interest subsidies, insurance, or direct appropriations." *See* 31 U.S.C. 7501(4).

Attachment P did not describe the specific scope of financial assistance covered. However, sections 6 and 7(a) of OMB Circular A-102 did provide a basis for the Department's determination of the scope of financial assistance covered by Attachment P. Section 6 of Circular A-102 states that its standards apply to grants to State and local governments and Federally recognized Indian tribal governments, and that agencies are "encouraged" to apply its standards to loan and loan guarantee programs "to the extent practicable." The term "grant" is defined in section 7(a) of the OMB Circular to exclude coverage for: "Technical assistance programs which provided services instead of money or other assistance in the form of general revenue sharing, loans, loan guarantees, insurance, or contracts which are entered into and administered under procurement laws and regulations."

2. *Applicability of the Single Audit Requirements.*

Recipient State and local governments that receive $100,000 or more in Federal financial assistance in any fiscal year must have a single audit for that year. Governments receiving at least $25,000 but less than $100,000 have the option of performing either a single audit or separate program audits required by the applicable Federal statutes and regulations. Governments receiving less than $25,000 in any fiscal year are exempt from a single audit as well as from other Federal audit requirements for that year. Attachment P did not set any financial assistance level as a cutoff for single audit requirements.

3. *Focus on Auditing "Major Federal Assistance Programs".*

Under the Act, auditing is focused on "major Federal assistance programs" for compliance testing and for reporting instances of noncompliance. The definition of "major Federal assistance program" refers to the total amount of expenditures by the State or local government from a Federal program for the fiscal year, as compared to all Federal expenditures for that government for the fiscal year, except for any program for which total expenditures of Federal financial assistance by the State or local government during the fiscal year exceed the larger of (i) $300,000, or (ii) 3 percent of such total expenditures for all programs, in the case of a State or local government for which such total expenditures for all programs exceed $100 million. (Under the Act, the auditor must determine and report whether there has been compliance with laws and regulations that could have a material effect on each "major Federal assistance program" rather than on the totality of Federal programs under evaluation.)

Attachment P did not include categories of assistance programs for auditing priority; rather, it delegated authority to auditors to choose a representative number of charges to Federal awards. Any audit test was required to be representative of (1) the total set of Federal awards received, and (2) all cost categories that materially affected the award.

4. *Plan for Corrective Action.*

Under 31 U.S.C. 7502(g), if the audit report indicates any material noncompliance or material weaknesses in internal control systems, the recipient government must prepare and submit a corrective action plan to the Federal cognizant agency assigned by OMB, in accordance with a schedule established by the cognizant agency. Such plans, together with the audit report, are to be submitted to appropriate Federal officials. The corrective action plan should indicate the government's plan to eliminate the noncompliance or material weakness, or reasons why corrective action is unnecessary. However, the recipient government may decide to not submit a corrective action plan if it chooses instead to submit a statement of why corrective action is not necessary.

Attachment P did not require a formal corrective action plan to respond to the results of the audit report. It merely required that audit reports include comments on any corrective action taken or planned by the grantee.

5. *Annual Audit Requirement.*

Under 31 U.S.C. 7502(b), single audits must be performed annually, unless the respective recipient government is required by constitution, statute, regulations or policy to conduct its audit less frequently. In this situation, the recipient government would conduct single audits on a biennial basis. After December 31, 1986, recipient governments must conduct such audits annually unless a recipient government codifies a requirement for biennial audits in its constitution or statutes by January 1, 1987. Audits conducted biennally under this provision must cover both years within the biennial period.

Attachment P required that single audits must be conducted at least on a biennial basis, but stated that they would "usually" be performed annually.

6. *Allocation of Audit Cost.*

Under 31 U.S.C. 7505(b), the amount charged to a program may not be more than a "reasonably proportionate share" of the audit cost. Under this standard, the proportion of the audit cost charged to Federal programs may not (in the absence of documentation demonstrating a higher actual cost) exceed the proportion of Federal expenditures to total expenditures. Attachment P stated that in the auditor's review of a representative number of charges for single audits to Federal funding sources, these auditing charges do not include costs properly allocated to other Federally assisted programs.

Under both 31 U.S.C. 7503 and Attachment P, audit activities in addition to those required under these authorities are not precluded, but such audits must build upon the single audit and not require duplicative audit functions.

III. OMB Circular A-128

HUD has revised 24 CFR Part 44 to repeat the substantive audit requirements of OMB Circular A-128. Circular A-128, issued on April 12, 1985, implements the Single Audit Act. Each recipient organization must comply with the audit requirements of the OMB Circular for fiscal years that begin after December 31, 1984, including any amendments to those requirements prescribed by future OMB action, as published. (OMB Circular A-128 provides for exceptions for public hospitals, public colleges and universities from State and local audits and the requirements of OMB Circular A-128 as long as these entities follow audit statutory requirements and OMB Circular A-110, "Uniform requirements for grants to universities, hospitals, and other nonprofit organizations".) To comply with the provisions of the OMB Circular, an audit report and, where required, a corrective action plan must be submitted to the Federal cognizant agency. However, the submission of a corrective action plan is not necessary if the recipient government instead submits a statement explaining why the corrective action plan is not necessary.

Included among the audit provisions in the OMB Circular are:

(a) Definitions of "financial assistance", "State", and "local government";

(b) Threshold standards for applicable levels of Federal financial assistance covered by the single audit requirements;

(c) Requirements for compliance review, testing, and reporting standards;

(d) Standards for the auditing of "major Federal assistance programs";

(e) Requirements for the annual performance of single audits; and

(f) Procedures for the audit reports and plans for corrective action, including provisions for the allocation of audit costs.

Under this rule, HUD audit requirements at 24 CFR Part 44 will contain all of the substantive audit requirements in OMB Circular A-128. If any of the audit requirements in OMB Circular A-128 are revised in the future, HUD will promulgate regulations to implement those revisions in Part 44.

IV. HUD Program Regulations Affected by the Single Audit Act and OMB Circular A-128

Critical to the identification of HUD program regulations affected by the Single Audit Act and OMB Circular A-128 is the Act's definition of "financial assistance". Given the broad scope of the definition, the Department has determined that the following HUD programs with codified regulations are affected by the audit requirements of the Act and the OMB Circular:

1. Fair Housing Assistance Program (24 CFR Part 111);
2. Mutual Mortgage Insurance and Rehabilitation Loans (24 CFR Part 203);
3. Multifamily Housing Mortgage Insurance (24 CFR Part 207);
4. Multifamily Housing Interest Reduction Payments and Mortgage Insurance (24 CFR Part 236);
5. Management and Disposition of HUD-Owned Multifamily Housing Projects (24 CFR Part 290);
6. Rental Rehabilitation Grant Program (24 CFR Part 511);
7. Programs Authorized under Title I of the Housing and Community Development Act of 1974, as Amended, and Codified at 24 CFR Part 570 (Entitlement Grants, the Secretary's Fund, the HUD-Administered Small Cities Program, Urban Development Action Grants, the State's Program, and Loan Guarantees);
8. Community Development Block Grant Program for Indian Tribes and Alaskan Native Villages (24 CFR Part 571));
9. Urban Homesteading Program (24 CFR Part 590) (see revised 24 CFR 590.27, which was promulgated in the **Federal Register** of June 24, 1985 (50 FR 25941, 25948));

10. Housing Development Grant Program (24 CFR Part 850);

11. Section 8 Housing Assistance Payments Program (24 CFR Parts 880, 881, 882, 883, 884, and 886);

12. Public Housing Development Program (24 CFR Part 941);

13. Comprehensive Improvement Assistance Program (24 CFR Part 968);

14. Annual Contributions for Operating Subsidies for Public Housing Projects (24 CFR Part 990); and

15. Solar Energy and Energy Conservation Bank (24 CFR Part 1800). (Revisions to the Solar Bank rule to implement HUD audit requirements in 24 CFR Part 44 were promulgated at 24 CFR 1800.125(b) and 1800.135(b), in the **Federal Register** of June 14, 1985 (50 FR 25010, 25020.))

Except as otherwise noted, these affected HUD program regulations are revised under this interim rule to refer to audit requirements for State and local governments under 24 CFR Part 44.

Concerning the programs authorized under Title I of the Housing and Community Development Act of 1974, as amended, the audit requirements in § 570.509 (which are contained in 24 CFR Part 570, Subpart J) are applicable to all of the following programs at 24 CFR Part 570 with the exception of the State's program (24 CFR Part 570, Subpart I):

1. Entitlement grants program (Subpart D);
2. Secretary's Fund program (Subpart E);
3. Small Cities program (Subpart F);
4. Urban Development Action Grant program (Subpart G); and
5. Loan Guarantees program (Subpart M).

In addition, the Indian Community Development Block Grant Program regulations at 24 CFR 571.500 refer to 24 CFR Part 570, Subpart J for applicable regulations on grant administration, including audit requirements.

In a letter dated September 29, 1981, the Director of OMB exempted States participating in the State's program from the requirements of OMB Circulars A-87 and A-102 (including the audit requirements of Attachment P to Circular A-102), provided that States apply equivalent procedures for cost accountability and audits. However, since grants made under the State's program are within the Act's definition of "Federal financial assistance", the State's program is not exempt from the requirements of the Single Audit Act.

Under the definition of "financial assistance" in OMB Circular A-128, audit requirements for the Department's single family and multifamily mortgage insurance programs apply to State and local governments when they act as mortgagees. The insurance contract provides that HUD will pay in insurance claim to a mortgagee upon the assignment of the mortgage or conveyance of the property to HUD after a default. (For a definition of mortgagee under the National Housing Act, see 12 U.S.C. 1707.) This interim rule adds provisions to 24 CFR Parts 203 and 207 to recognize that public mortgagees under the Department's single family and multifamily mortgage insurance programs, respectively, and their responsibilities under 24 CFR Part 44.

There are other HUD activities without codified regulations that are affected by the audit requirements of the Single Audit Act and OMB Circular A-128. Included among these HUD program activities are technical assistance grants provided by the HUD Office of Policy Development and Research and Congregate Housing contracts to eligible recipient governments. The primary factor for HUD's determination that this or any other uncodified activity involves recipient government responsibilities under the Single Audit Act will be the definition of "financial assistance" under section 7501(4) of the Act.

HUD is publishing this revision to Part 44, implementing the Single Audit Act and OMB Circular A-128, as an interim rule. The rule repeats the substantive audit requirements of Circular A-128, and because the OMB Circular was published for comment in the **Federal Register** (49 FR 50134, December 26, 1984) before being made effective, there is ample justification for making this rule effective without an additional comment period. There does exist, however, the possibility that public interest might be expressed with reference to the programs of the Department of Housing and Urban Development to which the rule is being made applicable. For that reason, the Department is inviting public comment for a period of sixty days, and will take these comments into account in publishing a final rule.

V. Miscellaneous

This rule does not constitute a "major rule" as that term is defined in section 1(b) of Executive Order 12291 on Federal Regulation issued by the President on February 17, 1981. The rule does not: (1) Have an annual effect on the economy of $100 million or more; (2) cause a major increase in costs or prices for consumers, individual industries, Federal, State, or local agencies or geographic regions; or (3) have significant adverse effect on competition, employment, investment, productivity, innovation or on the ability of United States-based enterprises to compete with foreign-based enterprises in domestic or export markets. This rule implements the significant audit requirements in the Single Audit Act and in OMB Circular A-128 that supercede Attachment P of OMB Circular A-102. It does not impose additional audit requirements.

Consistent with the provisions of 5 U.S.C. 605 (the Regulatory Flexibility Act), the Secretary has determined that this rule would not have a significant economic impact on a substantial number of small entities, because the audit requirements in this rule follow the Single Audit Act and OMB Circular A-128. The audit requirements of these authorities apply to State and local governments that meet certain threshold requirements for Federal assistance. In addition, section 19 of the OMB Circular is intended to encourage the use of small and minority audit firms to implement the Act.

A Finding of No Significant Impact with respect to the environment has been made in accordance with HUD regulations in 24 CFR Part 50, which implement section 102(c) of the National Environmental Policy Act of 1969. The Finding of No Significant Impact is available for public inspection during regular business hours at the Office of the Rules Docket Clerk at Room 10276, Department of Housing and Urban Development, 451 Seventh Street SW., Washington, DC 20410.

Information collection requirements contained in this regulation have been approved by the Office of Management and Budget under the provisions of the Paperwork Reduction Act of 1980 (Pub. L. 96-511) and have been assigned OMB control number 2535-0094.

This rule is listed as item number 216 in the Department's Semiannual Agenda of Regulations published on April 29, 1985 (50 FR 17286) under Executive Order 12291 and the Regulatory Agenda.

The Catalog of Federal Domestic Assistance program numbers affected by this rule are 14.108, 14.110, 14.112, 14.115, 14.116, 14.117, 14.119, 14.120, 14.121, 14.122, 14.123, 14.124, 14.125, 14.126, 14.127, 14.129, 14.134, 14.135, 14.137, 14.138, 14.139, 14.140, 14.156, 14.161, 14.162, 14.163, 14.164, 14.165, 14.166, 14.167, 14.169, 14.170, 14.172, 14.173, 14.174, 14.218, 14.219, 14.221, 14.222, 14.223, 14.225, 14.227, 14.228, 14.230, 14.401, 14.550, 14.850, 14.851, and 14.852.

List of Subjects

24 CFR Part 44

Audit requirements: non-federal governmental entities, Reporting and recordkeeping requirements.

24 CFR Part 111

Fair housing, Cooperative agreements, Grant programs: housing and community development.

24 CFR Part 203

Home improvement, Loan programs: housing and community development, Mortgage insurance, Solar energy.

24 CFR Part 207

Mortgage insurance, Rental housing, Mobile home parks.

24 CFR Part 236

Low and moderate income housing, Mortgage insurance, Rent subsidies, Taxes, Utilities, Projects.

24 CFR Part 290

Mortgage insurance, Low and moderate income housing.

24 CFR Part 511

Rental Rehabilitation grants, Administrative practice and procedure, Grant programs: housing and community development, Low and moderate income housing, Reporting and recordkeeping requirements.

24 CFR Part 570

Community development block grants, Grant programs: housing and community development, Loan programs; housing and community development, Low and moderate income housing, New communities, Pockets of poverty, Small cities.

24 CFR Part 850

Grant programs: housing and community development, Relocation assistance, Rental housing, Low and moderate income housing, Cooperatives.

24 CFR Part 880

Grant programs: housing and community development, Rent subsidies, Low and moderate income housing, New construction.

24 CFR Part 881

Grant programs; housing and community development, Rent subsidies, Low and moderate income housing.

24 CFR Part 882

Grant programs: housing and community development, Housing, Mobile homes, Rent subsidies, Low and moderate income housing.

24 CFR Part 883

Grant programs: housing and community development, Rent subsidies, New construction and substantial rehabilitation, Low and moderate income housing.

24 CFR Part 884

Grant programs: housing and community development, Rent subsidies, Rural areas, Low and moderate income housing.

24 CFR Part 886

Grant programs: housing and community development, Low and moderate income housing, Rent subsidies.

24 CFR Part 941

Loan programs: housing and community development, Public housing, Prototype costs, Cooperative agreements, Turnkey.

24 CFR Part 968

Loan programs: housing and community development, Public housing, Reporting and recordkeeping requirements, Substantial rehabilitation.

24 CFR Part 990

Grant programs: housing and community development, Low and moderate income housing, Public housing.

Accordingly, the Department of Housing and Urban Development (HUD) amends Title 24, Code of Federal Regulations as follows:

1. Title 24, Part 44 is revised to read as follows:

PART 44—NON-FEDERAL GOVERNMENTAL AUDIT REQUIREMENTS

Sec.
44.1 Purpose.
44.2 Definitions.
44.3 Scope of audit.
44.4 Frequency of audit.
44.5 Internal control and compliance reviews.
44.6 Subrecipients.
44.7 Relationship to other audit requirements.
44.8 Cognizant agency responsibilities.
44.9 Illegal acts or irregularities.
44.10 Audit reports.
44.11 Audit resolution.
44.12 Audit workpapers and reports.
44.13 Audit costs.
44.14 Sanctions.
44.15 Auditor selection.
44.16 Small and minority audit firms.
44.17 Reporting.
44.18 HUD audits.
Appendix to Part 44—Definition of Major Program as provided in the Single Audit Act of 1984.

Authority: Single Audit Act of 1984 (31 U.S.C. 7501-7507); sec. 7(d) of the Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

§ 44.1 Purpose.

(a) This part implements the general audit requirements for recipient organizations in OMB Circular A-128 "Audits of State and local governments." The OMB Circular was issued under the Single Audit Act of 1984, 31 U.S.C. 7501-7507. OMB Circular A-128 supersedes Attachment P, "Audit requirements," of Circular A-102, "Uniform requirements for grants to State and local governments."

(1) This part repeats all substantive audit requirements in OMB Circular A-128. If any of the substantive audit requirements in OMB Circular A-128 are revised in the future, HUD shall promulgate regulations to conform this part to those revisions.

(2) The difference between the language of this part and of OMB Circular A-128 generally reflects only the substitution of certain terms and phrases reflecting the implementation of the Circular into the codification for HUD regulations. For example, in certain sections of this part, references to "Federal agencies" in the Circular have been changed to "HUD" and references to "this Circular" are replaced with "OMB Circular A-128".

(b) The Single Audit Act requires State or local governments that receive $100,000 or more a year in Federal funds to have an audit made for that year. Section 7505 of the Act requires the Director of the Office of Management and Budget (OMB) to prescribe policies, procedures and guidelines to implement the Act. It specifies that OMB shall designate "cognizant" Federal agencies, determine criteria for making appropriate charges to Federal programs for the cost of audits, and provide procedures to assure that small firms or firms owned and controlled by disadvantage individuals have the opportunity to participate in contracts for single audits.

(c) Concerning the applicability of the audit requirements of the Single Audit Act, the Act requires:

(1) State or local governments that receive $100,000 or more a year in Federal financial assistance shall have an audit conducted in accordance with the Act's requirements;

(2) State or local governments that receive between $25,000 and $100,000 a year shall have an audit conducted in accordance with the Act's requirements, or in accordance with Federal laws and

regulations governing the programs they participate in; and

(3) State or local governments that receive less than $25,000 a year shall be exempt from compliance with the Act and other Federal audit requirements. These State and local governments shall comply with audit requirements prescribed by State or local law or regulation.

(d) The Act does not exempt State or local governments from maintaining records of Federal financial assistance or from providing access to such records to Federal agencies, as provided in Federal law or in OMB Circular A-102.

(e) The Act is applicable to State and local governments with respect to any fiscal year that begins after December 31, 1984.

§ 44.2 Definitions.

"Cognizant agency" means the Federal agency assigned by OMB to carry out the responsibilities described in this part, which incorporates OMB Circular A-128.

"Federal financial assistance" means assistance provided by a Federal agency in the form of grants, contracts, cooperative agreements, loans, loan guarantees, property, interest subsidies, insurance, or direct appropriations, but does not include direct Federal cash assistance to individuals. It includes awards received directly from Federal agencies, or indirectly through other units of State and local governments.

"Federal agency" has the same meaning as the term "agency" in section 551(1) of Title 5, United States Code.

"Generally accepted accounting principles" has the meaning specified in the generally accepted government auditing standards.

"Generally accepted government auditing standards" means the *Standards For Audit of Government Organizations, Programs, Activities, and Functions*, developed by the Comptroller General, dated February 27, 1981.

"HUD" means the Department of Housing and Urban Development.

"Independent auditor" means:

(1) A State or local government auditor who meets the independence standards specified in generally accepted government auditing standards; or

(2) A public accountant who meets such independence standards.

"Indian tribe" means any Indian tribe, band, nation, or other organized group or community, including any Alaskan Native village or regional or village corporation (as defined in, or established under, the Alaskan Native Claims Settlement Act) that is recognized by the United States as eligible for the special programs and services provided by the United States to Indians because of their status as Indians.

"Internal controls" means the plan of organization and methods and procedures adopted by management to ensure that:

(1) Resource use is consistent with laws, regulations, and policies;

(2) Resources are safeguarded against waste, loss, and misuse; and

(3) Reliable data are obtained, maintained, and fairly disclosed in reports.

"Local government" means any unit of local government within a State, including a county, a borough, municipality, city, town, township, parish, local public authority, special district, school district, intrastate district, council of governments, and any other instrumentality of local government.

"Major Federal Assistance Program" is defined in the Appendix to this part.

"Public accountants" means those individuals who meet the qualification standards included in generally accepted government auditing standards for personnel performing government audits.

"State" means any State of the United States, the District of Columbia, the Commonwealth of Puerto Rico, the Virgin Islands, Guam, American Samoa, the Commonwealth of the Northern Mariana Islands, and the Trust Territory of the Pacific Islands, any instrumentality thereof, and any multi-State, regional, or interstate entity that has governmental functions and any Indian tribe.

"Subrecipient" means any person or government department, agency, or establishment that receives Federal financial assistance through a State or local government, but does not include an individual that is a beneficiary of such assistance. A subrecipient may also be a direct recipient of Federal financial assistance.

§ 44.3 Scope of audit.

(a) The audit shall be made by an independent auditor in accordance with generally accepted government auditing standards covering financial and compliance audits.

(b) The audit shall cover the entire operations of a State or local government or, at the option of that government, it may cover departments, agencies or establishments that received, expended, or otherwise administered Federal financial assistance during the year. However, if a State or local government receives $25,000 or more in General Revenue Sharing Funds in a fiscal year, it shall have an audit of its entire operations. A series of audits of individual departments, agencies, and establishments for the same fiscal year may be considered a single audit.

(c) Public hospitals and public colleges and universities may be excluded from State and local audits and from the requirements of this part. However, if such entities are excluded, audits of these entities shall be made in accordance with statutory requirements and the provisions of Circular A-110, "Uniform requirements for grants to universities, hospitals, and other nonprofit organizations."

(d) The auditor shall determine whether:

(1) The financial statements of the government, department, agency or establishment present fairly its financial position and the results of its financial operations in accordance with generally accepted accounting principles;

(2) The organization has internal accounting and other control systems to provide reasonable assurance that it is managing Federal financial assistance programs in compliance with applicable laws and regulations; and

(3) The organization has complied with laws and regulations that may have a material effect on its financial statements and on each major Federal assistance program.

§ 44.4 Frequency of audit.

Audits shall be made annually unless the State or local government has adopted, by January 1, 1987, a constitutional or statutory requirement for less frequent audits. For those governments, the cognizant agency shall permit biennial audits, covering both years, if the government so requests. It shall also honor requests for biennial audits by governments that have an administrative policy calling for audits less frequent than annual, but only for fiscal years beginning before January 1, 1987.

§ 44.5 Internal control and compliance reviews.

The independent auditor shall determine and report on whether the organization has internal control systems to provide reasonable assurance that it is managing Federal assistance programs in compliance with applicable laws and regulations.

(a) *Internal control review.* In order to provide the above-described assurance, the auditor shall conduct a study and evaluation of internal control systems used in administering Federal assistance programs. The study and evaluation shall be made whether or not the auditor intends to place reliance on such systems. As part of this review, the auditor shall:

(1) Test whether these internal control systems are functioning in accordance with prescribed procedures, and

(2) Examine the recipient's system for monitoring subrecipients and obtaining and acting on subrecipient audit reports.

(b) *Compliance review.* The auditor shall determine whether the organization has complied with laws and regulations that may have a material effect on each major Federal assistance program.

(1) In order to determine which major programs are to be tested for compliance, State and local governments shall identify in their accounts all Federal funds received and expended and the programs under which they were received. This shall include funds received directly from Federal agencies and through other State and local governments.

(2) The review shall include the selection and testing of a representative number of charges from each major Federal assistance program. The selection and testing of transactions shall be based on the auditor's professional judgment considering such factors as the amount of expenditures for the program and the individual awards; the newness of the program or changes in its conditions; prior experience with the program, particularly as revealed in audits and other evaluations (e.g., inspections, program reviews); the extent to which the program is carried out through subrecipients; the extent to which the program contracts for goods or services; the level to which the program is already subject to program reviews or other forms of independent oversight; the adequacy of the controls for ensuring compliance; the expectation of adherence or lack of adherence to the applicable laws and regulations; and the potential impact of adverse findings.

(i) In making the test of transactions, the auditor shall determine whether the amounts reported as expenditures were for allowable services or benefits, and whether the records show that those who received services or benefits were eligible to receive them.

(ii) In addition to transaction testing, the auditor shall determine whether matching requirements, levels of effort and earmaking limitations were met; whether Federal financial reports and claims for advances and reimbursements contain information that is supported by the books and records from which the basic financial statements have been prepared; and whether amounts claimed or used for matching were determined in accordance with OMB Circular A-87, "Cost principles for State and local governments," and Attachment F of OMB Circular A-102.

(iii) The principal compliance requirements of the largest Federal aid programs may be ascertained by referring to the *Compliance Supplement for Single Audits of State and Local Governments,* issued by OMB and available from the Government Printing Office. For those programs not covered in the Compliance Supplement, the auditor may ascertain compliance requirements by researching the statutes, regulations, and agreements governing individual programs.

(3) Transactions related to other Federal assistance programs that are selected in connection with examinations of financial statements and evaluations of internal controls shall be tested for compliance with Federal laws and regulations that apply to such transactions.

(Approved by the Office of Management and Budget under OMB control number 2535-0094).

§ 44.6 Subrecipients.

State and local governments that receive Federal financial assistance and provide $25,000 or more of it in a fiscal year to a subrecipient shall:

(a) Determine whether State or local subrecipients have met the audit requirements of this part and whether subrecipients covered by OMB Circular A-110, "Uniform requirements for grants to universities, hospitals, and other nonprofit organizations," have met those requirements;

(b) Determine whether the subrecipient spent Federal assistance funds provided in accordance with applicable laws and regulations. This may be accomplished by reviewing an audit of the subrecipient made in accordance with this part, Circular A-110, or through other means (e.g., program reviews) if the subrecipient has not yet had such an audit;

(c) Ensure that appropriate corrective action is taken within six months after receipt of the audit report in instances of noncompliance with Federal laws and regulations;

(d) Consider whether subrecipient audits necessitate adjustment of the recipient's own records; and

(e) Require each subrecipient to permit independent auditors to have access to the records and financial statements as necessary to comply with this part.

§ 44.7 Relationship to other audit requirements.

(a) The Single Audit Act provides that an audit made in accordance with OMB Circular A-128 shall be in lieu of any financial or financial compliance audit required under individual Federal assistance programs. To the extent that a single audit provides HUD with information and assurances the Department needs to carry out its overall program responsibilities, such information shall be used. However, any additional audits that are necessary to carry out responsibilities under Federal law and regulation shall be planned and carried out in such a manner as to avoid duplication.

(b) HUD audit requirements in this part do not limit the authority of the Department to conduct or contract for audits and evaluations of Federal financial assistance programs, nor do these audit requirements limit the authority of the HUD Inspector General or other Federal audit officials.

(c) HUD audit requirements in this part do not authorize any State or local government or subrecipient thereof to constrain HUD in any manner, from carrying out additional audits.

(d) If HUD conducts or contracts for audits in addition to the audits conducted by recipients under this part, the Department shall, consistent with other applicable laws and regulations, arrange for funding the cost of such additional audits.

§ 44.8 Cognizant agency responsibilities.

(a) The Single Audit Act provides for cognizant Federal agencies to oversee the implementation of this part. OMB will assign cognizant agencies for States and their subdivisions, and for larger local governments and their subdivisions. HUD may participate with an assigned cognizant agency, in order to fulfill the cognizant responsibilities. Smaller governments not assigned a cognizant agency will be under the general oversight of the Federal agency that provides them the most funds, whether directly or indirectly.

(b) If HUD is designated as a cognizant agency, it will:

(1) Ensure that audits are made and reports are received in a timely manner and in accordance with audit requirements of this part.

(2) Provide technical advice and liaison to State and local governments and independent auditors.

(3) Obtain or make quality control reviews of selected audits made by non-Federal audit organizations, and provide the results, when appropriate, to other interested organizations.

(4) Promptly inform other affected Federal agencies and appropriate Federal law enforcement officials of any reported illegal acts or irregularities. They should also inform State or local law enforcement and prosecuting authorities, if not advised by the recipient, of any violation of law within their jurisdiction.

(5) Advise the recipient of audits that have been found not to have met the requirements in this part. In such instances, the recipient will be expected to work with the auditor to take corrective action. If corrective action is not taken, HUD shall notify the recipient and Federal awarding agencies of the facts and make recommendations for follow-up action. Major inadequacies or repetitive substandard performance of independent auditors shall be referred to appropriate professional bodies for disciplinary action.

(6) Coordinate, to the extent practicable, audits made by or for Federal agencies that are in addition to the audits conducted under this part, so that the additional audits build upon such audits.

(7) Oversee the resolution of audit findings that affect the programs of more than one agency.

§ 44.9 Illegal acts or irregularities.

If the auditor becomes aware of illegal acts or other irregularities, prompt notice shall be given to recipient management officials above the level of involvement. The recipient, in turn, shall promptly notify the cognizant agency of the illegal acts or irregularities and of proposed and actual actions, if any. Illegal acts and irregularities include such matters as conflicts of interest, falsification of records or reports, and misappropriations of funds or other assets.

§ 44.10 Audit reports.

(a) Audit reports shall be prepared at the completion of the audit. The audit report shall state that the audit was made in accordance with the provisions of HUD requirements at 24 CFR Part 44. The report shall be made up of at least:

(1) The auditor's report on financial statements and on a schedule of Federal assistance; the financial statements; and a schedule of Federal assistance, showing the total expenditures for each Federal assistance program as identified in the *Catalog of Federal Domestic Assistance*. Federal programs or grants that have not been assigned a catalog number shall be identified under the caption "other Federal assistance."

(2) The auditor's report on the study and evaluation of internal control systems must identify the organization's significant internal accounting controls, and those controls designed to provide reasonable assurance that Federal programs are being managed in compliance with laws and regulations. It must also identify the controls that were evaluated, the controls that were not evaluated, and the material weaknesses identified as a result of the evaluation.

(3) The auditor's report on compliance containing:

(i) A statement of positive assurance with respect to those items tested for compliance, including compliance with law and regulations pertaining to financial reports and claims for advances and reimbursements;

(ii) Negative assurance on those items not tested;

(iii) A summary of all instances of noncompliance; and

(iv) An identification of total amounts questioned, if any, for each Federal assistance award, as a result of noncompliance.

(b) The three parts of the audit report may be bound into a single report, or presented at the same time as separate documents.

(c) All fraud, abuse, or illegal acts or indications of such acts, including all questioned costs found as the result of these acts that auditors become aware of, should normally be covered in a separate written report submitted in accordance with paragraph (f).

(d) In addition to the audit report, the recipient shall provide comments on the findings and recommendations in the report, including a plan for corrective action taken or planned and comments on the status of corrective actions taken on prior findings. If corrective action is not necessary, a statement describing the reason it is not should accompany the audit report.

(e) The reports shall be made available by the State or local government for public inspection within 30 days after the completion of the audit.

(f) In accordance with generally accepted government audit standards, reports shall be submitted by the auditor to the organization audited and to those requiring or arranging for the audit. In addition, the recipient shall submit copies of the reports to each Federal department or agency that provided Federal assistance funds to the recipient. Subrecipients shall submit copies to recipients that provided them Federal assistance funds. The reports shall be sent within 30 days after the completion of the audit, but no later than one year after the end of the audit period, unless a longer period is agreed to with the cognizant agency.

(g) Recipients of more than $100,000 in Federal funds shall submit one copy of the audit report, within 30 days after issuance, to a central clearing house, designated by OMB. The clearing house will keep completed audits on file and follow up with State and local governments that have not submitted required audit reports.

(h) Recipients shall keep audit reports on file for three years after their issuance.

(Approved by the Office of Management and Budget under OMB control number 2535–0094.)

§ 44.11 Audit resolution.

(a) As described in § 44.8, the cognizant agency shall be responsible for monitoring the resolution of audit findings that affect the programs of more than one Federal agency. Resolution of findings that relate to the programs of a single Federal agency will be the responsibility of the recipient and that agency. Alternative arrangements may be made on a case-by-case basis by agreement among the agencies concerned.

(b) Resolution shall be made within six months after receipt of the report by the Federal departments and agencies. Corrective action should proceed as rapidly as possible.

§ 44.12 Audit workpapers and reports.

Workpapers and reports shall be retained for a minimum of three years from the date of the audit report, unless the auditor is notified in writing by the cognizant agency to extend the retention period. Audit workpapers shall be made available upon request to the cognizant agency or its designee or the General Accounting Office, at the completion of the audit.

(Approved by the Office of Management and Budget under OMB control number 2535–0094.)

§ 44.13 Audit costs.

The cost of audits made in accordance with the audit requirements of this part are allowable charges to Federal assistance programs.

(a) The charges may be considered a direct cost or an allocated indirect cost, determined in accordance with the provisions of OMB Circular A–87, "Cost principles for State and local governments."

(b) Generally, the percentage of costs charged to Federal assistance programs for a single audit shall not exceed the percentage that Federal funds expended represent of total funds expended by the recipient during the fiscal year. The percentage may be exceeded, however, if appropriate documentation demonstrates higher actual cost.

§ 44.14 Sanctions.

No cost may be charged to Federal assistance programs for audits that are required by this part, but are not conducted in accordance with the audit requirements of this part. In case of a recipient's continued inability or unwillingness to have a proper audit, Federal agencies shall consider other appropriate sanctions, including:

(a) Withholding a percentage of assistance payments until the audit is completed satisfactorily;

(b) Witholding or disallowing overhead costs; and

(c) Suspending the Federal assistance agreement until the audit is made.

§ 44.15 Auditor selection.

In arranging for audit services, State and local governments shall follow the procurement standards prescribed by Attachment O of OMB Circular A-102, "Uniform requirements for grants to State and local governments." The standards provide that, while recipients are encouraged to enter into intergovernmental agreements for audit and other services, analysis should be made to determine whether it would be more economical to purchase the services from private firms. In instances where use of such intergovernmental agreements are required by a State statute (e.g., audit services) the State statute will take precedence.

§ 44.16 Small and minority audit firms.

Small audit firms, and audit firms owned and controlled by socially and economically disadvantaged individuals, shall have the maximum practicable opportunity to participate in contracts awarded to fulfill the audit requirements of this part. (As used in this section, the term "small audit firms" includes the term "audit firms controlled by socially and economically disadvantage individuals".) Recipients of Federal assistance shall take the following steps to further this goal:

(a) Assure that small audit firms are used to the fullest extent practicable;

(b) Make information on forthcoming opportunities available to, and arrange time schedules for the audit so as to encourage and facilitate participation by, small audit firms;

(c) Consider in the contract process whether firms competing for larger audits intend to subcontract with small audit firms;

(d) Encourage contracting with small audit firms that have traditionally audited government programs and, in cases where this is not possible, assure that these firms are given consideration for audit subcontracting opportunities;

(e) Encourage contracting with consortiums of small audit firms when a contract is too large for an individual small firm; and

(f) Use the services and assistance, as appropriate, of the Small Business Administration in the solicitation and utilization of small audit firms.

§ 44.17 Reporting.

HUD shall report to the Director of OMB on or before March 1, 1987, and annually thereafter, on the effectiveness of State and local governments in carrying out the requirements of the OMB Circular. The report shall identify each State or local government or Indian tribe that, in the opinion of HUD, has failed to comply with OMB Circular or with this part.

§ 44.18 HUD audits.

(a) The Secretary of HUD or the Secretary's authorized representative shall have access to all books, accounts, records, reports, files and other papers or property of a recipient organization pertaining to Federal assistance supplied by HUD to the recipient organization, for the purpose of making specific suveys, audits, examinations, excerpts and transcripts.

Appendix to Part 44—Definition of Major Program as Provided in the Single Audit Act of 1984

"Major Federal Assistance Program," for State and local governments having Federal assistance expenditures between $100,000 and $100 million dollars, means any program for which Federal expenditures during the applicable year exceed the larger of $300,000, or 3 percent of such total expenditures.

Where total expenditures of Federal assistance exceed $100 million, the following criteria apply:

Total expenditures of Federal financial assistance for all programs		Major Federal assistance program means any program that exceeds—
More than—	But less than—	
$100 million	$1 billion	$3 million.
$1 billion	$2 billion	$4 million.
$2 billion	$3 billion	$7 million.
$3 billion	$4 billion	$10 million.
$4 billion	$5 billion	$13 million.
$5 billion	$6 billion	$16 million.
$6 billion	$7 billion	$19 million.
Over $7 billion		$20 million.

PART 111—FAIR HOUSING ASSISTANCE PROGRAM

2. The authority citation for 24 CFR Part 111 is revised to read as set forth below, and any authority citation following any section in Part 111 is removed:

Authority: Title VIII of the Civil Rights Act of 1968 (42 U.S.C. 3601–19); sec. 7(d) of the Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

3. In § 111.108, a new paragraph (d) is added, to read as follows:

§ 111.108 Program administration.

* * * * *

(d) All State and local agencies that receive financial assistance under the Fair Housing Assistance Program shall conduct audits in accordance with 24 CFR Part 44.

PART 203—MUTUAL MORTGAGE INSURANCE AND REHABILITATION LOANS

4. The authority citation for 24 CFR Part 203 continues to read as follows:

Authority: Sections 203 and 211 of the National Housing Act (12 U.S.C. 1709 and 1715b); sec. 7(d) of the Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

5. In § 203.7, a new paragraph (c) is added, to read as follows:

§ 203.7 Governmental institutions and national mortgage associations.

* * * * *

(c) Since the insuring of mortgage notes or other evidence of indebtedness under the National Housing Act constitutes "financial assistance" for purposes of audit requirements set out in 24 CFR Part 44, State and local governments (as defined in § 44.2) that receive mortgage insurance as mortgagees shall conduct audits in accordance with HUD audit requirements at 24 CFR Part 44.

PART 207—MULTIFAMILY HOUSING MORTGAGE INSURANCE

6. The authority citation for 24 CFR Part 207 continues to read as follows:

Authority: Sections 207 and 211 of the National Housing Act (12 U.S.C. 1713 and 1715b); sec. 7(d) of the Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

7. A new undesignated center heading and § 207.250 are added, to read as follows:

Subpart A—Eligibility Requirements

* * * * *

Audit Requirements

§ 207.250 Audit requirements for State and local governments as mortgagees.

Since the insuring of mortgage notes or other evidence of indebtedness under the National Housing Act constitutes "financial assistance" for purposes of audit requirements set out in 24 CFR Part 44. State and local governments (as defined in § 44.2) that receive mortgage insurance as mortgagees shall conduct audits in accordance with HUD audit requirements at 24 CFR Part 44.

PART 236—MORTGAGE INSURANCE AND INTEREST REDUCTION PAYMENTS FOR RENTAL PROJECTS

8. The authority citation for 24 CFR Part 236 continues to read as follows:

Authority: Secs. 211 and 236, National Housing Act (12 U.S.C. 1715b and 1715z-1); sec. 7(d) of the Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

9. A new Subpart E of 24 CFR Part 236 and § 236.901 are added, to read as follows:

Subpart E—Audit of State and Local Governments

§ 236.901 Audit.

Where a State or local government receives interest reduction payments under § 236(b) of the National Housing Act, it shall conduct audits in accordance with HUD audit requirements at 24 CFR Part 44.

PART 290—MANAGEMENT AND DISPOSITION OF HUD-OWNED MULTIFAMILY HOUSING PROJECTS

10. The authority citation for 24 CFR Part 290 is revised to read as set forth below, and any authority citation following any section in Part 290 is removed:

Authority: Secs. 202, 203, and 204, Housing and Community Development Amendments of 1978 (12 U.S.C. 1715z-1b, 1701z-11, and 1701z-12); secs. 207 and 211, National Housing Act (12 U.S.C. 1713 and 1715b); sec. 7(d), Department of HUD Act (42 U.S.C. 3535(d)).

11. A new § 290.9 is added, to read as follows:

§ 290.9 State and Local Government Audits.

Where State or local governments receive financial assistance as defined in 24 CFR 44.2, audits shall be conducted in accordance with HUD audit requirements at 24 CFR Part 44.

PART 511—RENTAL REHABILITATION GRANT PROGRAM

12. The authority citation for 24 CFR Part 511 is revised to read as set forth below, and any authority citation following any section in Part 511 is removed:

Authority: Section 17 of the United States Housing Act of 1937 (42 U.S.C. 1437o); sec. 7(d) of the Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

13. In § 511.73, paragraph (b) is revised to read as follows:

§ 511.73 Audit.

* * * * *

(b) *Audit.* The financial management systems used by local governments as grantees and, where applicable, State recipients shall provide for audits in accordance with 24 CFR Part 44.

PART 570—COMMUNITY DEVELOPMENT BLOCK GRANTS

14. The authority citation for 24 CFR Part 570 is revised to read as set forth below, and any authority citation following any section in Part 570 is removed.

Authority: Title I of the Housing and Community Development Act of 1974 (42 U.S.C. 5301–5320); sec. 7(d) of the Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

15. In § 570.496, a new paragraph (g) is added, to read as follows:

§ 570.496 Program requirements.

* * * * *

(g) *Audits.* Where States and units of general local government receive financial assistance under this part, the audit requirements in 24 CFR Part 44 shall apply.

16. In § 570.509, paragraph (b) is revised to read as follows:

§ 570.509 Audit.

* * * * *

(b) The recipient financial management systems shall provide for audits to be made by the recipient or at its direction, in accordance with 24 CFR Part 44. Where audit reports have been completed in accordance with § 44.10, these reports shall be used in conjunction with the performance review procedures of § 570.909.

PART 850—HOUSING DEVELOPMENT GRANTS

17. The authority citation for 24 CFR Part 850 is revised to read as set forth below, and any authority citation following any section in Part 850 is removed:

Authority: Section 17 of the United States Housing Act of 1937 (42 U.S.C. 1437o); sec. 7(d) of the Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

18. Section 850.73 is revised to read as follows:

§ 850.73 Audit.

* * * * *

(b) *Grantee audits.* The grantee's financial management system shall provide for audits to be conducted by the grantee or at its direction, in accordance with audit requirements in 24 CFR Part 44. Audit reports will be used in conjunction with the performance review procedures of § 850.101.

* * * * *

PART 880—SECTION 8 HOUSING ASSISTANCE PAYMENTS PROGRAM FOR NEW CONSTRUCTION

19. The authority citation for 24 CFR Part 880 continues to read as follows:

Authority: Sections 3, 5, and 8 of the United States Housing Act of 1937 (42 U.S.C. 1437a, 1437c, and 1437f); sec. 7(d) of the Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

20. A new § 880.211 is added, to read as follows:

§ 880.211 Audit.

Where a State or local government is the eligible owner of a project or a contract administrator under § 880.505 receiving financial assistance under this part, the audit requirements in 24 CFR Part 44 shall apply.

PART 881—SECTION 8 HOUSING ASSISTANCE PAYMENTS PROGRAM FOR SUBSTANTIAL REHABILITATION

21. The authority citation for 24 CFR Part 881 continues to read as follows:

Authority: Sections 3, 5, and 8 of the United States Housing Act of 1937 (42 U.S.C. 1437a, 1437c, and 1437f); sec. 7(d) of the Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

22. A new § 881.211 is added, to read as follows:

§ 881.211 Audit.

Where a State or local government is the eligible owner of a project or a contract administrator under § 881.505 receiving financial assistance under this part, the audit requirements in 24 CFR Part 44 shall apply.

PART 882—SECTION 8 HOUSING ASSISTANCE PAYMENTS PROGRAM—EXISTING HOUSING

23. The authority citation for 24 CFR Part 882 continues to read as follows:

Authority: Sections 3, 5, and 8 of the United States Housing Act of 1937 (42 U.S.C. 1437a, 1437c, and 1437f); sec. 7(d) of the Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

24. Section 882.124 is added, to read as follows:

§ 882.124 Audit.

PHAs receiving financial assistance under this part are subject to audit requirements in 24 CFR Part 44.

PART 883—SECTION 8 HOUSING ASSISTANCE PAYMENTS PROGRAM—STATE HOUSING AGENCIES

25. The authority citation for 24 CFR Part 883 continues to read as follows:

Authority: Sections 3, 5, and 8 of the United States Housing Act of 1937 (42 U.S.C. 1437a, 1437c, and 1437f); sec. 7(d) of the Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

26. A new § 883.313 is added, to read as follows:

§ 883.313 Audit.

Where housing assistance under the section 8 Program is provided for projects developed by State agencies, these agencies shall follow audit requirements in 24 CFR Part 44.

PART 884—SECTION 8 HOUSING ASSISTANCE PAYMENTS PROGRAM, NEW CONSTRUCTION SET-ASIDE FOR SECTION 515 RURAL RENTAL HOUSING PROJECTS

27. The authority citation for 24 CFR Part 884 continues to read as follows:

Authority: Sections 3, 5, and 8 of the United States Housing Act of 1937 (42 U.S.C. 1437a, 1437c, and 1437f); sec. 7(d) of the Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

28. A new § 884.124 is added, to read as follows:

§ 884.124 Audit.

Where a State or local government is the eligible owner of a project, or is a contract administrator under §§ 884.119 or 884.120, receiving financial assistance under this part, the audit requirements in 24 CFR Part 44 shall apply.

PART 886— HOUSING ASSISTANCE PAYMENTS PROGRAM—SPECIAL ALLOCATION

29. The authority citation for 24 CFR Part 886 continues to read as follows:

Authority: Sections 3, 5, and 8 of the United States Housing Act of 1937 (42 U.S.C. 1437a, 1437c, and 1437f); sec. 7(d) of the Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

30. A new § 886.131 is added, to read as follows:

§ 886.131 Audit.

Where a State or local government is the eligible owner of a project, or is a contract administrator under § 886.120, receiving financial assistance under this part, the audit requirements in 24 CFR Part 44 shall apply.

31. A new § 886.336 is added, to read as follows:

§ 886.336 Audit.

Where a State or local government is the eligible owner of a project receiving financial assistance under this part, the audit requirements in 24 CFR Part 44 shall apply.

PART 941—PUBLIC HOUSING DEVELOPMENT

32. The authority citation for 24 CFR Part 941 is revised to read as set forth below, and any authority citation following any section in Part 941 is removed:

Authority: Sections 4, 5, and 9 of the United States Housing Act of 1937 (42 U.S.C. 1437b, 1437c, and 1437g); sec. 7(d) of the Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

33. A new § 941.209 is added, to read as follows:

§ 941.209 Audit.

All PHAs that receive funds under this part for the development of lower-income housing shall comply with audit requirements in 24 CFR Part 44.

PART 968—COMPREHENSIVE IMPROVEMENT ASSISTANCE PROGRAM

34. The authority citation for 24 CFR Part 968 is revised to read as set forth below, and any authority citation following any section in Part 968 is removed:

Authority. Sections 5 and 14 of the United States Housing Act of 1937 (42 U.S.C. 1437c and 1437l); sec. 7(d) of the Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

35. Section 968.9 is revised to read as follows:

§ 968.9 Other program requirements.

* * * * *

(j) *Audits.* PHAs that receive financial assistance under this part shall comply with audit requirements in 24 CFR Part 44.

PART 990—ANNUAL CONTRIBUTIONS FOR OPERATING SUBSIDIES

36. The authority citation for 24 CFR Part 990 continues to read as follows:

Authority: Section 9 of the United States Housing Act of 1937 (42 U.S.C. 1437g); sec. 7(d) of the Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

§§ 990.104, 990.105 and 990.108 [Amended]

37. 24 CFR Part 990 is amended by removing "biennial" from §§ 990.104(a), 990.105(a)(2), and 990.108(a).

38. A new § 990.117 is added, to read as follows:

§ 990.117 Audit.

PHAs that receive financial assistance under this part shall comply with audit requirements in 24 CFR Part 44.

Dated: September 20, 1985.

John J. Knapp,

Acting Secretary.

[FR Doc. 85–23058 Filed 9–26–85; 8:45 am]

BILLING CODE 4210-32-M

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24 CFR Parts 215, 236, 813, 880, 881, 883, 884 and 913

[Docket No. R-85-1246; FR-2052]

Technical Amendments to Definition of Income

AGENCY: Office of the Secretary, (HUD).

ACTION: Final rule.

SUMMARY: This rule implements section 102(b)(3) of the Housing and Community Development Technical Amendments Act of 1984 ("1984 technical amendments"), which added a deduction from income for certain handicapped assistance expenses in the determination of an assisted family's adjusted income. This rule also implements section 102(b)(9) of the 1984 technical amendments, which extended eligibility for the Housing Voucher program, administered under section 8(o) of the United States Housing Act of 1937 ("1937 Act"), to families with incomes equal to or above 50 percent of area median income if their incomes are not above 80 percent of area median income and they are displaced by Rental Rehabilitation program activities. Other minor changes are made to 24 CFR Parts 215, 236, 813 and 913, which prescribed the definition of Annual Income and Adjusted Income used in determining rental payments of tenants in the Rent Supplement, Section 236, Section 8 and Public Housing programs, and to Parts 880, 881, 883 and 884 with respect to adjustment of utility allowances.

EFFECTIVE DATE: November 1, 1985, except for amendments to § 913.102 and 913.106, which will become effective upon subsequent notice in the **Federal Register.**

FOR FURTHER INFORMATION CONTACT: For Rent Supplement, Section 236, and Section 8 programs administered under 24 CFR Parts 880, 881 and 883–886—James J. Tahash, Director, Program Planning Division, Office of Multifamily Managment, Department of Housing and Urban Development, 451 Seventh Street, SW., Washington, D.C. 20410, telephone (202) 426–3944; for Section 8 programs administered under 24 CFR Part 882 (Existing Housing, Moderate

Rehabilitation and the Housing Voucher Demonstration Program) and for public and Indian Housing programs—Edward Whipple, Chief, Rental and Occupancy Branch, Office of Public and Indian Housing, telephone (202) 426–0744. (These are not toll-free telephone numbers.)

SUPPLEMENTARY INFORMATION:

1. Handicapped Assistance Expenses

Section 102(b)(3) of the 1984 technical amendments amended the definition of adjusted income found in section 3 of the 1937 Act, which governs the Section 8 and Public Housing programs and which is being applied administratively by HUD to the Rent Supplement and Section 236 programs for the sake of uniformity. The amendment expanded the deduction for elderly families for medical expenses to include handicapped assistance expenses and applied the deduction for handicapped assistance expenses to nonelderly families as well. The revised statute permits a deduction calculated as follows:

> (c) the amount by which the aggregate of the following expenses of the family exceeds 3 percent of annual family income: (i) medical expenses for any elderly family; and (ii) reasonable attendant care and auxiliary apparatus expenses for each handicapped member of any family, to the extent necessary to enable any member of such family (including such handicapped member) to be employed.

This rule amends the definition sections of Parts 215, 236, 813 and 913 to add a definition of Handicapped Assistance Expenses and to revise the definition of Adjusted Income to allow a deduction for Handicapped Assistance Expenses. The statute provides that the combination of Handicapped Assistance Expenses and Medical Expenses is to be deducted only to the extent this total exceeds three percent of income. For Elderly families, the rule applies the threshold to the combined total of Medical and Handicapped Assistance Expenses. For nonelderly families, such as a family with a handicapped member who is neither the head of household or spouse, the rule applies the three percent threshold to the Handicapped Assistance Expenses only, since such families are ineligible for a deduction of Medical Expenses.

Under the income definition rules for the Section 8 and Public Housing programs before their revision in May 1984, expenses for attendant care for handicapped or disabled family members were permitted as medical expenses, or (in the Section 8 program) as unusual expenses, or (in the Public Housing program) as expenses for care of an incapacitated family member. When attendant care expenses were deducted as unusual expenses or as care for an incapacitated family member, the expenses were permitted to be deducted to the extent of the employment income derived by the family member who was thereby relieved of attendant care responsibilities. This rule preserves that limitation.

Although the statute specifically addresses the deduction of certain expenses for each "handicapped" member of a family, the rule extends the definition of Handicapped Assistance Expense to cover those expenses for each "disabled" member of a family, also. The definitions of Disabled Person and Handicapped Person, as used by HUD in, for example, 24 CFR Parts 813 and 913, are effectively prescribed by section 3 of the 1937 Act. A Disabled Person is a person who is "under a disability as defined in section 223 of the Social Security Act or in section 102 of the Developmental Disabilities Service and Facilities Construction Amendments of 1970." A Handicapped Person is defined a few sentences later in section 3 of the 1937 Act, as a person having an "impairment which is expected to be of a long-continued and indefinite duration, substantially impedes such person's ability to live independently, and is of such a nature that such ability could be improved by more suitable housing conditions." The sentence that defines a Disabled Person groups such persons with Handicapped Persons as individuals eligible to be considered an Elderly Family. The two definitions generally apply to the same persons, and there appears to be no reason to refuse a deduction for the attendant care or auxiliary apparatus expenses of a Disabled Person who has never officially been classified as a Handicapped Person. Therefore, HUD has concluded that Congress intended to apply the term "handicapped member of the family" in a general sense, to include persons who have been classified as either disabled or handicapped. (This determination will obviate the need to consider whether persons classified as "disabled" also qualify as "handicapped".)

2. Revision of Income Limit for Housing Vouchers

Section 102(b)(9) of the 1984 technical amendments amended section 8(o)(3) of the 1937 Act to broaden the category of families with income greater than 50 percent of area median income that are eligible for Housing Vouchers under the demonstration Housing Voucher program. Originally, the statute provided that Housing Vouchers could only be issued to families (1) with incomes no greater than 50 percent of area median income, or (2) that had been continuously assisted under the 1937 Act. The 1984 technical amendments extended eligibility to families with incomes between 50 and 80 percent of median who are displaced by Rental Rehabilitation program activities under section 17(c) of the 1937 Act.

Eligibility of a family for a Housing Voucher under section 8(o)(3) of the 1937 Act is related to the income limit restrictions imposed by section 16(b) of the 1937 Act. Section 16(b) limits the number of units that can be leased with assistance under the 1937 Act to families that have incomes greater than 50 percent of area median income, adjusted by family size. For units first available for occupancy on or after October 1, 1981, no more than five percent may be leased to families with incomes greater than 50 percent of area median income. The five percent limit applies to the aggregate of units nationwide. Generally, no families with incomes greater than 50 percent of area median can be admitted to these units without prior HUD approval. However, approval can be granted for such applicants to be admitted to more than five percent of the units in a particular project, if HUD finds it to be justified.

Section 813.105 is the provision that implements section 16(b) of the 1937 Act. However, as published in the **Federal Register** on May 10, 1984 (49 FR 19925), § 813.105 did not specifically address the interaction of section 8(o)(3) eligibility and the section 16(b) income limit restriction. A revision to an earlier notice of funding availability for Housing Vouchers in support of the Rental Rehabilitation program was published on February 28, 1985 (50 FR 8196), to inform the public of the change made by the 1984 technical amendments in eligibility for the Housing Voucher program. The February Notice also stated that Part 813 would be amended to provide for implementation of section 16 of the 1937 Act as it applies to the revised Housing Voucher program. (For other Notices affecting the Housing Voucher program, see 49 FR 28458 (July 12, 1984) and 50 FR 19475 (May 8, 1985).)

This rule now amends § 813.105 by adding a new paragraph (d) applicable only to Housing Vouchers (and redesignating old paragraphs (d) and (e)). Under the new § 813.105(d)(1), a Housing Voucher may be issued to a family with income above 50 percent of area median income without prior permission from HUD only if the family was assisted under the 1937 Act in a unit in a public housing project that was demolished or disposed of with HUD approval, or in a unit that was assisted under a project-based Section 8 Housing Assistance Payments Contract (*i.e.*, under 24 CFR Part 880, 881, 883, 884 or 886) that is being terminated at the sole discretion of the owner. For any other family with an income over 50 percent of median, HUD must authorize issuance of a Housing Voucher. Section 813.105(d)(2) states the only two grounds for HUD to grant permission to issue a voucher to an over-50 percent of median income family: the family has continuously received assistance under the 1937 Act, or it has an income no greater than 80 percent of area median income and is being displaced from its unit by rental rehabilitation activities. If a family moves from its unit in a building undergoing such rehabilitation activities not because it is required by the owner to move but because its post-rehabilitation rent is higher than the family is willing to pay, the family is not considered to be displaced for purposes of this provision.

The reporting provision of § 813.105, which is redesignated from paragraph (e) to paragraph (f), is revised to reflect the need for data on how many housing vouchers are issued to families moving into units that first came under HAP contract after October 1, 1981, and, of these, how many vouchers are issued to families with incomes in excess of 50 percent of median.

3. Technical and Conforming Changes

In the interim rule published on July 23, 1984 (49 FR 29580) to define income for the Rent Supplement and Section 236 programs, a provision was included that directed owners to provide an analysis of utility allowances when they request HUD to approve a rent increase, or when a utility rate change would result in a cumulative increase of 10 percent or more in the current utility allowances. The 10 percent cumulative change part of that requirement had been in the previous rule. The submission of utility data along with a rent increase application was added to assure that the utility component of rent not be overlooked when rent levels are determined.

Part 813 does not include any comparable provision. The parts governing project-based Section 8 programs administered by private owners do contain provisions requiring owners to recommend whether adjustment of utility allowances is appropriate whenever the owner submits a request for an adjustment in the rents and at "other times if appropriate." These sections (880.610, 881.610, 883.711, and 884.220) are being amended in this rule to conform to the utility adjustment provisions of Parts 215 and 236 (see §§ 215.45(e) and 236.55(d)). One change is to make the requirement more specific: owners must submit not just a "recommendation" on whether to adjust utility allowances, but an "analysis" of the project's utility allowances, including information on rates and utility consumption. The other change is to specify a time other than at rent increase approval when it is "appropriate" to recommend an adjustment: when a utility rate change would result in a cumulative increase of 10 percent or more in the previously approved utility allowances.

On the other hand, changes have already been made in the Section 8 and Public Housing rules (but not in the corresponding Rent Supplement and Section 236 rules) as a result of a recent statutory requirement that HUD consult with the Department of Agriculture in determining its definition of income under section 3 of the 1937 Act. (See 50 FR 25949, June 24, 1985.) To conform the definition of income in Parts 215 and 236 with the definition in Parts 813 and 913, this rule makes a minor change in the definition of Net Family Assets and revises §§ 215.21 (b)(2) and (b)(3) and 236.3 (b)(2) and (b)(3) to permit deduction of a depreciation allowance in the determination of net income from a business, and to include in income cash or assets withdrawn from a business, unless they were reimbursement for the family's investment in the business.

In response to questions and concerns that have arisen with respect to § 913.106(b)(8), which includes in a family's annual income the income of an absent service member whose dependents are living in the unit, the phrase "other person" is being changed to "other Family member." This change is intended to clarify that the pay of an absent service member is included in the family's annual income when the service member is a family member (e.g., a son of the head of household) and the service member's children reside in the assisted household; however, the pay of an absent service member is not included in the family's annual income, even if his children do live in the household, if the service member is not a family member (such as a divorced father). In the latter case, of course, to the extent the former husband provides support for the household, these payments would be included in the family's annual income. This change is being made to all the comparable sections (§§ 813.106(b)(8), 913.106(b)(8), 215.21(b)(8), and 236.3(b)(8)).

It came to our attention that in § 913.106 and comparable sections, the term Income was used in some cases instead of the defined term Annual Income. This failure to use the defined term was an oversight, which is being corrected in this rule by the addition of the word Annual in §§ 813.106 (b) and (d), 913.106 (b) and (d), 215.21 (b) and (d), and 236.3 (b) and (d).

The citation, in the definition of Annual Income to the provisions of the Domestic Volunteer Service Act of 1973 that prohibit inclusion of benefits under that Act as income for purposes of other governmental programs was incorrect. It is being corrected in §§ 813.106(d)(3)(iii), 913.106(d)(3)(iii), 215.21(d)(3)(iii) and 236.3(d)(3)(iii).

Use of Final Rule

This rule is being published as a final rule without prior notice and comment. Providing prior notice and comment is unnecessary and would be contrary to the public interest for several reasons. The handicapped assistance expense deduction and the application of income limits to the Housing Voucher program are mandated by statute, are relatively straightforward in their application (given the history of similar provisions), and benefit some applicants and participants while not being harmful to any. The rule treats the new handicapped assistance expense the way a similar component of the medical expense and of the unusual/dependent care expense categories was treated under previous definitions of income. The treatment of income limits with reference to applicants for Housing Vouchers is very similar to the treatment of income limits applied to applicants for the Section 8 Existing Housing Certificate program. These provisions adopt established practice and are therefore not controversial.

Another reason for immediate implementation is that revisions to forms and instructions for most of the Section 8 programs, as well as for the Rent Supplement and Section 236 programs, have been developed to implement these statutory provisions at the same time as for implementation of the major changes required by rules published in May and July of 1984 (see citations above). To avoid confusion, this simultaneous development has produced one set of forms and instructions to implement the totality of changes in income definition from the 1984 rules to the present. Since the 1984 rules already require recalculations of rental payments and rebates for many tenants, a second revision occasioned by the delay involved in development of first, a proposed and then a final rule, would require a great burden on PHAs and owners and would be harmful to some tenants.

The other changes made by this rule are minor and technical in nature and would not be likely to draw significant public comment.

Findings and Certifications

A Finding of No Significant Impact with respect to the environment has been made in accordance with HUD regulations in 24 CFR Part 50 that implement section 102(2)(C) of the National Environmental Policy Act of 1969, 42 U.S.C. 4332, in connection with the rules that are being amended herein. The Finding of No Significant Impact is available for public inspection and copying during regular business hours in the Office of the Rules Docket Clerk, Room 10276, 451 Seventh Street SW., Washington, D.C. 20410.

This rule does not constitute a "major rule" as that term is defined in section 1(b) of the Executive Order of Federal Regulation issued by the President on February 17, 1981. Analysis of the rule indicates that it does not: (1) Have an annual effect on the economy of $100 million or more, (2) cause a major increase in costs or prices for consumers, individual industries, Federal, State or local government agencies or geographic regions, or (3) have a significant adverse effect on competition, employment, investment, productivity, innovation, or on the ability of United States-based enterprises to compete with foreign-based enterprises in domestic or export markets.

Under 5 U.S.C. 605(b) (the Regulatory Flexibility Act), the undersigned hereby certifies that this rule does not have a significant economic impact on a substantial number of small entities, because it makes only minor changes in the income definitions used by owners and PHAs in administration of assisted housing programs.

This rule was listed as sequence number 103 under the Office of Housing in the Department's Semiannual Regulatory Agenda published on April 29, 1985 (50 FR 17285, 17289) under Executive Order 12291 and the Regulatory Flexibility Act.

The information collection requirements contained in this rule were submitted to the Office of Management and Budget for review under the provisions of the Paperwork Reduction Act of 1980, 44 U.S.C. 3501-3520. All requirements have been approved and have been assigned OMB control numbers 2502-0315, 2502-0204, and 2502-0161.

The Catalog of Domestic Assistance numbers are 14.103, 14.149 and 14.156.

List of Subjects

24 CFR Part 215

Grant programs—housing and community development, Rent subsidies.

24 CFR Part 236

Low and moderate income housing, Mortgage insurance, Rent subsidies.

24 CFR Part 813

Lower income housing.

24 CFR Part 880

Grant programs—housing and community development, Rent subsidies, Low and moderate income housing, New construction.

24 CFR Part 881

Grant programs—housing and community development, Rent subsidies, Low and moderate income housing, Substantial rehabilitation.

24 CFR Part 883

Grant programs—housing and community development, Rent subsidies, New construction and substantial rehabilitation.

24 CFR Part 884

Grant programs—housing and community development, Rent subsidies, Rural areas, Low and moderate income housing.

24 CFR Part 913

Public housing.

Accordingly, 24 CFR Parts 215, 236, 813, 880, 881, 883, 884 and 913 are amended as follows:

PART 215—RENT SUPPLEMENT PAYMENTS

1. The authority citation of Part 215 continues to read as follows:

Authority: Sec. 101(g), Housing and Urban Development Act of 1965 (12 U.S.C. 1701s); sec. 7(d), Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

2. In § 215.1, the definition of Net Family Assets is amended by adding the phrase "business or family" after the words "value of any"; a definition of Handicapped Assistance Expenses is added, in appropriate alphabetical order; and the definition of Adjusted Income is revised, to read as follows:

§ 215.1 Definitions.

* * * * *

Adjusted Income. Annual Income less the following allowances, determined in accordance with HUD instructions:

(a) $480 for each Dependent;

(b) $400 for any Elderly Family;

(c) For any Family that is not an Elderly Family but has a Handicapped or Disabled member other than the head of household or spouse, Handicapped Assistance Expenses in excess of three percent of Annual Income, but this allowance may not exceed the employment income received by Family members who are 18 years of age or older as a result of the assistance to the Handicapped or Disabled Person;

(d) For any Elderly Family

(1) That has no Handicapped Assistance Expenses, an allowance for Medical Expenses equal to the amount by which the Medical Expenses exceed three percent of Annual Income;

(2) That has Handicapped Assistance Expenses greater than or equal to three percent of Annual Income, an allowance for Handicapped Assistance Expenses computed in accordance with paragraph (c) of this section, plus an allowance for Medical Expenses that is equal to the Family's Medical Expenses;

(3) That has Handicapped Assistance Expenses that are less than three percent of Annual Income, an allowance for combined Handicapped Assistance Expenses and Medical Expenses that is equal to the amount by which the sum of these expenses exceeds three percent of Annual Income; and

(e) Child Care Expenses.

* * * * *

Handicapped Assistance Expenses. Reasonable expenses that are anticipated, during the period for which Annual Income is computed, for attendant care and auxiliary apparatus for a Handicapped or Disabled Family member, and that are necessary to enable a Family member (including the Handicapped or Disabled member) to be employed, provided that the expenses are neither paid to a member of the Family nor reimbursed by an outside source.

* * * * *

§ 215.21 [Amended]

3. Section 215.21 is revised by adding to the introductory language of paragraphs (b) and (d), before the word "Income", the word "Annual"; by removing from paragraph (b)(8) the phrase "other person" and substituting in its place the phrase "other Family member"; and by removing from paragraph (d)(3)(iii) the phrase "42 U.S.C. 4951-4993" and substituting in its place the phrase "42 U.S.C. 5044(g), 5058".

4. Paragraphs (b)(2) and (b)(3) of § 215.21 are revised to read as follows:

§ 215.21 Annual income.

* * * * *

(b) * * *

(2) The net income from operation of a business or profession. Expenditures for business expansion or amortization of capital indebtedness shall not be used as deductions in determining net income. An allowance for depreciation of assets used in a business or profession may be deducted, based on straight line depreciation, as provided in Internal Revenue Service regulations. Any withdrawal of cash or assets from the operation of a business or profession will be included in income, except to the extent the withdrawal is reimbursement of cash or assets invested in the operation by the Family;

(3) Interest, dividends, and other net income of any kind from real or personal property. Expnditures for amortization of capital indebtedness shall not be used as a deduction in determining net income. An allowance for depreciation is permitted only as authorized in paragraph (b)(2) of this section. Any withdrawal of cash or assets from an investment will be included in income, except to the extent the withdrawal is reimbursement of cash or assets invested by the Family. Where the Family has Net Family Assets in excess of $5,000, Annual Income shall include the greater of the actual income derived from all Net Family Assets or a percentage of the value of such Assets based on the current passbook savings rate, as determined by HUD;

* * * * *

PART 236—MORTGAGE INSURANCE AND INTEREST REDUCTION PAYMENTS FOR RENTAL PROJECTS

5. The authority citation for Part 236 continues to read as follows:

Authority: Secs. 211 and 236, National House Act (12 U.S.C. 1715b, 1715z-1); sec. 7(d), Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

6. In § 236.2, the definition of Net Family Assets is amended by adding the phrase "business or family" after the words "value of any"; a definition of Handicapped Assistance Expenses is added, in appropriate alphabetical order; and the definition of Adjusted Income is revised, to read as follows:

§ 236.2 Definitions.

Adjusted Income. Annual Income less the following allowances, determined in accordance with HUD instructions:

(a) $480 for each Dependent;

(b) $400 for any Elderly Family;

(c) For any Family that is not an Elderly Family but has a Handicapped or Disabled member other than the head of household or spouse, Handicapped Assistance Expenses in excess of three percent of Annual Income, but this allowance may not exceed the employment income received by Family members who are 18 years of age or older as a result of the assistance to the Handicapped or Disabled Person;

(d) For any Elderly Family

(1) That has no Handicapped Assistance Expenses, an allowance for Medical Expenses equal to the amount by which the Medical Expenses exceed three percent of Annual Income;

(2) That has Handicapped Assistance Expenses greater than or equal to three percent of Annual Income, an allowance for Handicapped Assistance Expenses computed in accordance with paragraph (c) of this section, plus an allowance for Medical Expenses that is equal to the Family's Medical Expenses;

(3) That has Handicapped Assistance Expenses that are less than three percent of Annual Income, an allowance for combined Handicapped Assistance Expenses and Medical Expenses that is equal to the amount by which the sum of these expenses exceeds three percent of Annual Income; and

(e) *Child Care Expenses.* * * *

Handicapped Assistance Expenses. Reasonable expenses that are anticipated, during the period for which Annual Income is computed, for attendant care and auxiliary apparatus for a Handicapped or Disabled Family member, and that are necessary to enable a Family member (including the Handicapped or Disabled member) to be employed, provided that the expenses are neither paid to a member of the Family nor reimbursed by an outside source.

* * * * *

§ 236.3 [Amended]

7. Section 236.3 is revised by adding to the introductory language of paragraphs (b) and (d), before the word "Income", the word "Annual"; by removing from paragraph (b)(8) the phrase "other person" and substituting in its place the phrase "other Family member"; and by removing from paragraph (d)(3)(iii) the phrase "42 U.S.C. 4951–4993" and substituting in its place the phrase "42 U.S.C. 5044(g), 5058".

8. Paragraphs (b)(2) and (b)(3) of § 236.3 are revised to read as follows:

§ 236.3 Annual income.

* * * * *

(b) * * *

(2) The net income from operation of a business or profession. Expenditures for business expansion or amortization of capital indebtedness shall not be used as deductions in determining net income. An allowance for depreciation of assets used in a business or profession may be deducted, based on straight line depreciation, as provided in Internal Revenue Service regulations. Any withdrawal of cash or assets from the operation of a business or profession will be included in income, except to the extent the withdrawal is reimbursement of cash or assets invested in the operation by the Family;

(3) Interest, dividends, and other net income of any kind from real or personal property. Expenditures for amortization of capital indebtedness shall not be used as a deduction in determining net income. An allowance for depreciation is permitted only as authorized in paragraph (b)(2) of this section. Any withdrawal of cash or assets from an investment will be included in income, except to the extent the withdrawal is reimbursement of cash or assets invested by the Family. Where the Family has Net Family Assets in excess of $5,000, Annual Income shall include the greater of the actual income derived from all Net Family Assets or a percentage of the value of such Assets based on the current passbook savings rate, as determined by HUD;

* * * * *

PART 813—DEFINITION OF INCOME, INCOME LIMITS, RENT AND REEXAMINATION OF FAMILY INCOME FOR THE SECTION 8 HOUSING ASSISTANCE PAYMENTS PROGRAMS AND RELATED PROGRAMS

9. The authority citation for Part 813 continues to read as follows:

Authority: Sections 3, 8 and 16, United States Housing Act of 1937 (42 U.S.C. 1437a, 1437f, and 1437n); section 7(d), Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

10. In § 813.102, the definition of Handicapped Assistance Expenses is added, in appropriate alphabetical order, and the definition of Adjusted Income is revised, to read as follows:

§ 813.102 Definitions.

Adjusted Income. Annual Income less the following allowances, determined in accordance with HUD instructions:

(a) $480 for each Dependent;

(b) $400 for any Elderly Family;

(c) For any Family that is not an Elderly Family but has a Handicapped or Disabled member other than the head of household or spouse, Handicapped Assistance Expenses in excess of three percent of Annual Income, but this allowance may not exceed the employment income received by Family members who are 18 years of age or older as a result of the assistance to the Handicapped or Disabled Person;

(d) For any Elderly Family

(1) That has no Handicapped Assistance Expenses, an allowance for Medical Expenses equal to the amount by which the Medical Expenses exceed three percent of Annual Income;

(2) That has Handicapped Assistance Expenses greater than or equal to three percent of Annual Income, an allowance for Handicapped Assistance Expenses computed in accordance with paragraph (c) of this section, plus an allowance for Medical Expenses that is equal to the Family's Medical Expenses;

(3) That has Handicapped Assistance Expenses that are less than three percent of Annual Income, an allowance for combined Handicapped Assistance Expenses and Medical Expenses that is equal to the amount by which the sum of these expenses exceeds three percent of Annual Income; and

(e) Child Care Expenses.

* * * * *

Handicapped Assistance Expenses. Reasonable expenses that are anticipated, during the period for which Annual Income is computed, for attendant care and auxiliary apparatus for a Handicapped or Disabled Family member, and that are necessary to enable a Family member (including the Handicapped or Disabled member) to be employed, provided that the expenses are neither paid to a member of the Family nor reimbursed by an outside source.

* * * * *

11. Section 813.105 is revised by redesignating paragraphs (d) and (e) as paragraphs (e) and (f), respectively; by adding a new paragraph (d); and by revising the redesignated paragraph (f), to read as follows:

§ 813.105 Admission to units available on or after October 1, 1981.

* * * * *

(d) *Specific limitation on Housing Vouchers.* (1) Except with the prior approval of HUD, no Housing Voucher shall be issued under section 8(o) of the 1937 Act to any Lower Income Family that is not a Very Low-Income Family unless the Family has been continuously assisted under the 1937 Act and, immediately before issuance of a Housing Voucher, the Family was residing in a public housing unit that is being demolished or disposed of with HUD approval, or was residing in a unit that was assisted under a Section 8 Housing Assistance Payments contract administered under Part 880, 881, 883, 884 or 886, that is being terminated at the sole discretion of the owner.

(2) A request by a PHA for HUD approval to grant a Housing Voucher to a Lower Income Family other than a Very Low-Income Family must state the basis for requesting the exception and provide supporting data. The only bases for granting exceptions are that either the Family has been continuously assisted under the 1937 Act, or the Family is determined to be a Lower Income Family and it is being displaced by rental rehabilitation activity under 24 CFR Part 511. For this purpose, a Family that lives in a project undergoing rental rehabilitation activities and whose post-rehabilitation rent would not be affordable is not considered displaced.

* * * * *

(f) *Reporting.* PHAs and Owners shall comply with HUD-prescribed reporting requirements that will permit HUD to maintain reasonably current data as to (1) the number of dwelling units that are subject to paragraph (a) of this section; (2) the number of units that are subject to paragraphs (c) and (d) of this section for which HAP contracts were first effective under Part 882, Subpart B of this chapter or under Section 8(o) of the 1937 Act on or after October 1, 1981 (including new HAP Contracts for Families for whom HAP Contracts had been in effect before that date for a different unit); (3) the number of Families occupying units described in clause (1) of this paragraph that were admitted to such units on or after July 1, 1984 and were not Very Low-Income Families when admitted, and (4) the number of Families occupying units described in clause (2) of this paragraph with Certificates or Vouchers issued on or after July 1, 1984 and were not Very Low-Income Families when such Certificates or Vouchers were granted.

(Information collection requirements contained in paragraph (b), (c)(2) and (d)(2) were approved by the Office of Management and Budget under control number 2502–0315. Information collection requirements contained in paragraph (f) were approved by the Office of Management and Budget under control number 2502–0204.)

§ 813.106 [Amended]

12. Section 813.106 is amended by adding to the introductory language of paragraphs (b) and (d), before the word "Income", the word "Annual"; by removing from paragraph (b)(8) the phrase "other person" and substituting in its place the phrase "other Family member"; and by removing from paragraph (d)(3)(iii) the phrase "42 U.S.C. 4951–4993" and substituting in its place the phrase "42 U.S.C. 5044(g), 5058".

PART 880—SECTION 8 HOUSING ASSISTANCE PAYMENTS PROGRAM FOR NEW CONSTRUCTION

13. The authority citation for Part 880 continues to read as follows:

Authority: Secs. 3, 5 and 8, United States Housing Act of 1937 (42 U.S.C. 1437a, 1437c, 1437f); sec. 7(d) Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

14. § 880.610 is revised to read as follows:

§ 880.610 Adjustment of utility allowances.

In connection with annual and special adjustments of contract rents, the owner must submit an analysis of the project's Utility Allowances. Such data as changes in utility rates and other facts affecting utility consumption should be provided as part of this analysis to permit appropriate adjustments in the Utility Allowances. In addition, when approval of a utility rate change would result in a cumulative increase of 10 percent or more in the most recently approved Utility Allowances, the project owner must advise the contract administrator and request approval of new Utility Allowances. Whenever a Utility Allowance for a unit is adjusted, the owner will promptly notify affected families and make a corresponding adjustment of the tenant rent and the amount of the housing assistance payment for the unit.

(Information collection requirements contained in this section have been approved by the Office of Management and Budget under OMB control number 2502–0161)

PART 881—SECTION 8 HOUSING ASSISTANCE PAYMENTS PROGRAM FOR SUBSTANTIAL REHABILITATION

15. The authority citation for Part 881 continues to read as follows:

Authority: Secs. 3, 5 and 8, United States Housing Act of 1937 (42 U.S.C. 1437a, 1437c, 1437f); sec. 7(d), Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

16. Section 881.610 is revised to read as follows:

§ 881.610 Adjustment of utility allowances.

In connection with annual and special adjustments of contract rents, the owner must submit an analysis of the project's Utility Allowances. Such data as changes in utility rates and other facts affecting utility consumption should be provided as part of this analysis to permit appropriate adjustments in the Utility Allowances. In addition, when approval of a utility rate change would result in a cumulative increase of 10 percent or more in the most recently approved Utility Allowances, the project owner must advise the contract administrator and request approval of new Utility Allowances. Whenever a Utility Allowance for a unit is adjusted, the owner will promptly notify affected families and make a corresponding adjustment of the tenant rent and the amount of the housing assistance payment for the unit.

(Information collection requirements contained in this section have been approved by the Office of Management and Budget under OMB control number 2502–0161)

PART 883—SECTION 8 HOUSING ASSISTANCE PAYMENTS PROGRAM-STATE HOUSING AGENCIES

17. The authority citation for Part 883 continues to read as follows:

Authority: Secs. 3, 5 and 8, United States Housing Act of 1937 (42 U.S.C. 1437a, 1437c, 1437f); sec. 7(d), Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

18. Section 883.711 is revised to read as follows:

§ 883.711 Adjustment of utility allowances.

In connection with annual and special adjustments of contract rents, the owner must submit an analysis of the project's Utility Allowances. Such data as changes in utility rates and other facts affecting utility consumption should be provided as part of this analysis to permit appropriate adjustments in the Utility Allowances. In addition, when approval of a utility rate change would result in a cumulative increase of 10 percent or more in the most recently approved Utility Allowances, the project owner must advise the Agency and request approval of new Utility Allowances. Whenever, a Utility Allowance for a unit is adjusted, the owner will promptly notify affected families and make a corresponding adjustment of the tenant rent and the amount of the housing assistance payment for the unit.

(Information collection requirements contained in this section have been approved by the Office of Management and Budget under OMB control number 2502–0161.)

PART 884—SECTION 8 HOUSING ASSISTANCE PAYMENTS PROGRAM, NEW CONSTRUCTION SET-ASIDE FOR SECTION 515 RURAL RENTAL HOUSING PROJECTS

19. The authority citation for Part 884 continues to read as follows:

Authority: Sections 3, 5 and 8, United States Housing Act of 1937 (42 U.S.C. 1437a, 1437c, 1437f); section 7(d), Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

20. Section 884.220 is revised to read as follows:

§ 884.220 Adjustment of utility allowances.

In connection with annual and special adjustments of contract rents, the owner must submit an analysis of the project's Utility Allowances. Such data as changes in utility rates and other facts affecting utility consumption should be provided as part of this analysis to permit appropriate adjustments in the Utility Allowances. In addition, when approval of a utility rate change would result in a cumulative increase of 10 percent or more in the most recently approved Utility Allowances, the project owner must advise the Secretary and request approval of new Utility Allowances. Whenever a Utility Allowance for a unit is adjusted, the owner will promptly notify affected families and make a corresponding adjustment of the tenant rent and the amount of the housing assistance payment for the unit.

(Information collection requirements contained in this section have been approved by the Office of Management and Budget under OMB control number 2502–0161.)

PART 913—DEFINITION OF INCOME, INCOME LIMITS, RENTS AND REEXAMINATION OF FAMILY INCOME FOR THE PUBLIC HOUSING AND INDIAN HOUSING PROGRAMS

21. The authority citation for Part 913 continues to read as follows:

Authority: Secs. 3, 6, and 16, United States Housing Act of 1937 (42 U.S.C. 1434a, 1437d, 1437n); sec. 7(d), Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

22. In 913.102, the definition of Handicapped Assistance Expenses is added, in appropriate alphabetical order, and the definition of Adjusted Income is revised, to read as follows:

§ 913.102 Definitions.

Adjusted Income. Annual Income less the following allowances, determined in accordance with HUD instructions:

(a) $480 for each Dependent;

(b) $400 for any Elderly Family;

(c) For any Family that is not an Elderly Family but has a Handicapped or Disabled member other than the head of household or spouse, Handicapped Assistance Expenses in excess of three percent of Annual Income, but this allowance may not exceed the employment income received by Family members who are 18 years of age or older as a result of the assistance to the Handicapped or Disabled Person;

(d) For any Elderly Family.

(1) That has no Handicapped Assistance Expenses, an allowance for Medical Expenses equal to the amount by which the Medical Expenses exceed three percent of Annual Income;

(2) That has Handicapped Assistance Expenses greater than or equal to three percent of Annual Income, an allowance for Handicapped Assistance Expenses computed in accordance with paragraph (c) of this section, plus an allowance for Medical Expenses that is equal to the Family's Medical Expenses;

(3) That has Handicapped Assistance Expenses that are less than three percent of Annual Income, an allowance for combined Handicapped Assistance Expenses and Medical Expenses that is equal to the amount by which the sum of these expenses exceeds three percent of Annual Income; and

(e) Child Care Expenses.

* * * * *

Handicapped Assistance Expenses. Reasonable expenses that are anticipated, during the period for which Annual Income is computed, for attendant care and auxiliary apparatus for a Handicapped or Disabled Family member and that are necessary to enable a Family member (including the Handicapped or Disabled member) to be employed, provided that the expenses are neither paid to a member of the Family nor reimbursed by an outside source.

* * * * *

§ 913.106 [Amended]

23. Section 913.106 is amended by adding to the introductory language of paragraphs (b) and (d), before the word "Income", the word "Annual"; by removing from paragraph (b)(8) the phrase "other person" and substituting in its place the phrase "other Family member"; and by removing from paragraph (d)(3)(iii) the phrase "42 U.S.C. 4951–4993" and substituting in its place the phrase "42 U.S.C. 5044(g), 5058".

Dated: September 20, 1985.

John J. Knapp,

Acting Secretary.

[FR Doc. 85–23059 Filed 9–26–85; 8:45 am]

BILLING CODE 4210-27-M

DEPARTMENT OF THE TREASURY

Bureau of Alcohol, Tobacco and Firearms

27 CFR Parts 170 and 252

[T.D. ATF-212; correction]

Distilled Spirits; Increase in Rate of Tax and Floor Stocks Tax

AGENCY: Bureau of Alcohol, Tobacco and Firearms, Treasury.

ACTION: Final rule (Treasury decision); correction.

SUMMARY: This document corrects errors made in FR Doc. 85-20120, published in the **Federal Register** on August 23, 1985 at 50 FR 34116, which implemented section 27 of the Deficit Reduction Act of 1984.

FOR FURTHER INFORMATION CONTACT: Robert G. Hardt or J.R. Whitley, Distilled Spirits and Tobacco Branch, (202) 566-7531.

SUPPLEMENTARY INFORMATION: 1. On page 34116 in the right-hand column in line 6 of the 2nd paragraph the word "distillled" should read "distilled".

2. On page 34118 in the middle column of § 170.62(b)(1)(iii) should read "Combined groups as defined in 26 CFR 1.1563-1(a)(4)".

3. On page 34119 in the left hand column in line 10 of § 170.64(a) the word "bonded" should read "bond".

4. On page 34119 in the left hand column in line 6 of § 170.64(c) add the words "of this section" after the words "paragraph (b)".

5. On page 34120 in the middle column in line 5 of § 170.69(a) insert a comma after the word "inventory".

6. On page 34120 in the middle column at the end of the 1st sentence of § 170.69(a), rename the period, and add ", except that the record of disposition supporting the inventory need not include the name of the consignee.".

7. On page 34120 in the right hand column line 23 § 170.69(c) should read "open bottles-______ proof"."

8. On page 34121 in the left hand column in § 170.70(c)(1), in line 5 the word "dividend" should read "divided".

9. On page 34121 in the left hand column in § 170.70(c)(1), in line 6 the number "10.134.4" should read "10,134.4".

10. On page 34121 in the left hand column in § 170.70(c)(2), in line 3 the number "12.667.97" should read "12,667.97".

11. On page 34121 in the right hand column in § 170.73(a) in line 11 add "of this section" after the words "paragraph (b)".

12. On page 34121 in the right hand column in § 170.73(b) in line 11 add a comma after the number "1986".

13. On page 34122 in the right hand column under GENERAL, in § 170.60, the section number and title should read:

"§ 170.76 Retention of Records".

14. On page 34123 in the left hand column in line 10 "(26 U.S.C. 6621;" should read "(26 U.S.C. 6621);".

Signed: September 20, 1985.

Stephen E. Higgins,

Director.

[FR Doc. 85-23101 Filed 9-26-85; 8:45 am]

BILLING CODE 4810-31-M

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 60

[TN-022; A-4-FRL-2903-9]

Standards of Performance for New Stationary Sources, Delegation of Authority to the State of Tennessee

AGENCY: Environmental Protection Agency.

ACTION: Notice of delegation of authority.

SUMMARY: On January 16, 1985, the State of Tennessee requested that EPA delegate authority for implementation and enforcement of one additional category of Standards of Performance for New Stationary Sources (NSPS). Since EPA's review of pertinent State laws and rules and regulations showed them to be adequate for the implementation and enforcement of this Federal standard, the Agency has made the delegation as requested.

EFFECTIVE DATE: The effective date of the delegation of authority is August 14, 1985.

ADDRESSES: Copies of the request for delegation of authority and EPA's letter of delegation are available for public inspection at EPA's Region IV office, 345 Courtland Street, NE, Atlanta, Georgia 30365.

All reports required pursuant to the newly delegated standard (listed below) should be submitted to the following address: Air Pollution Control Board, Tennessee Department of Health and Environment, T.E.R.R.A. Building, 150 Ninth Avenue North, Nashville, Tennessee 37219.

FOR FURTHER INFORMATION CONTACT: Kelly McCarty, at the EPA Region IV address listed above, and phone 404/881-3286 or FTS 257-3286.

SUPPLEMENTARY INFORMATION: Section 301, in conjunction with sections 101, and 111(c)(1) of the Clean Air Act, authorizes EPA to delegate authority to implement and enforce the standards set out in 40 CFR Part 60, NSPS.

On April 11, 1980, EPA initially delegated the authority for implementation and enforcement of the NSPS program to the State of Tennessee. On January 16, 1985, Tennessee requested a delegation of authority for implementation and enforcement of the NSPS for Subpart VV—Equipment Leaks of VOC in the Synthetic Organic Chemicals Manufacturing Industry.

After a thorough review of the request, the Regional Administrator determined that such a delegation was appropriated for these source categories with the conditions set forth in the original delegation letter of April 11, 1980. Tennessee sources subject to the requirements of Subpart VV of 40 CFR Part 60, will now be under the jurisdiction of the State of Tennessee.

The State definition of "existing source" differs considerably from, and could possibly conflict with, EPA's definition. However, the April 11, 1980, letter of delegation, condition 5, requires the State to notify EPA if it determines that the State version of a NSPS does not apply to a source which would be subject to the federal version.

The authority to make equivalency determinations pursuant to 40 CFR 60.484 is not delegated. All requests for equivalency determinations should be forwarded to EPA for action. Any determinations made by the State pursuant to paragraph 1200-3-16.43(3)(a)3. of the Tennessee air pollution control regulations, before EPA makes a decision, or inconsistent with EPA's decision, will not be recognized by EPA.

Dated: September 17, 1985.

John A. Little,

Deputy Regional Administrator.

[FR Doc. 85-23117 Filed 9-26-85; 8:45 am]

BILLING CODE 6560-50-M

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40 CFR Part 180

[PP 2F2623/R781; PH-FRL 2904-6]

Cypermethrin; Tolerance Extension

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: This rule extends tolerances for residues of the synthetic pyrethroid insecticide cypermethrin in or on certain raw agricultural commodities. This regulation to extend the maximum permissible level for residues of cypermethrin in or on these commodities was requested by ICI Americas, Inc.

EFFECTIVE DATE: Effective on September 27, 1985.

ADDRESS: Written objections, identified by the document control number [pp 2F2623/R781], may be submitted to the: Hearing Clerk (A–110), Environmental Protection Agency, Rm. 3708, 401 M St. SW., Washington, DC 20460.

FOR FURTHER INFORMATION CONTACT:

George T. LaRocca, Product Manager (PM) 15, Registration Division (TS–767C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460.

Office location and telephone number: Rm. 207, CM #2, 1921 Jefferson Davis Highway, Arlington, Va 22202, (703–557–2690).

SUPPLEMENTARY INFORMATION: EPA issued a final rule, published in the **Federal Register** of February 21, 1985 (50 FR 7172), which announced the Agency decision to extend the tolerances for residues of the insecticide cypermethrin [(±) *alpha*-cyano-(3-phenoxphenyl)methyl(±)*cis,trans*-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate] in or on the raw agricultural commodities cottonseed at 0.5 part per million (ppm); meat, fat, and meat byproducts of cattle, goat, hogs, horses, and sheep at 0.05 ppm; and milk at 0.05 ppm.

There were no comments received in response to the final rule extension.

The Agency published in the **Federal Register** of June 15, 1984 (49 Fr 24864), a notice announcing its decision to establish a tolerance for residues of cypermethrin on cottonseed, meat, fat, and meat byproducts of cattle, goats, hogs, horses, and sheep; and milk for a period extending to December 31, 1985, to cover residues existing from the conditional registration of cypermethrin. Based on additional information received in response to the June 15, 1984 notice, the Agency extended the conditional registration of cypermethrin to December 1, 1986 (see 50 FR 1112; January 9, 1985). The Agency also extended the tolerances for cypermethrin for the period extending to December 1, 1987, and the tolerance may be made permanent if registration is continued based on information received in 1986. However, ICI Americas, Inc. (ICI), was prohibited from further testing in the State of Maryland. The State of Maryland Department of Agriculture denied ICI's request for further testing of cypermethrin in Maryland until the safe use of cypermethrin can be demonstrated to the satisfaction of the State Secretary of Agriculture. The action by the State of Maryland precipitates the need for initiating a new study (including baseline data), making it impossible to meet the previous deadline of April 1986. In a letter dated June 12, 1985, ICI requested an extension of the subject petition until December 31, 1988, in order to conduct a new field monitoring study. On July 1, 1985, ICI submitted a letter from the State of Alabama authorizing ICI to conduct the study in that State.

The data submitted in the petition and other relevant material have been evaluated. The toxicological data considered in support of the tolerances, as well as the oncogenic risks associated with this use of cypermethrin, are discussed in related documents, published in the **Federal Register** of June 15, 1984 (49 FR 24864) and January 9, 1985 (50 FR 1112).

Based on a 1-year dog feeding study with a no-observed-effect level (NOEL) of 1.0 mg/kg/day [1] and using a safety factor of 100, the acceptable daily intake (ADI) has been calculated to be 0.01 mg/kg/day with a maximum permissible intake (MPI) of 0.6 mg/day for a 60-kg person. The tolerances represent a theoretical maximal residue contribution (TMRC) of 0.0307 mg/day in a 1.5-kg diet and represent 5.12 percent of the MPI.

There are no regulatory actions pending against the registration of cypermethrin. The metabolism of cypermethrin in plants and animals is adequately understood for purposes of the tolerances set forth below. An analytical method using electron capture gas-liquid chromatography is available for enforcement purposes.

Based on the above information, the Agency has determined that extending the tolerances for residues of the pesticide in or on the commodities will protect the public health. Therefore, as set forth below, the tolerances are extended to December 31, 1989, to cover residues existing from this continuing conditional registration of cypermethrin, and the tolerances may be made permanent if registration is continued based on information received in 1988.

Any person adversely affected by this regulation may, within 30 days after publication of this document in the **Federal Register**, file written objections with the Hearing Clerk, at the address given above. Such objections should specify the provisions of the regulation deemed objectionable and the grounds for the objections. If a hearing is requested, the objections must state the issues for the hearing and the grounds for the objections. A hearing will be granted if the objections are supported by grounds legally sufficient to justify the relief sought.

The Office of Management and Budget has exempted this rule from the requirements of section 3 of Executive Order 12291.

Pursuant to the requirements of the Regulatory Flexibility Act (Pub. L. 96–354, 94 Stat. 1164, 5 U.S.C. 601–612), the Administrator has determined that regulations establishing exemptions from tolerance requirements do not have a significant economic impact on a substantial number of small entities. A certification statement to this effect was published in the **Federal Register** of May 4, 1981 (46 FR 24950).

List of Subjects in 40 CFR Part 180

Administrative practice and procedure, Agricultural commodities, Pesticides and pests.

Dated: September 17, 1985.

Steven Schatzow,

Director, Office of Pesticide Programs.

Therefore, 40 CFR Part 180 is amended as follows:

1. The authority citation for 40 CFR Part 180 continues to read as follows:

Authority: 21 U.S.C. 346a.

§ 180.418 [Amended]

2. Section 180.418 *Cypermethrin; tolerances for residues* is amended by extending the effective date of December 31, 1987, to December 31, 1989.

[FR Doc. 85–23112 Filed 9–26–85; 8:45 am]

BILLING CODE 6560-50-M

FEDERAL COMMUNICATIONS COMMISSION

47 CFR Parts 0 and 1

Reduction in the Number of Commissioners From Seven to Five and Correcting a Typographical Error

AGENCY: Federal Communications Commission.

ACTION: Correction to final rule.

SUMMARY: This action (Erratum) corrects an error in paragraph 12 and the Appendix section of a Commission Order (FCC 85–256, 50 FR 26566, June 27, 1985) amending Parts 0 and 1 of the Commission's Rules. The Order, released May 31, 1985, amends various sections of the rules to (1) reflect the

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[1] The Agency used the 1-year dog feeding study NOEL to establish the ADI since the dog was the most sensitive species tested, i.e., gave the lowest NOEL.

reduction of the number of Commissioners; (2) delete references to the Telecommunications Committee and Telegraph and Telephone Committee; and (3) reflect the transfer of the functions of the Office of Opinions and Review to the Office of General Counsel.

This action also corrects an error in the Appendix section of a Commission Order (G–3) amending Part 0 of the Commission's Rules. This Order, released July 5, 1985, 50 FR 27952, July 9, 1985, makes certain editorial amendments which changed the Official title and authority of the Executive Director.

DATES: Effective October 28, 1985.

ADDRESS: Federal Communications Commission, Washington, D.C. 20554.

FOR FURTHER INFORMATION CONTACT: Steve Kaminer, (202) 632–6990; Donald L. McClure, (202) 254–6530, Office of General Counsel.

SUPPLEMENTARY INFORMATION:

List of Subjects in 47 CFR Part 1

Administrative practice and procedure.

Erratum

In the matter of amendments of §§ 0.1, 1.51(a)(3), 1.51(b), and 1.419 of the Commission's rules to reflect the reduction in the number of Commissioners from seven to five and to correct a typographical error; Amendment of §§ 0.91(h) and 0.201(a)(1) to delete references to the Telecommunications Committee and the Telegraph and Telephone Committee; Amendment of §§ 0.5(a)(11), 0.5(b) (2), (5) and 1.1205(b) to reflect the transfer of the questions of the Office of Opinions and Review to the Office of General Counsel and to make conforming changes to these Commission rules.

Released: September 24, 1985.

On May 31, 1985, the Commission released an Order (FCC 85–256) concerning the above-captioned matter. In this connection, paragraph 10 of the Order amended §§ 0.5(a)(11), 0.5(b)(2), 0.5(b)(5) and 1.1205(b). These amended sections reflected the transfer of functions from the Office of Opinions and Review to the Office of General Counsel. However, the amendment of § 0.5(b)(2) resulted in the unnecessary repetition of the phrase "General Counsel." Therefore, paragraph 12 of the Order and the Appendix are corrected to delete, in view of the previous amendment, the phrase "the Office of Opinions and Review."

§ 0.231 [Corrected]

On July 5, 1985, the Commission released an Order (G–3) concerning editorial amendments to Part 0 of the Commission's Rules. This Order, *inter alia*, amended relevant paragraphs of § 0.231. However, the amendment of the section title was neglected. Therefore, the section title of § 0.231 is corrected to insert, in lieu of "Executive Director", "Managing Director."

Federal Communications Commission.

William J. Tricarico,

Secretary.

[FR Doc. 85–23108 Filed 9–26–85; 8:45 am]

BILLING CODE 6712–01–M

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47 CFR Part 2, 74, 90, and 94

[Gen. Docket No. 84–689; RM–4426; FCC 85–388]

Allocating Spectrum for, and Establishing Other Rules and Policies Pertaining to, a Radiodetermination Satellite Service

AGENCY: Federal Communications Commission.

ACTION: Final rule.

SUMMARY: The Federal Communications Commission has allocated spectrum to provide for a new Radiodetermination Satellite Service (RDSS). The service will allow users to accurately determine their position and to relay and receive brief associated alphanumeric messages. This action is in response to favorable public comment on the FCC's Notice of Proposed Rulemaking for this proceeding.

EFFECTIVE DATE: October 31, 1985.

ADDRESS: Federal Communications Commission, 1919 M Street NW., Washington, D.C. 20554.

FOR FURTHER INFORMATION CONTACT: Melvin J. Murray, (202) 653–8168; Lawrence L. Petak, (202) 632–7025.

SUPPLEMENTARY INFORMATION:

List of Subjects

47 CFR Part 2

Frequency allocations.

47 CFR Part 90

Industrial radio service, Land transportation radio service, Public Safety radio service, Radiolocation radio service.

47 CFR Part 94

Radio.

47 CFR Part 74

Communications equipment, Education, Radio, Reporting and recordkeeping requirements, Research, Television.

Report and Order

In the Matter of Amendment of the Commission's Rules to Allocate Spectrum for, and to Establish other Rules and Policies Pertaining to, a Radiodetermination Satellite Service, FCC 85–388, Gen. Docket No. 84–689, RM–4426; In the Matter of Policies and Procedures for the Licensing of Space and Earth Stations in the Radiodetermination Satellite Service, Gen. Docket No. 84–690; In the Matter of the Application of Geostar Corporation For Authority to Construct, Launch and Operate Space Stations in the Radiodetermination Satellite Service File Nos. 2191–DSS–P/L–83, 2192–DSS–P/L–83, 2193–DSS–P/L–83, 2194–DSS–P/L–83; A request to allocate the 1606.8–1613.8 MHz band on a Primary Basis to the Radio Astronomy Service, RM–4839.

Adopted: July 25, 1985.
Released: September 13, 1985.

By the Commission: Commissioners Quello and Rivera dissenting in part and issuing statements.

Introduction

1. This *Report and Order* amends Part 2 of the Commission's Rules to allocate frequencies in the 1610–1626.5 MHz, 2483.5–2500 MHz and 5117–5183 MHz bands for use by a new radiodetermination satellite service (RDSS). Also, certain rule sections in Parts 74, 90, and 94 are amended to indicate the reallocation of the 2483.5–2500 MHz band and to provide "grandfathering" for certain stations in the band. This *Report and Order* addresses issues raised in this proceeding concerning the proposed allocations. Issues concerning technical standards and licensing policies and procedures in Gen. Docket No. 84–690 will be treated in a further proceeding.

Background

2. On July 12, 1984, the Commission adopted a *Notice of Proposed Rulemaking (Notice)* in this proceeding, proposing the allocation of three frequency bands to establish a radiodetermination satellite service.[1] This *Notice* was adopted in response to a petition for rulemaking filed with the Commission by the Geostar Corporation (Geostar) in 1983. The 1610–1626.5 MHz band was to provide for the radiodetermination user uplink to geostationary satellites. A second band, 2483.5–2500 MHz, was proposed to provide for the radiodetermination user downlink. A third band, 5117–5183 MHz,

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[1] A combined *Notice of Proposed Rulemaking* in Gen. Docket 84–689 and 84–690, FCC 83–319, 49 FR 36512, was adopted on July 12, 1984. Issues concerning the proposed allocations were assigned to General Docket 84–689; whereas, General Docket 84–690 addressed the matter of policies and procedures for the licensing of space and earth stations in the radiodetermination satellite service.

was to be used as a communications link from each satellite to a central control facility to handle the computational functions for the system. Geostar further requested 16 megahertz of spectrum in the range 6425 to 7075 MHz to provide for an uplink to transmit command and message traffic from the central control facility to each of the geosynchronous satellites within the system. In the *Notice* we suggested the use of the 6525–6541.5 MHz band, which is allocated to fixed and fixed-satellite (earth-to-space) services, for this purpose. Because the uplink operation is allowable, by definition, in this band, no allocation change was proposed.

3. In addition to the allocation issues, we proposed certain entry policies and application processing procedures in General Docket No. 84–690.[2] Concurrent filing dates were established for submitting comments and reply comments in General Docket 84–689 and General Docket 84–690. Also applications from the Geostar Corporation (File Nos. 2191–DSS–P/LA–83, 2192–DSS–P/LA–83, 2193–DSS–P/LA–83, and 2194–DSS–P/LA–83) were accepted for filing.[3] We stated that applications for radiodetermination satellite systems would be processed simultaneously with consideration of the rulemaking issues and a schedule was set for filing other applications to be considered concurrently with Geostar's applications. Inasmuch as the time for filing comments or petitions on Geostar's applications and for submitting other RDSS applications has been extended several times and because we desire to proceed in a timely fashion in this proceeding, we have chosen in this *First Report and Order* to address only those issues related to our specific allocation proposal. Other issues not addressed herein will be treated in a subsequent item.

Public Interest Findings

4. In response to our *Notice* we received many comments from various companies; local, state, and federal government agencies; and individuals that support the adoption of an allocation to provide for the proposed service. It is apparent from these comments that there is an outstanding need for the type of service discussed in the *Notice*. In particular, the proposed RDSS would provide to the end user certain capabilities that are not currently available by means of any other radio service.[4] Accurate positioning information could be attained within a fraction of a second using the type of service that Geostar has proposed. Related alphanumeric messages, as well as the positioning information, could be relayed to any other party or parties via the RDSS satellite system. It appears that this service could provide for a number of innovative applications that heretofore have not been possible. Moreover, we believe RDSS has the potential to assist in the administration and to improve the efficiency of a number of services that affect the general public. In Appendix B, we have summarized some of the various applications indicated for RDSS and have shown how a number of services intend to employ RDSS in their operations.

5. Relatively few comments questioned the public interest benefits offered by RDSS. Principal objection came from those licensees which presently are using the 2483.5–2500 MHz band and would be displaced by the adoption of the proposed rules. These specific concerns are addressed later. Negative comment was filed by the Federal Aviation Administration, which mentioned that the allocation should be postponed until the RDSS "design has matured to the point that its performance implications can be clearly established and spectrum efficiency has been seriously considered." Although we share some of the FAA's concern, we believe the allocation of the spectrum in question is in the public interest and should be made. Specifically, we note that the vast majority of comments support our proposal. Additionally, due to our policy of encouraging multiple entrants the spectrum will be used efficiently and the public will be able to choose among several competitors the system providing the best performance. Finally, the specific bands proposed for allocation to the RDSS provide minimal impact on existing users in the 2483.5–2500 MHz band and are compatible with known future plans for use of the 1.6 and 5 GHz bands.

6. The United States Coast Guard (USCG) also objected to the proposal in that as an aid to navigation RDSS should not be allowed for maritime use. Specifically, the USCG under United States Code (14 U.S.C. 81) has authority to establish aids to navigation. It points out that § 66.01(d) of Part 66 of Title 33, Code of Federal Regulations, provides "with the exception of shore based radar stations, operation of electronic aids to navigation as private aids will not be authorized." Accordingly, it requests that the RDSS be denied as a navigation aid to boats. While we believe RDSS may be used aboard boats for non-navigational purposes, we recognize that a boat owner could decide to use the system for navigation. It the USCG believes this to be of serious concern, it is suggested that appropriate information be provided to the boating public discouraging such use and emphasizing the legal consequences of such use.

7. Other opposing comments came from entities proposing the use of technology different from Geostar's. The Omninet Corporation, while recognizing public need for RDSS, suggests that the proposed allocation be used for voice communication as well as radiodetermination service. It claims that "the public interest is best served by adopting a flexibile regulatory regim that will permit market-place forces to regulate the mix of services to be offere in the RDSS bands." Omninet states tha it intends to file an RDSS application using a design which will employ frequency division multiplexing to establish 5 kHz user-channels and will allocate bandwidth for users on a random access basis as required by traffic demands. Omninet states that it will fully address the comparative benefits of its system design with respect to the Commission's policies of multiple entry and efficient use of scarc spectral resources when it files its application.[5]

8. Another entity filing comments, Transit Communications, Inc. (TCI), states that its system design, entitled "GPS-compatible Mobile Satellite" would more efficiently use the spectrum compared to Geostar. Its system would make use of the federal government's Global Positioning System (GPS) to acquire positioning information which could then be relayed with or without alphanumeric messages via satellite to another party. TCI also intends to file applications in conjunction with this proceeding.[6]

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[2] *Id.*

[3] See *Public Notice*, 49 FR 36155, published September 14, 1984.

[4] The Global Positioning System (also known as Navstar) which is being developed for Federal government use will have provisions for civil access. However, that system would not provide to nongovernment parties the position accuracy or associated message capability envisaged for the private RDSS.

[5] On April 5, 1985, the OMNINET Corporation filed an application with the Commission requesting a license to operate a radiodetermination satellite system.

[6] TCI filed an application on April 30, 1985, with the Commission requesting a license to operate a land mobile satellite system (General Docket No. 84–1234). It did not file an application in the radiodetermination satellite service. (General Docket 84–689 and 84–690).

9. Since we have indicated that we will consider all applications whether or not they are compatible with Geostar's design, we are not going to address herein the particular points raised by Geostar's competitors. All applicants proposing a system design incompatible with Geostar's were required to demonstrate how multiple entry would be accomplished and how their proposed design would better serve the public interest. The procedures developed in the *Notice* and subsequent *Orders* will be followed in the final determination of which applicants will receive authorization to construct, launch, and operate satellites in the radiodetermination satellite service. Accordingly, subsequent documents will discuss and address the issues raised by Omninet and TCI.

10. Under Section 1 of the Communications Act of 1934 the Commission is charged with the responsibility of establishing radio communication services that will promote safety of life and property. We feel that the comments submitted in this docket point to a conclusion that the establishment of a radiodetermination satellite service would be in the public interest. We perceive the primary benefits to the public to be increased safety to human life, reduced transportation and labor costs, and improvements in navigational service. Moreover, it appears there are numerous applications for RDSS, only a few of which have been summarized herein. In summary, we have determined that it is in the public interest to adopt the proposed allocations for the establishment of the radiodetermination satellite service.

Discussion

11. In the paragraphs that follow we address the individual allocations adopted herein and the allocation status of other services affected by this action.

The 1610–1626.6 MHz Band

We are adding a new U.S. footnote, as proposed in the *Notice*, to this band to indicate that the radiodetermination satellite service will be using these frequencies in the Earth-to-space direction. The 1610.6–1613.8 MHz band segment is used by the radio astronomy service, as provided in Footnote 734 to the Table of Frequency Allocations in the international Radio Regulations and our domestic regulations, on a secondary basis for observing the hydroxyl spectral line. Footnote 734 urges that all practicable steps be taken to protect radio astronomy from harmful interference. Recognizing the potential interference threat that RDSS transceivers could cause to the radio astronomy service, in the *Notice* we solicited specific comment on what possibilities there may be for sharing. Apparently in anticipation of this potential problem, the National Academy of Sciences submitted a petition for rulemaking in July 1984, requesting that the Commission upgrade the allocation status of the radio astronomy service in thge 1610.6–1613.8 MHz band from secondary to primary.[7] The comments and reply comments received in response to RM–4839 are summarized in Appendix C.

12. In comments in this proceeding dated November 13, 1984, the National Academy of Sciences (NAS) stated it has met with Geostar to address how both the radio astronomy service and the radiodetermination satellite service would cooperatively share the allocation in the 1610 MHz region. Proposed rules were developed by Geostar and NAS to permit use of the RDSS within regions where radio astronomy observations are made. In brief, RDSS licensees would restrict their transmissions to occur within the first 200 milliseconds following the one second time marks of Coordinated Universal Time when users enter Radio Astronomy Regions during a period of radio astronomy observations in the 1606.8–1613.8 MHz band.[8] Further, NAS and Geostar reached agreement on an emission limitation for out-of-band radiation. NAS wants the limitation applied to terrestrial and airborne vehicles and spacecraft, excluding only pedestrian "hand-held" units. However, Geostar believes the requirement to extend this limitation to ground-based vehicular users adds an additional regulatory burden that would increase transceiver costs and is unnecessary to protect radio astronomy observations.

13. We commend Geostar and NAS for reaching a preliminary agreement concerning a potential conflict on the use of this band. Although the issue concerning whether vehicular users should also be subject to their proposed emission limtiation is still unresolved, we choose not to address this issue now. we believe the agreement reached between Geostar and NAS concerning how to time-share this band can serve as a basis whereby RDSS service can be offered without serious interference problems between the two services. This matter of sharing between the two services will be further addressed when the technical standards and licensing policies and procedures are adopted and RDSS licensees are selected. We intend to incorporate technical standards which will permit the radio astronomy service to continue to make observations.

14. We believe our acknowledgement that certain geographical areas used for radio astronomy observations, designated as "quiet zones," need continued protection from harmful interference fulfills our obligation to take all practicable steps to protect the radio astronomy service in the 1606.8–1613.8 MHz bank. Moreover, we support the preliminary agreement reached by Geostar and NAS as a means whereby radio astronomy may be protected from harmful interference. Accordingly, we believe that elevation of radio astronomy's allocation status to primary is unnecessary. Therefore, we are denying the petition filed by NAS.

The 2483.5–2500 MHz Band

15. As proposed, we are reallocating this band to RDSS to provide for space-to-Earth transmissions.[9] The band will serve to provide a downlink to users from the various geosynchronous satellites involved in the RDSS network. In the *Notice*, we proposed the relocation of all terrestrial fixed and mobile operations in this band.[10] However, we did invite comments on whether some other accommodation might be possible to avoid the proposed relocation. In the event the relocation proposal was adopted, we asked for comments on the amount of time that should be given to existing licensees for relocation and also on the feasibility and desirability of requiring radiodetermination satellite system licensees to compensate terrestrial licensees for the costs for such relocation.

16. Comments objecting to our proposal came from those broadcast and private radio licensees that would be affected. The broadcasters mention that frequency agile equipment that can tune all three channels in the 2450–2500 MHz

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[7] The petition, RM–4839, from the National Academy of Sciences was placed on public notice August 10, 1984, and comments were received from the National Astronomy and Ionosphere Center, the National Science Foundation, National Radio Astronomy Observatory and the Geostar Corporation.

[8] The agreement reached between Geostar and NAS is reproduced in Appendix D.

[9] Use of this band by RDSS is subject to footnote 752 of the international radio regulations, which states that radio services operating within the 2400–2500 MHz band must accept harmful interference which may be caused by industrial, scientific, and medical (ISM) applications.

[10] At the time of the *Notice* there were about 70 mobile and fixed stations used for electronic news-gathering operations (ENG) and for studio-to-transmitter links. Also, there were about 21 private radio licensees operating in this band under Parts 90 and 94.

band is just now coming into the marketplace. (These channels are 2450–2467 MHz; 2467–2484 MHz; and 2484–2500 MHz.) Heretofore, broadcasters primarily have employed equipment that has been able to tune to only the lower seven channels in Band A, i.e., 1990–2110 MHz. However, because of the increased use of these lower channels for ENG, which has resulted in increasing incidences of interference, a number of broadcasters are beginning to acquire equipment that permits use of the upper three channels, i.e., 2450–2500 MHz. Also, in some cases channels 2450–2467 MHz and 2484–2500 MHz are being employed in two-way fixed links to interconnect various broadcast facilities. (Because the three channels are contiguous in frequency, the middle channel 2467–2484 MHz, is generally not used so as to provide greater isolation between the two links.) Further, broadcasters point out that although the three channels are shared with ISM equipment, it is channel, 2484–2500 MHz, that suffers the least interference from these ISM sources. For these reasons, broadcasters are objecting to the proposed reallocation.

17. Several comments in opposition to the proposed reallocation were also submitted from private radio licensees, representing primarily petroleum interests that use the 2483.5–2500 MHz band in their operations. One such licensee, Superior Oil, mentions that these frequencies are used for voice-grade communications and real-time data acquisition in support of drilling operations both on shore and off-shore. It contends that a frequency shift would entail a system redesign which could be quite costly. The API contends that the Commission underestimated the impact to current users in the 2483.5–2500 MHz band by failing to recognize that each authorization may have multiple transmitters in use. As an example, it points out that the Gulf Oil Communications Corp. holds two authorizations in the 2.5 GHz band and uses 22 transmitters in conjunction with these licenses. It also indicates that it could cost up to $1500 per transmitter to modify existing equipment to operate on other frequencies. This cost includes associated administrative, installation and engineering functions. API points out that the reallocation would disrupt the paired frequency channeling plan that now exists under Section 94.65(e). It states that channels in the 2483.5–2500 MHz band are paired with channels in the band 2459.1–2475.1 MHz. Accordingly, it requests that the Commission create a new channeling plan of "mixing" narrow (400 kHz) and wide (800 kHz) channels in the 2450–2483.5 MHz band in the event the 2483.5–2500 MHz band is reallocated. It also believes that Part 94 licensees should be permitted to operate on a secondary basis until such time as harmful interference is caused to the proposed radiodetermination satellite service. In its reply comments, Geostar concurred with API to the extent that non-RDSS use of the 2483.5–2500 MHz band be permitted for an indefinite period on a secondary basis.

18. We acknowledge the operational and economic impact that would result to existing broadcast and private radio licensees if they were to be required to be relocated in frequency as proposed. We believe fixed and temporary fixed operations, by their nature, are unlikely to pose a serious interference threat to RDSS licensees since interference would occur generally when an RDSS user attempts operation within the direct beamwidth of such a station. For this reason, we are grandfathering all fixed and temporary fixed stations in the 2483.5–2500 MHz band on a primary basis to RDSS licensees.[11]

19. In contrast, we believe mobile operations, by their nature, may pose more of a threat to RDSS operations and may create larger zones of interference. However, we desire to take an approach that strikes a balance between the equities of the existing licensees and the needs, as they develop, of the RDSS providers. For this reason, we are grandfathering all existing mobile licensees in the 2483.5–2500 MHz band also on a primary basis with one condition. If interference from these grandfathered mobiles is determined to be unacceptable, we are providing RDSS licensees the option of paying the reasonable and prudent cost of modifying an existing licensee's operation. We believe the primary status afforded both RDSS and mobile licensees in this band provides a firm basis for negotiations among affected parties and we expect a good faith effort by all parties to resolve problems that arise.

20. We are accordingly reallocating the band 2483.5–2500 MHz to the radiodetermination satellite service as proposed with the foregoing provisions for existing licensees. All existing stations licensed as of July 25, 1985, or on a subsequent date as a result of submitting an application for license on or before July 25, 1985, are herein grandfathered and may continue operations, subject only to license renewal, on a primary basis with the RDSS service. Applications for additional terrestrial operations filed after July 25, 1985 will be dismissed as not in compliance with the new allocation for this band.

21. We note that none of the reply comments addressed API's suggestion to rechannelize the 2450–2483.5 MHz band. Equipment manufacturers have indicated that the 24.4 MHz pairing arrangement currently employed could not be reduced appreciably without large increases in equipment costs. Nevertheless, we believe API's suggestion has sufficient merit and we intend to address this issue in a future rulemaking item.

The 5117–5183 MHz Band

22. The 5117–5183 MHz band is herein allocated for use by the RDSS for space-to-Earth transmissions. We proposed the following US footnote to be added to the *Table of Frequency Allocations:*

> The sub-band 5117–5183 MHz is also allocated for space-to-Earth transmissions in the fixed satellite service for use in conjunction with the radiodetermination satellite service operating in the bands 1610–1626.5 MHz and 2483.5–2500 MHz. The total power flux density at the earth's surface shall in no case exceed xx dBW/m² per Hz for all angles of arrival.

23. In our *Notice* we requested recommendations on what level of power flux density should be set to limit harmful emissions to other possible users in the band. Geostar provided the only response. It suggests that RDSS be limited to a power flux density at the earth's surface of 159 −dbW/m² per 4 kHz for all angles of arrival. It claims this limit will provide a more than sufficient level of protection to any future Microwave Landing System (MLS) operations in the 5117–5183 MHz band. We believe this level to be a reasonable limit. We therefore adopt it.

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[11] Temporary fixed stations, which are used almost exclusively in support of petroleum operations, are licensed for a general area of operation rather than a specific transmitter site, and normally stay at one location for approximately 6 months to one year. Such stations are distinguished from permanent fixed stations only in that the transmitter is periodically moved to an unspecified location within the licensed area of operation. Otherwise, the operational characteristics and interference potential of temporary fixed stations are similar to those of permanent fixed stations. Few coordination problems are anticipated between permanent fixed stations and the RDSS. However, coordination would be somewhat more difficult for temporary fixed stations since RDSS licensees would not have exact information regarding the location of such temporary systems. Therefore, we are requiring that temporary fixed licensees notify RDSS licensees directly whenever the station is moved to a new location. We believe such an arrangement will allow both temporary and permanent fixed systems to co-exist with the RDSS.

International Coordination

24. In August 1984, the Commission forwarded information on the RDSS system proposed by Geostar to the International Frequency Registration Board (IFBR) of the International Telecommunication Union (ITU). The information was subsequently advanced published in October 1984. Comments from other nations have now been received and copies of each have been sent to the IFBR. We anticipate that the coordination process will commence shortly. Also, the United States plans to seek international recognition of the use of bands adopted herein for RDSS at the 1987 Mobile WARC (World Administrative Radio Conference).[12]

Final Regulatory Flexibility Analysis

25. This proceeding allocates spectrum for the establishment of a new radiodetermination satellite service (RDSS). No comments were received which addressed the Regulatory Flexibility Analysis put forth in the *Notice*. Further, no significant alternatives which would accomplish our stated objective were suggested, nor are we aware of any. The allocations herein adopted are intended to provide for the establishment of the new RDSS service, which promises to meet a significant outstanding public need as evidence in the comments submitted to date in this proceeding.

Paper Work Reduction Act Statement

26. The decision contained herein has been analyzed with respect to the Paperwork Reduction Act of 1980 and found to contain no new or modified form, information, collection, and/or recordkeeping, labeling, disclosure or record retention requirements, and will not increase or decrease burden hours imposed on the public.

Ordering Clauses

27. Accordingly, it is ordered, effective October 31, 1985, that Parts 2, 74, 90, and 94 of the Commission's Rules (47 CFR Parts 2, 74, 90, and 94) are amended as set forth in Appendix E attached hereto. The authority for this action is found in sections 4(i) and 303(r) of the Communications Act of 1934, as amended, 47 U.S.C. 154(i) and 303(r).

28. It is further ordered that the petition filed by the National Academy of Sciences, RM-4839, is denied.

Federal Communications Commission.
William J. Tricarico,
Secretary.

Appendix A

Parties Filing Comments and/or Reply Comments in Gen. Docket No. 84-689

Aircraft Owners and Pilots Association (AOPA)
Air Line Pilots Association
American Hospital Supply Corporation
American Red Cross
Arnautical, Inc.
Association of American Railroads (AAR)
Association of Maximum Service Telecasters (AMST)
Boise Cascade Corporation
Brookes and Gatehouse, Inc. (B&G)
Cable Airport (CA)
Cadec Systems, Inc. (Cadec)
CBS Inc.
Central Committee and Telecommunications of the American Petroleum Institute (API)
Chicago and North Western Transportation
City of Los Angeles
Communications Satellite Corporation
Contract Freighters, Inc. (CFI)
Cypress College
Dona Ana County Sheriff's Department
Drug Enforcement Administration
Eaton Yachts of Essex, Connecticut
Ellis, Victor R.
Embry—Riddle Aeronautical University
Emerald Communications Company
Emery Air Freight Corporation (Emery)
Federal Aviation Administration (FAA)
Florida Radio-Phone Company
Flying Magazine
General Electric Company
Geostar Corporation
Guilford Transportation Industries, Inc.
Heacock, Lowell E.
Hearst Corporation
IESC Technologies, Inc.
Inglis, Andrew F.
International Association of Natural Resources Pilots
Jack Elliot Associates
J Boats, Inc.
Jobson, Theron S.
Koss, Christopher D.
Lahr, H. Ray
Lawrenz, James R.
Leaseway Transportation
Los Angeles County Fair
Los Angeles, County Department of Communications
L.Y. Ltd.
M/A-Com, Inc.
Marine Navigation, Inc. (MAR NAV)
Michigan Department of Transportation, Traffic and Safety Division
Mobile Data International Inc. (MDI)
Mobile Satellite Corporation
Montana Forest and Conservation Experiment Station (University of Montana)
Mt. San Antonio College
National Academy of Sciences (NAS)
National Association of Broadcasters (NAB)
National Broadcasting Company, Inc.
National Ocean Industries Association (NOIA)
National Ski Patrol System, Inc.
National Telephone Cooperative Association (NTCA)
North American Van Lines, Inc.
Northrup Corporation
Office of the State Engineer of Colorado
Omninet Corporation
Outlet Communications, Inc.
Overland Express, Inc.
Penn Jersey Piper Sales, Inc.
Piper Aircraft Corporation
Radio-Televison News Directors Association
Satellite Financial Systems Corporation
Scripps Howard Broadcasting Company
Seashore Recovery and Salvage
Seatronics, Inc.
Sherk, Jerome
Skylink Corporation
Soaring Society of America, Inc.
Society of Broadcast Engineers (SBE)
Soderlind, Paul A.
SONY
Southeastern Colorado Water Conservancy District
Spacecast Satellite Paging
Sperry Corporation
Stevenson, James F.
Story, Paul G., M.D.P.C.
Superior Oil
Transocean Air Lines, Inc.
Transit Communications, Inc.
United Airlines
United States Bureau of the Census
Untied States Department of Transportation
Utilities Telecommunications Council (UTC)
Valentine, Lee Swenson, M.D.
Vertex, Inc.
Weaver, Russell
Westar Insurance Brokers, Inc.
Western Airlines, Inc.
WPRV-TV
York Center Fire Department

Parties Filing Comments and/or Reply Comments in Petition RM-4839

Geostar Corporation
National Astronomy and Ionosphere Center
National Radio Astronomy Observatory
National Science Foundation

Appendix B

Summary of comments indicating applications for RDSS and how existing services plan to use RDSS in their operations.

1. *Safety of life.* Dr. Lee Valentine, a medical practitioner in the Pittsburgh area, indicates that there could be a 30% to 40% reduction in all deaths related to motor vehicle accidents if helicopters were able to fly victims to hospitals using a navigational aid such as RDSS. He mentions that in the northeast U.S. helicopters transporting patients are useless 50% of the time because instrument control of helicopters is not safe from the hospital to the scene and back. Dr. Valentine states that use of RDSS would allow all-weather, round-the-clock, point-to-point operation of air mobile ambulances. He estimates that several tens of thousands of young lives could annually be saved.

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[12] For further information refer to the *Second Notice of Inquiry* in Gen. Docket 84-607, FCC 85-210, 50 FR 19803, adopted April 25, 1985.

2. Paul G. Story, M.D., a medical practitioner in Montrose, Colorado, indicates that many persons come to the mountains and backcountry areas of Colorado each year for recreation. Consequently, he sees numerous injuries from minor aggravations to major disasters. Some injuries result in permanent damage or death only because of the relative isolation of the injured and time required to notify authorities and bring help. Dr. Story claims that these tragedies could be alleviated if each such person were equipped with a RDSS transceiver that could be used to solicit help and provide precise geographical coordinates to the search-and-rescue team. Dr. Story cites a number of cases where persons have died and have attributed them to not having received medical help shortly after an accident. He feels that the RDSS would be able to save lives and lessen injury in the future.

3. The State of Michigan, Department of Transportation, Traffic and Safety Division, states that the effectiveness of highway accident analysis work and subsequent remedial measures is highly dependent upon accurately locating highway accidents in relation to the roadway geometrics and major features such as intersections, bridges and rail crossings. Presently, many accident scenes are located by the reporting officer's judgment of the distance to the nearest major feature. Some are located by odometer, pacing, or actual direct measurement. However, the State of Michigan asserts that the locating of an accident site to within one to seven meters would be extremely beneficial in highway safety work and that in reducing the time spent locating an accident will, no doubt, result in the saving of a number of lives. Moreover, traffic flow could be restored more quickly, according to the State of Michigan.

4. The Fire Department of York Center, Illinois, states that RDSS would be extremely important in locating firemen who have encountered difficulty in the line of duty. As examples, it mentions such problems experienced during a large apartment-complex fire and in open country assignments.

5. *Law enforcement.* A number of comments pointed out the benefits to be derived from RDSS in the area of law enforcement. The comments maintain that knowing the location of enforcement vehicles and individual officers at all times could contribute to improved management of existing resources. In cases of emergency situations, dispatching could be handled more expediently, as well as more efficiently. Also, an improvement in the safety of individual law enforcement officers who often work alone could be made using RDSS. Further, it is felt that many lives could be saved in cases of assault by being able to respond within a much shorter period of time than is now possible. The Drug Enforcement Agency (DEA) of the U.S. Department of Justice urges the prompt approval of RDSS as the proposed service would have direct application to DEA's law enforcement operations. The proposed RDSS has the potential to significantly enhance the efficiency and effectiveness of DEA operations. In particular, the proposed RDSS can potentially be used in the tracking and location of packages, individuals, vehicles, aircraft, and vessels. Additionally, the navigation, location and safety of DEA ground, sea, and air resources could be improved.

6. *Aviation.* Parties representing aviation interests cite the improvements to safety and operation that implementation of the service would bring. Navigation would be improved where present line-of-sight coverage is not available. This includes such areas as mountainous terrain and offshore oceanic areas. RDSS would be able to provide accurate approach guidance to landing without the need for ground-based equipment at individual runways. Also, the service would likely contribute to a decrease in the probability of mid-air collisions and avoidance of collisions with obstructions to the airspace. Moreover, downed aircraft could be quickly located through use of the service. From an economic view, airlines would be able to further conserve fuel consumption by being able to redirect certain aircraft to fly at those altitudes where the wind velocity more effectively assists the flight or to alternate routes that would be more fuel efficient.

7. *Navigation.* The Northrup Corporation indicates that the proposed RDSS could provide a unique, low cost, space-based navigation system with an accuracy that would substantially improve efficiency and the safety of its company aircraft. The Occidental Petroleum Corporation states that the proposed RDSS would provide navigation and communication services far more accurate and efficient than the existing methods for the operation of its large fleet of company aircraft, land vehicles and marine equipment. It adds that the safety aspects provided by a RDSS are of such inestimable value to life and property so as to be beyond description.

8. *Transportation.* North American Van Lines, Inc. states that it requires a computer-assisted, vehicle-scheduling communications system in which RDSS could assist. It claims that by having the capability to track all of its vehicles throughout the United States it would be provided with the needed information to improve management of its resources. Further, to be able to communicate with a particular vehicle, regardless of its location or status, would contribute to an improvement in efficiency in serving the public.

9. Overload Express, Inc. supports the RDSS proposal indicating that the proposed service would enable it to continuously monitor the locations of all its vehicles and maintain nearly constant communication with each driver. Such a tracking system would significantly reduce the frequency of "empty" or "deadhead" miles (i.e., mileage driven without a load) and would enable the carrier to respond more quickly and efficiently to customer needs.

10. *Railroads.* The Association of American Railroads points out that RDSS could supplement existing rail traffic control systems to provide for enhanced security and efficiency of rail operations. At present there are over 168,000 miles of railroad lines not presently covered by traffic control systems. RDSS offers an effective means to extend traffic control systems so as to assure coverage of all trackage. On single track lines RDSS would offer a service that could improve rail traffic management. In order for two trains on a single tract to pass each other, one of them must move to a siding and wait until the other one arrives at that point. The precision of the RDSS would permit a train's time of arrival at the siding to be calculated precisely, minimizing the delay while one train waits for another. Furthermore, a train could be supplied with information via a RDSS transceiver on the speed it must average in order to arrive at a siding at a given time, and use this information to minimize fuel consumption.

11. *Resource Management.* The Montana Forest and Conservation Experiment Station indicates that the proposed RDSS promises to be a very useful and economical system for the consistent determination of position, communication and data transfer. Its work requires obtaining adequate position determination for data collection points, inventory plots, mapping, development work and other surveys on western forest and range lands. In addition, it states that the increased security that the proposed RDSS holds for field workers and

recreational users of western wildlands is very significant.

12. In its comments the Office of the State Engineer of Colorado points out that the proposed RDSS could provide more effective water resources management. The implementation of a real-time data collection system enhanced with two-way interrogation would be extremely valuable in the areas of dam safety and in flood warning. The radiodetermination capability of the proposed service would assist the Office in defining the location of water resources supplies, storage structures, diversions, wells, and points of water use and consumption.

Appendix C

Summary of comments related to Petition RM–4839, filed by the National Academy of Sciences.

1. On August 10, 1984, a petition filed by the National Academy of Sciences (RM–4839) was placed on public notice. The petition requested the Commission to change the allocation status of the radio astronomy service in the 1606.8–1613.8 MHz band. Radio astronomers observe OH lines in this frequency range from different regions of our galaxy. To protect these observations from wide-spread interference which could be generated from a RDSS system or systems, a change in allocation status was requested from secondary to primary.

2. Supporting comments in response to the petition, RM–4839, were submitted by the National Astronomy Observatory (NRAO), the National Astronomy and Inonsphere Center, and the National Science Foundation (NSF). The NRAO states that the 1612 MHz line is an important frequency for studying the motions of stars. Also, the 1612 MHz observations provide information on the structure and mass of the central region of our galaxy from the mapping of the ordered motion of the red-giant stars. It is from such measurements that radio astronomers hope to detect a black hole at the galaxy's center.

3. The National Astronomy and Ionosphere Center operates the world's largest radio telescope in Puerto Rico. Its equipment provides the highest instantaneous sensitivity anywhere in the world for studies of the hydroxyl (OH) molecule. It requests improved protection from air-borne usage of the 1612 MHz frequency by upgrading the existing allocation from secondary to primary. It claims that the 1612 MHz line is the dominant emission line for maturally occurring masers around infrared stars. Characteristics of these stars cannot be inferred from observations in any other frequency band.

4. NSF states that if the radio astronomy service has a lesser allocation status than other services in the band, then adequate safeguards will not be developed and radio astronomy research will be excluded from the 1612 MHz band. It urges adoption of the petition submitted by CORF.

5. The Geostar Corporation opposed the petition claiming the public interest would best be served if the Commission were to maintain the present primary allocation of the 1610–1626.5 MHz band for aeronautical radio navigation with the proposed U.S. footnote for the radiodetermination service and decline to adopt a primary allocation for a third hydroxyl frequency line. It suggests that the pending rulemaking proceeding regarding the radiodetermination satellite service is the appropriate forum for resolving conflicts between the RDSS service and the radio astronomy service. It accordingly suggests the dismissal of the NAS petition.

5. Geostar indicates that because radio astronomers rely on data summations gained from lengthy observations, it is possible that Geostar pulses, which will arrive at observations within certain knowable intervals, can be factored out with minimal signal loss.

6. In reply comments the National Academy of Sciences reiterates its position that a primary allocation in the 1606.8–1613.8 MHz band is necessary to protect radioastronomy observations of the hydroxyl lines. It indicates it is encouraged by Geostar's attitude of cooperation to protect the radio astronomy service but cannot assume that any agreement reached in bilateral discussions between Geostar and the radio astronomy community can be extended to future applicants to provide RDSS functions in the band 1610–1626.5 MHz.

Subsequent to the comment period on the petition, in a letter received December 19, 1984 enclosing comments filed by Geostar in the radiodetermination satellite service rulemaking, Geostar supported the NAS request so long as a sharing arrangement agreed by Geostar and representatives of the radio astronomy service were implemented in the Commission's Rules.

Appendix D—Agreement Reached Between Geostar and the National Academy of Sciences for Sharing Between the Radiodetermination Satellite Service and the Radio Astronomy Service in the 1606.8–1613.8 MHz Band

Emission Limitations

The mean power density of airborne and spacecraft RDSS emissions at a frequency which is removed from the assigned frequency by more than 50% shall be attenuated below the mean power density at the assigned center frequency as specified in the following equation (attenuation greater than 75 decibels is not required):

$$A=12+0.2(P-50)$$

where,

A=attenuation (in decibels) below the mean power density level, and

P=percent of assigned bandwidth removed from the carrier frequency

Radio Astronomy Coordination Procedures

All RDSS licensees will automatically restrict user transmissions to occur only within the first 200 milliseconds following the one second time marks of Coordinated Universal Time when users enter Radio Astronomy Regions (RARs) during a period of radio astronomy observations in the 1606.8–1613.8 MHz band. RARs are defined by a circle with a radius of 150 kilometers from the coordinates provided below for airborne transmissions, and by a circle with a radius of 25 kilometers from the coordinates provided below for ground-based transmissions. Any segment of a RAR which is part of a Standard Consolidated Statistical Area is not subject to coordination and transmission restriction limitations.

RDSS licensees will establish an observation notification procedure through the Electromagnetic Spectrum Management Unit, National Science Foundation, Washington, DC 20550, that satisfactorily provides for the restriction of user transmissions as described above during periods of radio astronomy observations in the frequency band 1606.8–1613.8 MHz.

RDSS licensees shall reimburse radio observatories for the cost of any special equipment or services required by their observation notification procedure.

Radio Astronomy Sites

The following radio astronomy sites are the centers of RARs as described above:

Name	Latitude	Longitude
Hat Creek, California	40°49′ N.	121°28′ W.
Owens Valley, California	37°13′ N.	118°17′ W.
VLA, New Mexico	34°04′ N.	107°37′ W.
Fort Davis, Texas	30°38′ N.	103°56′ W.
Green Bank, West Virginia	38°26′ N.	79°49′ W.
Arecibo, Puerto Rico	18°21′ N.	66°45′ W.

Appendix E

Parts 2, 74, 90 and 94 of Chapter I of Title 47 of the Code of Federal Regulations are proposed to be amended as follows:

The authority citations in Parts 2, 74, 90 and 94 continues to read:

Authority: Sec. 4, 303, 48 Stat. 1066, 1082 as amended; 47 U.S.C. 154, 303.

PART 2—FREQUENCY ALLOCATIONS AND RADIO TREATY MATTERS; GENERAL RULES AND REGULATIONS

§ 2.106 Table of frequency allocations. [Amended]

1. In § 2.106, columns 4 and 5 of the allocation table for the band 1610–1626.5 MHz are amended by adding a new footnote US 306 as follows:

UNITED STATES TABLE

Government allocation (MHz) (4)	Non-Government allocation (MHz) (5)
* * *	* *
1610–1626.5 AERONAUTICAL RADIO-NAVIGATION 722 732 733 734 US39 US40 US208 US260 US306	1610–1626.5 AERONAUTICAL RADIO-NAVIGATION 722 732 733 734 US39 US40 US208 US260 US306

*   *   *   *   *

United States Footnotes

*   *   *   *   *

US306 The band 1610–1626.5 MHz is also allocated for use by the radiodetermination satellite service in the Earth-to-space direction.

2. In § 2.106, columns 5 and 6 are amended by deleting auxiliary broadcasting, private operational-fixed microwave and private land mobile from the 2483.5–2500 MHz band. A new footnote NG147 is added to provide for the grandfathering of existing auxiliary broadcasting, private operational-fixed and private land mobile stations in the 2483.5–2500 MHz band. Further, the radiodetermination satellite service is added to the 2483.5–2500 MHz band on a primary basis as follows:

UNITED STATES TABLE

Government	Non-Government	FCC use designators	
Allocation (MHz)	Allocation (MHz)	Rule part(s)	Special-use frequencies
(4)	(5)	(6)	(7)
*	*	*	* *
2450–2483.5	2450–2483.5 FIXED MOBILE Radiolocation	AUXILIARY BROADCASTING (74) PRIVATE OPERATIONAL-FIXED (94)	2450 ± 50 MHz Industrial, scientific and medical frequency.
752 US41	752 US41	PRIVATE LAND MOBILE (90)	
2483.5–2500	2483.5–2500 RADIODETERMINATION SATELLITE (space-to-Earth)		
752 US41	752 US41 NG147		

*   *   *   *   *

NG Footnotes

*   *   *   *   *

NG147 Stations in the broadcast auxiliary service and private radio services licensed as of July 25, 1985, or on a subsequent date following as a result of submitting an application for license on or before July 25, 1985, may continue to operate on a primary basis with the radiodetermination satellite service.

3. In § 2.106 columns 4 and 5 are amended for the band 5000–5250 MHz by adding a new footnote US307 as follows:

UNITED STATES TABLE

Government	Non-Government	FCC use designators	
Allocation (MHz)	Allocation (MHz)	Rule part(s)	Special-use frequencies
(4)	(5)	(6)	(7)
*	*	*	* *
5000–5250 AERONAUTICAL RADIONAVIGATION 733 796 797 US211 US260 US307	5000–5250 AERONAUTICAL RADIONAVIGATION 733 796 797 US211 US260 US307	AVIATION (87)	
*	*	*	* *

*   *   *   *   *

United States Footnotes

*   *   *   *   *

US307 The sub-band 5117–5183 MHz is also allocated for space-to-Earth transmissions in the fixed satellite service for use in conjunction with the radiodetermination satellite service operating in the bands 1610–1626.5 MHz and 2483.5–2500 MHz. The total power flux density at the Earth's surface shall in no case exceed −159 dBW/m² per 4 kHz for all angles of arrival.

PART 74—EXPERIMENTAL, AUXILIARY, AND SPECIAL BROADCAST, AND OTHER PROGRAM DISTRIBUTIONAL SERVICES

1. In § 74.602 the table of channel frequencies following paragraph (a) is amended by deleting the last frequency range, 2483–2500, in the first column labeled Band A MHz. Also, the ninth frequency range, 2467–2484, in the first column is amended by revising it to read as follows: 2467–2483.5.

2. Section 74.602(a)(2) is amended by adding a new footnote NG147 to read as follows:

§ 74.602 Frequency assignment.

(a) * * *

(1) * * *

(2) The following notes to the Table of Frequency Allocations contained in § 2.106 apply to the shared use of the frequency bands shown above.

NG 123 * * *

NG147 Stations in the broadcast auxiliary service and private radio services licensed as of July 25, 1985, or on a subsequent date following as a result of submitting an application for license on or before July 25, 1985, may continue to operate on a primary basis to the radiodetermination satellite service.

*   *   *   *   *

PART 90—PRIVATE LAND MOBILE RADIO SERVICES

1. Section 90.17(c) is amended by revising paragraph (18) to read as follows:

§ 90.17 Local Government Radio Service.

*   *   *   *   *

(c) * * *

(18) Available only on a shared basis with stations in other services, and subject to no protection from interference due to the operation of industrial, scientific, or medical (ISM) devices. In the 2483.5–2500 MHz band, no applications for new stations or modification to existing stations to increase the number of transmitters will be accepted. Existing licensees as of July 25, 1985, or on a subsequent date following as a result of submitting an application for license on or before July 25, 1985, are grandfathered and their operation is co-primary with the Radiodetermination Satellite Service.

2. Section 90.19(e) is amended by revising paragraph (25) to read as follows:

§ 90.19 Police Radio Service.

* * * * *

(e) * * *

(25) Available only on a shared basis with stations in other services, and subject to no protection from interference due to the operation of industrial, scientific, or medical (ISM) devices. In the 2483.5–2500 MHz band, no applications for new stations or modification to existing stations to increase the number of transmitters will be accepted. Existing licensees as of July 25, 1985, or on a subsequent date following as a result of submitting an application for license on or before July 25, 1985, are grandfathered and their operation is co-primary with the Radiodetermination Satellite Service.

3. Section 90.21(c) is amended by revising paragraph (13) to read as follows:

§ 90.21 Fire Radio Service.

* * * * *

(c) * * *

(13) Available only on a shared basis with stations in other services, and subject to no protection from interference due to the operation of industrial, scientific, or medical (ISM) devices. In the 2483.5–2500 MHz band, no applications for new stations or modification to existing stations to increase the number of transmitters will be accepted. Existing licensees as of July 25, 1985, or on a subsequent date following as a result of submitting an application for license on or before July 25, 1985, are grandfathered and their operation is co-primary with the Radiodetermination Satellite Service.

4. Section 90.23(c) is amended by revising paragraph (13) to read as follows:

§ 90.23 Highway Maintenance Radio Service.

* * * * *

(c) * * *

(13) Available only on a shared basis with stations in other services, and subject to no protection from interference due to the operation of industrial, scientific, or medical (ISM) devices. In the 2483.5–2500 MHz band, no applications for new or modification to existing stations to increase the number of transmitters will be accepted. Existing licensees as of July 25, 1985, or on a subsequent date following as a result of submitting an application for license on or before July 25, 1985, are grandfathered and their operation is co-primary with the Radiodetermination Satellite Service.

5. Section 90.53(b) is amended by revising paragraph (24) to read as follows:

§ 90.53 Frequencies available.

* * * * *

(b) * * *

(24) Available only on a shared basis with stations in other services, and subject to no protection from interference due to the operation of industrial, scientific, or medical (ISM) devices. In the 2483.5–2500 MHz band, no applications for new or modification to existing stations to increase the number of transmitters will be accepted. Existing licensees as of July 25, 1985, or on a subsequent date following as a result of submitting an application for license on or before July 25, 1985, are grandfathered and their operation is co-primary with the Radiodetermination Satellite Service.

6. Section 90.63(d) is amended by revising paragraph (19) to read as follows:

§ 90.63 Power Radio Service.

* * * * *

(d) * * *

(19) Available only on a shared basis with stations in other services, and subject to no protection from interference due to the operation of industrial, scientific, or medical (ISM) devices. In the 2483.5–250 MHz band, no applications for new or modification to existing stations to increase the number of transmitters will be accepted. Existing licensees as of July 25, 1985, or on a subsequent date following as a result of submitting an application for license on or before July 25, 1985, are grandfathered and their operation is co-primary with the Radiodetermination Satellite Service.

7. Section 90.65(c) is amended by revising paragraph (32) to read as follows:

§ 90.65 Petroleum Radio Service.

* * * * *

(c) * * *

(32) Available only on a shared basis with stations in other services, and subject to no protection from interference due to the operation of industrial, scientific, or medical (ISM) devices. In the 2483.5–2500 MHz band, no applications for new or modification to existing stations to increase the number of transmitters will be accepted. Existing licensees as of July 25, 1985, or on a subsequent date following as a result of submitting an application for license on or before July 25, 1985, are grandfathered and their operation is co-primary with the Radiodetermination Satellite Service.

8. Section 90.67(c) is amended by revising paragraph (22) to read as follows:

§ 90.67 Forest Products Radio Service.

* * * * *

(c) * * *

(22) Available only on a shared basis with stations in other services, and subject to no protection from interference due to the operation of industrial, scientific, or medical (ISM) devices. In the 2483.5–2500 MHz band, no applications for new or modification to existing stations to increase the number of transmitters will be accepted. Existing licensees as of July 25, 1985, or on a subsequent date following as a result of submitting an application for license on or before July 25, 1985, are grandfathered and their operation is co-primary with the Radiodetermination Satellite Service.

9. Section 90.69(c) is amended by revising paragraph (8) to read as follows:

§ 90.69 Motion Picture Radio Service.

* * * * *

(c) * * *

(8) Available only on a shared basis with stations in other services, and subject to no protection from interference due to the operation of industrial, scientific, or medical (ISM) devices. In the 2483.5–2500 MHz band, no applications for new or modification to existing stations to increase the number of transmitters will be accepted. Existing licensees as of July 25, 1985, or on a subsequent date following as a result of submitting an application for license on or before July 25, 1985, are grandfathered and their operation is co-primary with the Radiodetermination Satellite Service.

10. Section 90.71(c) is amended by revising paragraph (6) to read as follows:

§ 90.71 Relay Press Radio Service.

* * * * *

(c) * * *

(6) Available only on a shared basis with stations in other services, and subject to no protection from interference due to the operation of industrial, scientific, or medical (ISM) devices. In the 2483.5–2500 MHz band, no applications for new or modification to existing stations to increase the number of transmitters will be accepted. Existing licensees as of July 25, 1985, or on a subsequent date following as a

result of submitting an application for license on or before July 25, 1985, are grandfathered and their operation is co-primary with the Radiodetermination Satellite Service.

11. Section 90.73(d) is amended by revising paragraph (23) to read as follows:

§ 90.73 Special Industrial Radio Service.

* * * * *

(d) * * *

(23) Available only on a shared basis with stations in other services, and subject to no protection from interference due to the operation of industrial, scientific, or medical (ISM) devices. In the 2483.5–2500 MHz band, no applications for new or modification to existing stations to increase the number of transmitters will be accepted. Existing licensees as of July 25, 1985, or on a subsequent date following as a result of submitting an application for license on or before July 25, 1985, are grandfathered and their operation is co-primary with the Radiodetermination Satellite Service.

12. Section 90.79(d) is amended by revising paragraph (17) to read as follows:

§ 90.79 Manufacturers Radio Service.

* * * * *

(d) * * *

(17) Available only on a shared basis with stations in other services, and subject to no protection from interference due to the operation of industrial, scientific, or medical (ISM) devices. In the 2483.5–2500 MHz band, no applications for new or modification to existing stations to increase the number of transmitters will be accepted. Existing licensees as of July 25, 1985, or on a subsequent date following as a result of submitting an application for license on or before July 25, 1985, are grandfathered and their operation is co-primary with the Radiodetermination Satellite Service.

13. Section 90.81(d) is amended by revising paragraph (9) to read as follows:

§ 90.81 Telephone Maintenance Radio Service.

* * * * *

(d) * * *

(9) Available only on a shared basis with stations in other services, and subject to no protection from interference due to the operation of industrial, scientific, or medical (ISM) devices. In the 2483.5–2500 MHz band, no applications for new or modification to existing stations to increase the number of transmitters will be accepted. Existing licensees as of July 25, 1985, or on a subsequent date following as a result of submitting an application for license on or before July 25, 1985, are grandfathered and their operation is co-primary with the Radiodetermination Satellite Service.

14. Section 90.89(c) is amended by revising paragraph (15) to read as follows:

§ 90.89 Motor Carrier Radio Service.

* * * * *

(c) * * *

(15) Available only on a shared basis with stations in other services, and subject to no protection from interference due to the operation of industrial, scientific, or medical (ISM) devices. In the 2483.5–2500 MHz band, no applications for new or modification to existing stations to increase the number of transmitters will be accepted. Existing licensees as of July 25, 1985, or on a subsequent date following as a result of submitting an application for license on or before July 25, 1985, are grandfathered and their operation is co-primary with the Radiodetermination Satellite Service.

15. Section 90.91(c) is amended by revising paragraph (16) to read as follows:

§ 90.91 Railroad Radio Service.

* * * * *

(c) * * *

(16) Available only on a shared basis with stations in other services, and subject to no protection from interference due to the operation of industrial, scientific, or medical (ISM) devices. In the 2483.5–2500 MHz band, no applications for new or modification to existing stations to increase the number of transmitters will be accepted. Existing licensees as of July 25, 1985, or on a subsequent date following as a result of submitting an application for license on or before July 25, 1985, are grandfathered and their operation is co-primary with the Radiodetermination Satellite Service.

16. Section 90.93(c) is amended by revising paragraph (8) to read as follows:

§ 90.93 Taxicab Radio Service.

* * * * *

(c) * * *

(8) Available only on a shared basis with stations in other services, and subject to no protection from interference due to the operation of industrial, scientific, or medical (ISM) devices. In the 2483.5–2500 MHz band, no applications for new or modification to existing stations to increase the number of transmitters will be accepted. Existing licensees as of July 25, 1985, or on a subsequent date following as a result of submitting an application for license on or before July 25, 1985, are grandfathered and their operation is co-primary with the Radiodetermination Satellite Service.

17. Section 90.95(d) is amended by revising paragraph (13) to read as follows:

§ 90.95 Automobile Emergency Radio Service.

* * * * *

(d) * * *

(13) Available only on a shared basis with stations in other services, and subject to no protection from interference due to the operation of industrial, scientific, or medical (ISM) devices. In the 2483.5–2500 MHz band, no applications for new or modification to existing stations to increase the number of transmitters will be accepted. Existing licensees as of July 25, 1985, or on a subsequent date following as a result of submitting an application for license on or before July 25, 1985, are grandfathered and their operation is co-primary with the Radiodetermination Satellite Service.

18. Section 90.103(c) is amended by revising paragraph (9) to read as follows:

§ 90.103 Radiolocation Service.

* * * * *

(c) * * *

(9) This band is allocated to the radiolocation service on a secondary basis to other fixed or mobile services and most accept any harmful interference that may be experienced from such services or from the industrial, scientific, and medical (ISM) equipment. Operating in accordance with Part 18 of this chapter. In the 2483.5–2500 MHz band, no applications for new or modification to existing stations to increase the number of transmitters will be accepted. Existing licensees as of July 25, 1985, or on a subsequent date following as a result of submitting an application for license on or before July 25, 1985, are grandfathered and their operation is co-primary with the Radiodetermination Satellite Service.

PART 94—PRIVATE OPERATIONAL—FIXED MICROWAVE SERVICE

1. Section 94.61(b) is amended by revising limitation (4) to read as follows:

§ 94.61 Applicability.

* * * * *

(b) * * *

(4) Frequencies in this band are shared with mobile and radiolocation stations in other services, and must accept harmful interference that may be experienced from operations of industrial, scientific, or medical (ISM) equipment operating on 2450 MHz. In the 2483.5–2500 MHz band, no applications for new stations or modifications to existing stations to increase the number of transmitters will be accepted. Existing licensees as of July 25, 1985, or on a subsequent date following as a result of submitting an application for license on or before July 25, 1985, are grandfathered and their operation is co-primary with the radiodetermination Satellite Service. However, all grandfathered temporary fixed licensees are required to notify directly each Radiodetermination Satellite Service licensee concerning present and proposed locations of operation.

Statement of Commissioner James H. Quello Dissenting in Part

Re: Amendment of the Commission's Rules to Allocate Spectrum for, and to Establish Other Rules and Policies Pertaining to, a Radiodetermination Satellite Service (RDSS) (General Docket No. 84–689).

I strongly support the establishment of a radiodetermination satellite service. A review of the comments reveals the potential array of public interest benefits offered by this service, such as safety to human life, reduced transportation and labor costs, and improvements in navigational services. Further, RDSS provides to the end user certain capabilities that currently are not available by any other means. For these reasons, I agree with the necessity to establish RDSS.

The Report and Order, however, also grandfathers all existing mobile licensees in the 2483.5–2500 MHz band on a co-primary basis with RDSS licensees and provides that RDSS licensees have the option of paying the "reasonable and prudent cost of modifying an existing licensee's operation" if interference from these mobile operations is unacceptable. I dissent to these provisions.

I object first to the concept of co-primary status. The Report and Order clearly establishes the public interest served by RDSS. If the needs met by RDSS are this great, then RDSS should be granted primary status and all other licensees should be given secondary status. Further, we are establishing a dangerous precedent by having the new user of the spectrum pay to relocate existing licensees. As the demand for spectrum increases and new services are developed warranting the reallocation of spectrum, the Commission may find itself increasingly burdened with resolving disputes associated with allocating the costs of relocation. Further, in some cases the delivery of new services that would fulfill public needs may be thwarted due to the costs associated with relocating existing licensees. Accordingly, I am dissenting in part to this Report and Order.

Statement of Commissioner Henry M. Rivera Dissenting in Part

Re: Report and Order to Allocate Spectrum for a New Radiodetermination Satellite Service (RDSS).

This *Report and Order* allocating spectrum for a new Radiodetermination Satellite Service (RDSS) contains a novel, innovative, but also illegal and undesirable approach to the spectrum allocations process. After reaching the conclusion that RDSS is in the public interest, the majority concludes that existing private radio and broadcast mobile licensees in the reallocated band need not move until the cost of modifying or replacing their current equipment is paid for by future RDSS licensees. This procedure establishes a far-reaching precedent which will change the fundamental nature of the Commission's spectrum allocation process without the benefit of a public review or record on the desirability of the administrative ramifications of the change.

The majority's action has some intuitive appeal. However, this pay-to-play spectrum acquisition mechanism contravenes language in Sections 301 and 304 of the Communications Act, frustrating the congressional determination that the spectrum belongs to the government and that a licensee's right to use the spectrum is limited to the specified term of the license. Furthermore, I fear that the result the majority reaches establishes a policy that will be harmful to future spectrum allocation, because it will mire the Commission in an insoluable tar-pit of factual disputes by existing spectrum users resisting spectrum reallocation.

I agree with the majority that the reallocation of spectrum to permit the creation of the RDSS is in the public interest. I also agree that to implement this public interest determination it is appropriate for the Commission to modify the licenses of existing users in the subject bands, specifying that their future use of the band will be secondary and that, as secondary users, they must terminate operation if their continued use of the band harmful interference to RDSS.

I part company with the majority, however, on the question of how this decision should be implemented. The majority proposes to allow existing users of these bands to remain "co-equal primary" until such time as the RDSS licensees reach agreements with the existing users about paying the cost of modifying or replacing their current equipment to permit the existing licenses to operate in other bands. As the majority sees it, RDSS providers should be willing to make these payments if RDSS use of the spectrum is more valuable than that of existing licenses. The requirement that the newcomers pay the cost of displacing these current users will, therefore, validate the majority's assumption that the proposed use is superior to the existing use. If the Commission's determination that the RDSS is the most desirable use is wrong, the RDSS providers will not be willing to make the payments necessary to clear the band.

The majority's cost-benefit equation omits one critical paramenter. Nowhere in its algorithm is there a term for factoring in the *public* interest. In fact, the algorithm specified in the Communications Act provides that the public interest be the dominant term in making such calculations. As Congress envisioned it, when the *public* interest weights in favor of the spectrum being put to a particular use, *private* considerations are subordinate factors which cannot determine the outcome of the Commission's deliberations.[1] Therefore, as attractive as the principal of reimbursing displaced licensees may be, I cannot acquiesce in the majority's decision absent amendment of the Communications Act.

In drafting the Communications Act, Congress took care, in several places, to insure that a grant of a license in and of itself did not entitle the licensee to any right beyond Section 316 hearing rights.[2]

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[1] These subordinate factors have been held to require careful consideration by the Commission in the form of a hearing right pursuant to Section 316 of the Act or our careful documentation of their scope in the course of notice and comment rulemaking. *See e.g., Carroll Broadcasting* v. *FCC*, 258 F.2d 440 (D.C. Cir. 1958). These *private* considerations have also been the basis of a Commission decision to delay implementation of a spectrum reallocation to a new service until the Commission has taken action to accommodate the displaced pre-existing users. *See e.g., DBS Report and Order*, 90 FCC 2d 676 (1982).

[2] The first section of Title III, governing regulation of radio communications, specifies that: It is the purpose of this Act * * * to provide for the use of [channels of radio communications], but not the ownership thereof, by persons with limited periods of time. . . . and no such license shall be construed to create any right, beyound the terms, conditions, and periods of that license.

The statute's drafters sought to insure that future licensees would be aware of the limited nature of their right by requiring applicants to sign an explicit waiver to rights beyond the term of their licneses.[3] Section 309, governing the process of applying for use of the spectrum, reinforces these limitations on licensees' rights.[4]

These statutory limitations embody a plain congressional intent. Congress intended to preserve control over use of the spectrum, to ensure that ownership of the spectrum remained with the government and not licensees, and to perserve for the Commission the freedom to terminate the use of the spectrum by certain licensees in order to permit its reassignment to higher public interest uses.

In the past, compensation has been required in three instances. However, each of those instances is distinguishable from the precedent established by today's action. First, in our Direct Broadcast Satellite (DBS) decision, we decided to let new licensees gain access to the spectrum prior to the expiration of a five year transition period if they paid the cost of relocating the existing licensees.[5] The second instance in which the Commission has authorized relocation cost involved Broadcast Corporation of Georgia (WVEU-TV).[6] The third case in which the Commission authorized payment involved a situation where the requested assignment of a particular channel required a change of frequency by an existing station in the same service.[7]

While the equitable considerations involved in these prior Commission actions involving compensation may be similar to the case at hand,[8] the policy considerations are dramatically different. First, in the *Channel 69* case the Commission was not making a choice between private land mobile and broadcast utilization of the same spectrum. The television station was authorized on UHF channel 69 while the land mobile stations were authorized on an adjacent band of spectrum. Both services utilized their respective channels extensively throughout the country. The Commission failed to foresee the destructive interference that would take place if both users located their transmitters within close physical proxy. This problem arose after the fact and did not preclude effectuation of the Commission's spectrum allocation decision generally. The significance of the co-primary status in the *Channel 69* case is that the Commission's public interest determination was that *both* uses of the spectrum were in the public interest—now and in the future. In the RDSS reallocation proceeding before us today, we are concluding that the RDSS use of the spectrum is a superior public interest use, warranting a clearing of the band for RRDS.

The case law interpreting the Commission's obligations to protect the rights of existing licensees is sparse. Early cases seem to establish the principle that the ethers remain the government's property and a licensee's use of it is subject to modification without compensation as the public interest demands.[9] As indicated in footnote 1, some cases have recognized a Commission responsibility to consider the economic impact of its actions on existing licensees.[10] These uncertain or

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[3] Section 304 of the Act specifies: No station license shall be granted by the Commission until the applicant therefore shall have signed a waiver of any claim to use of any particular frequency or of the electromagnetic spectrum as against the regulatory powers of the United States because of previous use of the same, whether by license or otherwise.

[4] Section 309 specifies that: Such station licenses as the Commission may grant shall be in such general form as it may prescribe, but each license shall contain, in addition to other provisions, a statement of the following conditions to which such licensee shall be subject: (1) The station license shall not vest in a licensee any right to operate the station nor any right in the use of the frequencies designated in the license beyond the term thereof nor in any other manner than authorized therein; . . . .

[5] In the Commission's DBS proceeding, spectrum was reallocated from terrestrial microwave users to the new DBS service. *DBS Report and Order*, 90 FCC 2d 676 (1982). The existing terrestrial licensees were given a five year period to vacate the band after which their primary status would be reduced to secondary and they would be required to protect the DBS service. In response to concerns that early DBS entrants might need the spectrum prior to completion of the five year transitional period, the Commission concluded that DBS licensees "would have a strong incentive to compensate the FS users for the costs of moving to other frequency bands during this period." The distinction between that decision and the decision here is important. After the transition period the new user gained access to the spectrum regardless of the new user's ability or willingness to pay to relocate existing users. This mechanism was intended to act as an incentive to encourage early movement out of the band by the existing users. *DBS Report and Order*, 90 FCC 2d 676 at paragraph 67, note 60.

[6] *Memorandum Opinion and Order, Mar. 8, 1984.* In this case, a construction permit was issued for Channel 69, Atlanta, Georgia (Channel 69). The CP holder specified a tower site heavily used by land mobile radio service licensees operating on UHF channel 70. When the Channel 69 transmitter was activited for program test, extensive interference was experienced by the Channel 70 land mobile radio licensees. The Commission concluded that the most efficient alternative was to require the land mobile licensees to re-channelize with greater frequency separation from Channel 69. Upon comparing the equities of a later coming spectrum user causing destructive interference to existing spectrum users, the Commission concluded that fairness dictated that the Channel 69 permittee bear the cost of relocating the existing Channel 70 land mobile licensees.

[7] *Jahnke* v. *FCC*, D.C. Circuit No. 80-2448 (March 16, 1982). In this case an applicant for a new FM broadcast radio station negotiated an agreement with an existing FM station licensee which called for a channel change by the existing station at the applicant's expense to resolve a technical incompatability. The Commission then granted the requested application subject to this reimbursement agreement. The applicant subsequently filed a request for extension of the construction permit. The Commission agreed to grant the six-month extension, provided that within 30 days the applicant made definite arrangements to pay the agreed to rechannelization expense. When the applicant failed \o do so, the extention was denied. On appeal challenging this denial, the Court concluded that the Commission had the authority to impose such a condition on the construction permit. In so concluding, the Court ruled that:

"Although the scope of the Commission's authority is limited to actions in the public, not private, interest, it is established that private losses to existing stations occasioned by licensing new stations may have an adverse effect on the provision of broadcast service to the public. (Citations omitted.) While it is true that the Commission lacks authority to create or enforce any legal obligation to [the applicant] to reimburse [the licensee], the Commission does have the power to determine the issuance of a license or permit to [the applicant] without his having reimburse [the licensee] would adversely effect the public interest. *See Regents of University System* v. *Carroll*, 338 U.S. 586, 596-97 and note. 12 (1950)." *Jahkne* v. *FCC*, *Judgment*, D.C. Cir. No. 80-2448 (March 16, 1982), slip opinion at 3.

This case is inapplicable to the situation at hand for two reasons. First, the Commission was allocating cost between two co-equal primary users, both of whose continued existence in this band the Commission determined was in the public interest. The Commission was not conditioning the initiation of a primary use found to be in the public interest on payment to secondary users whose continued use of the spectrum the Commission no longer believed was warranted. Second, as in the DBS case, the licensee here voluntarily undertook reimbursement of the existing licensee, but was not required to do so in order to use the spectrum.

[8] Commission enforcement of obligations voluntarily undertaken by applicants or the adjudication of the conflicting interest among adjacent channel co-primary users raises wholly different rights or equities than are raised by the displacement of licensees by higher public interest users in spectrum reallocation proceedings. I reiterate, we do not have such a situation here.

[9] "We are well satisfied that there is a vital difference between the rights of one whose property (in coal land such as was considered in *Pennsylvania Coal Company* v. *Mahone*, 360 U.S. 393, 43 S.Ct. 158, 67 L.Ed. 322) is confiscated by judicial decree and the rights of one to use the air, which right is dependent upon a government permit limited both in extent and time. The former is vested. The latter is permissive. *American Bond and Mortgage Co. et al.* v. *United States*, 52 F.2d 318, 320 (1932).

[10] *See e.g., Carroll Broadcasting* v. *FCC*, F.2d 440 (D.C. Cir. 1958); *FCC* v. *Sanders Brothers Radio Station*, 309 U.S. 470 (1940); *FCC* v. *National Citizens Committee for Broadcasting*, 436 U.S. 775, 805 note 24 (1978). These cases have consistently

Continued

inapplicable judicial pronouncements must be weighed against specific obligations placed upon the Commission by the Communications Act.[11] Nowhere does that Act authorize the Commission to delegate its spectrum allocation responsibilities to the private sector via auction, displacement payment or any other mechanism.[12] In those recent instances where the Commission has concluded that private or marketplace determination of the best use of the spectrum was preferable to the Commission's spectrum allocation process, the Commission has concluded that amendment of the Communications Act is necessary.[13]

The practical effect of today's decision will be to modify the public interest determination made by the Commission with a marketplace determination made by RDSS licensees. These private party interests will determine to what use this spectrum is finally put.[14]

In addition to my concern over the legality of the majority's actions, I am deeply concerned over the policy ramifications of the precedent we are establishing. As the numbers of spectrum users and the number of instances in which the Commission must displace or repack existing licensees in order to make room for new uses increases, the Commission will be confronted increasingly with situations in which the allocation of displacement costs on the new user may prevent initiation of the new use.[15]

While administrative considerations should not be a significant component of our public interest calculus, the administrative ramifications of the precedent set today provides another basis for concluding that this decision is flawed. In a relatively simple conflict between 41 land mobile radio licensees and UHF Channel 69, Atlanta, the Commission consumed an enormous amount of administrative time and energy determining what constituted reasonable costs for relocation of the land mobile licensees. The Commission also experienced extreme difficulty determining what amount of degradation was sufficient to justify a particular land mobile licensee demanding reimbursement for the cost of relocation. If this Commission was stymied by the 42 parties involved in the Channel 69 dispute, the number of parties involved in a nationwide band reallocation or channel splitting has the potential for creating a factual dispute so great that disposition will become impossible.

Even in the instant case, the majority has far from resolved the outstanding cost issues. Because it acts without the benefit of rulemaking on the question of this new payment process, the majority has no basis for concluding which RDSS applicants should bear the cost for relocating which licensees currently in their band. Should the first licensee or all the RDSS applicants share the cost? If the four RDSS applicants currently pending pay to clear the band, should later filed applicants make payments to the early entrants for their investment? Will the Commission place an assessment against each applicant to cover the relocation cost or must the applicants negotiate a private arrangement to divide the cost involved? Will the Commission adjudicate disputes over the actual cost of relocation or must the parties seek local judicial determinations? If a displaced licensee has a choice between moving into a more crowded band at somewhat less cost or a less crowded band at a higher cost, who will decide to which band the displaced licensee should relocate? Does degradation of service justify a payment? How much degradation will serve as the trigger? Conversely, how much degradation must be tolerated by existing licensees before relocation payment rights are triggered?

Under this decision, the Commission will be required to answer these questions. It is unfortunate that we will be attempting to resolve these questions for this group of applicants without the benefit of public notice and comment. It is even more unfortunate for members of the public that the majority is establishing this precedent without careful consideration of how this decision should be applied in foreseeable upcoming reallocation decisions.

While moving toward a market-oriented spectrum allocation mechanism may be consistent with the marketplace regulatory philosophy, it is inconsistent with the Commission's statutory mandate. The Commission must seek congressional revision of its organic statute before continuing down this path, it should also conduct a comprehensive public proceeding to systematically develop the new administrative machinery necessary to make such a marketplace-based system work. The failure to do so, here, is therefore, both illegal and undesirable. Accordingly, to this part of this *Report and Order*, I dissent.

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required only that the Commission consider those economic impacts in the hearing prior to granting a license or as part of the rulemaking record prior to amending the rules. None of these cases required the Commission to compensate displaced licensee or arrange payment of such compensation.

[11] The Commission is assigned responsibility for allocating the spectrum; to wit:

Sec. 303.* * * The Commission from time to time, as the public convenience, interest or necessity require shall—

*   *   *   *   *

(c) Assign bands of frequency to the various classes of stations, and assign frequencies for each individual station and determine the power which each station shall use in the time during which it may operate; * * *

(f) Make such regulations not inconsistent with law as it may deem necessary to prevent interference between stations and to carry out the provisions of this Act: *provided, however*, that changes in frequencies, authorized power, or in the times of operation of any station, shall not be made without consent of the station licensee unless, after a public hearing, the Commission shall determine that such changes will promote public convenience or interest or will serve public necessity, or the provisions of this Act will be more fully complied with; * * *

[12] *Western Air Lines, Inc.* v. *Civil Aeronautics Board*, 194 F.2d 211, 214 (9th Cir. 1952); *Shreveport Engraving Co.*, v. *U.S.*, 143 F.2d 222 (5th Cir.), *cert. denied*, 323 U.S. 749 (1944).

[13] Cf. Letter of May 1, 1985 to Honorable John C. Danforth, Chairman Senate Committee on Commerce from FCC Chairman Mark S. Fowler and attached "Auction Licensing Act of 1985." *Communications Daily* (May 2, 1985)

[14] If RDSS licensees determine that payment of the pre-existing private land mobile and broadcast users is sufficiently in their economic interest, they will pay the cost of relocating these pre-existing licensees in order to clear the band the Commission now allocates for their primary use. If the RDSS licensees conclude that, given the cost of clearing the band, initiation of the service is no longer in their economic interest, the pre-existing licensees will not be paid and will continue to make use of the band for purposes for which the Commission has determined the band should no longer be used.

[15] This may arise, for example, in the proceeding where the Commission proposes to reallocate spectrum currently occupied by television broadcast licensees or common carrier mobile radio service providers to the private land mobile radio services for the purpose of satisfying the need for public safety communications. *See* Private Radio Bureau Staff *Report on Future Public Safety Telecommunications Requirements, Order* in PR Docket No. 84-232, FCC 85-329 (August 1, 1985). The precedent established today would require the public safety licensees to pay the pre-existing users of the broadcast spectrum for the displacement or diminution of their use of the spectrum. A close analogy will arise in the future if the Commission decides to split existing channels to accommodate heavier use of existing bands that have become overcrowded. The new user accommodated by this channel splitting could, consistent with today's decision, be required to pay the cost of re-channelizing the existing licensee's equipment.

[FR Doc. 85-22585 Filed 9-26-85; 8:45 am]

BILLING CODE 6712-01-M

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47 CFR Part 76

Oversight of the Radio and TV Broadcast Rules; Correction

AGENCY: The Federal Communications Commission.

ACTION: Correction to Final Rule.

SUMMARY: In the Order, Oversight of the Radio and TV Broadcast Rules, Mimeo No. 7001, published in the **Federal Register** on September 23, 1985 at 50 FR 38529, there is an error in the Alphabetical Index for Part 76 which omits the listing "Must carry requirements". It is changed to add the omitted listing.

ADDRESS: Federal Communications Commission, Washington DC 20554.

FOR FURTHER INFORMATION CONTACT: Steve Crane, Mass Media Bureau, (202) 632-5414.

SUPPLEMENTARY INFORMATION:

List of Subjects in 47 CFR Part 76

Cable television service.

Erratum

In the matter of Oversight of the Radio and TV Broadcast Rules.

Released: September 24, 1985.

In the above captioned *Order*, (Mimeo No. 7001) released September 18, 1985 and published in the **Federal Register** on September 23, 1985 at 50 FR 38529, the Alphabetical Index to Part 76 (47 CFR Part 76) omitted the listing of the Must Carry requirement.

It is added herein to follow the listing, Monitoring, CATV system, and will read as follows:

Must carry requirements...76.7, 76.55, 76.57, 76.59, 76.61, 76.64

Federal Communications Commission.

William J. Tricario,

Secretary.

[FR Doc. 85-23110 Filed 9-26-85; 8:45 am]

BILLING CODE 6712-01-M

DEPARTMENT OF TRANSPORTATION

National Highway Traffic Safety Administration

49 CFR Part 571

[Docket No. 74-09; Notice 19]

Federal Motor Vehicle Safety Standards; Child Restraint Systems; Correction

AGENCY: National Highway Traffic Safety Administration (NHTSA), DOT.

ACTION: Correction of final rule.

SUMMARY: NHTSA published a notice in the **Federal Register** on April 17, 1985, which added a new Figure 6 to Standard No. 213, *Child Restraint Systems*. Subsequently, the agency published a notice in the August 21, 1985 edition of the **Federal Register**, which added two new figures at the end of Standard No. 213. These two new figures were erroneously designated Figures 6 and 7. This notice corrects that error by designating the figures added in the August 21, 1985 rule as Figures 7 and 8. No new obligations or requirements are imposed on any party as a result of this correction.

EFFECTIVE DATE: February 18, 1986.

FOR FURTHER INFORMATION CONTACT: Stephen Kratzke, Office of Chief Counsel, National Highway Traffic Safety Administration, 400 Seventh Street, SW, Washington, DC 20590 (202-426-2992).

SUPPLEMENTARY INFORMATION: This agency published a final rule amending the inversion test in Standard No. 213, *Child Restraint Systems* (49 CFR 571.213) at 50 FR 15154, April 17, 1985. Among other things, that rule added a new Figure 6 at the end of Standard No. 213. Subsequently, the agency published a final rule amending the requirements of Standard No. 213 applicable to the buckles used in child restraint systems (50 FR 33722, August 21, 1985). That rule added two new figures at the end of Standard No. 213, erroneously designating them as Figures 6 and 7.

This oversight in the August 21 final rule gives rise to a situation where the next edition of the Code of Federal Regulations would show two Figure 6's at the end of Standard No. 213. Additionally, it would be unclear to which Figure 6 the text of Standard No. 213 was referring. To avoid such confusion, this notice designates the two figures added to Standard No. 213 in the August 21 rule as Figures 7 and 8, and makes the corresponding changes in the text of the standard.

Publication of this correction imposes no duties or obligations on any party nor does it alter existing obligations. This notice simply ensures that the public will have an accurate version of Standard No. 213 in the Code of Federal Regulations. Accordingly, the agency finds for good cause that notice and opportunity for comment on this correction are unnecessary.

List of subjects in 49 CFR Part 571

Imports, Motor vehicle safety, Motor vehicles, Rubber and rubber products, Tires.

In consideration of the foregoing, the version of 49 CFR 571.213 published at 50 FR 33722, August 21, 1985, is amended as follows:

PART 571—[AMENDED]

1. The authority citation for Part 571 continues to read as follows:

Authority: 15 U.S.C. 1392, 1401, 1403, and 1407; delegation of authority at 49 CFR 1.50.

2. In § 571.213, paragraph S6.2.1. is corrected to read as follows:

S6.2 Buckle release test procedure.

* * * * *

S6.2.1. Before conducting the testing specified in S6.1, place the loaded buckle on a hard, flat, horizontal surface. Each belt end of the buckle shall be pre-loaded in the following manner. The anchor end of the buckle shall be loaded with a two pound force in the direction away from the buckle. In the case of buckles designed to secure a single latch plate, the belt latch plate end of the buckle shall be pre-loaded with a two pound force in the direction away from the buckle. In the case of buckles designed to secure two or more latch plates, the belt latch plate ends of the buckle shall be loaded equally so that the total load is two pounds, in the direction away from the buckle. For pushbutton-release buckles, the release force shall be applied by a conical surface (cone angle not exceeding 90 degrees). For pushbutton-release mechanisms with a fixed edge (referred to in Figure 7 as "hinged button"), the release force shall be applied at the centerline of the button, 0.125 inches away from the movable edge directly opposite the fixed edge, and in the direction that produces maximum releasing effect. For pushbutton-release mechanisms with no fixed edge (referred to in Figure 7 as "floating button"), the release force shall be applied at the center of the release mechanism in the direction that produces the maximum releasing effect. For all other buckle release mechanisms, the force shall be applied on the centerline of the buckle lever or finger tab in the direction that produces the maximum releasing effect. Measure the force required to release the buckle. Figure 7 illustrates the loading for the different buckles and the point where the release force should be applied, and Figure 8 illustrates the conical surface used to apply the release force to pushbutton-release buckles.

3. Paragraph S6.2.4 is corrected to read as follows:

S6.2.2 * * *

S6.2.3 * * *

S6.2.4 While applying the force specified in S6.2.3, and using the device shown in Figure 8 for pushbutton-release buckles, apply the release force in the manner and location specified in S6.2.1. for that type of buckle. Measure the force required to release the buckle.

* * * * *

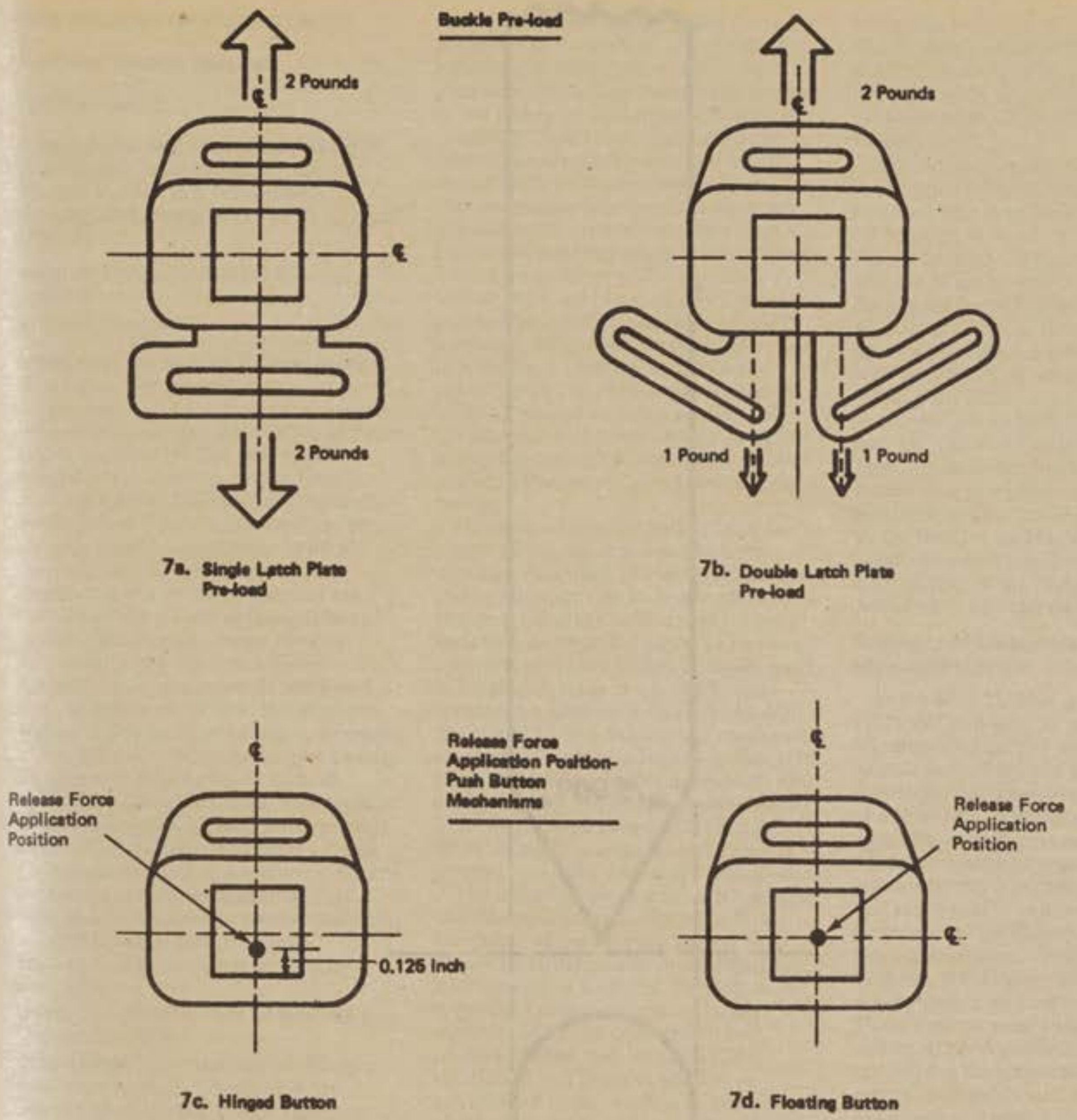
4. Figure 6, Pre-impact Buckle Release Force Test Set-up, and Figure 7, Release Force Application Device—Push Button Release Buckles, are redesignated as Figures 7 and 8, respectively, appearing as follows:

Issued: September 23, 1985.

Diane K. Steed,

Administrator.

BILLING CODE 4910-59-M



7a. Single Latch Plate Pre-load

7b. Double Latch Plate Pre-load

7c. Hinged Button

7d. Floating Button

Figure 7. Pre-impact Buckle Release Force Test Set-up

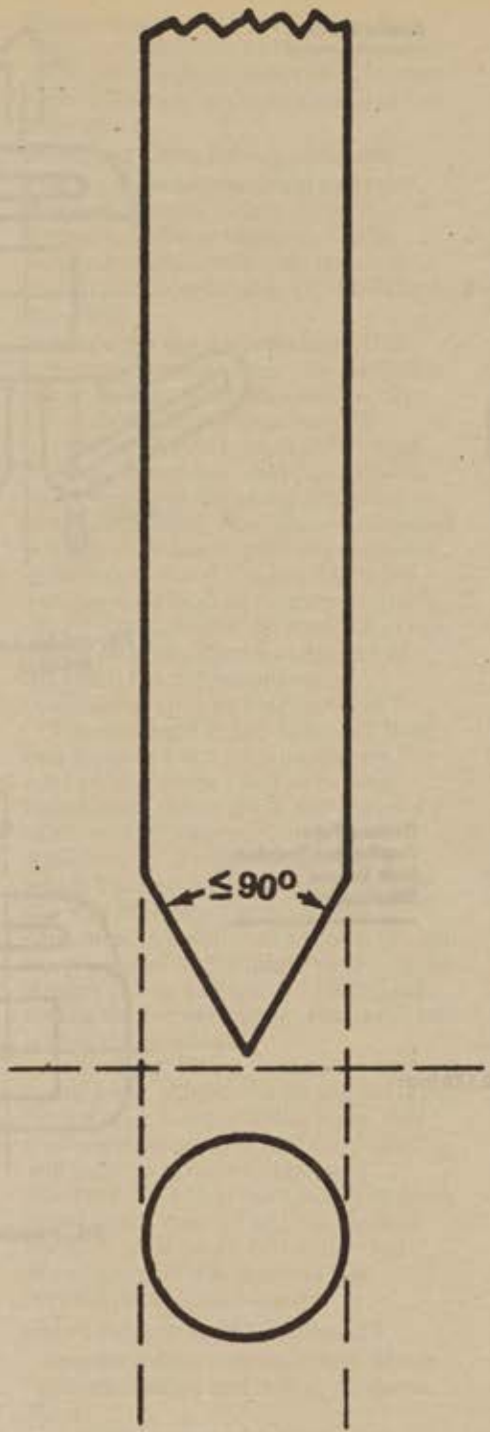


Figure 8. Release Force Application Device — Push Button Release Buckles

[FR Doc. 85-23083 Filed 9-26-85; 8:45 am]

BILLING CODE 4910-59-C

DEPARTMENT OF THE INTERIOR

Fish and Wildlife Service

50 CFR Part 17

Endangered and Threatened Wildlife and Plants; Determination That the Warner Sucker Is a Threatened Species and Designation of Its Critical Habitat

AGENCY: Fish and Wildlife Service, Interior.

ACTION: Final rule.

SUMMARY: The Service determines the Warner sucker (*Catostomus warnerensis*) to be a threatened species, with critical habitat. This action is being taken because: (1) The range and numbers of this species have been reduced substantially; (2) predation by exotic fishes has reduced survival of juvenile suckers, especially in lake habitats; and (3) instream water diversions and artificial barriers are restricting movement and migration of suckers within and among streams. Historically, the Warner sucker occurred in several natural lakes and their tributary streams in the Warner Valley of south-central Oregon. Portions of the following habitats in Lake County, Oregon, are designated as critical habitat: Twelvemile Creek, Twentymile Creek, the spillway canal north of Hart Lake, Snyder Creek, and Honey Creek. A determination that the Warner Sucker is threatened and designation of its critical habitat provide the species with the protection pursuant to the Endangered Species Act of 1973, as amended.

DATE: The effective date of this rule is October 28, 1985.

ADDRESSES: The complete file for this rule is available for inspection, by appointment, during normal business hours at the U.S. Fish and Wildlife Service, Lloyd 500 Building, 500 NE. Multnomah Street, Suite 1692, Portland, Oregon 97232.

FOR FURTHER INFORMATION CONTACT: Mr. Wayne S. White, Chief, Division of Endangered Species, at the above address (503/231-6131 or FTS 429-6131).

SUPPLEMENTARY INFORMATION:

Background

The Warner sucker (*Catostomus warnerensis*) is endemic to the streams and lakes of the Warner Basin in south-central Oregon. It was first described by Snyder (1908). This species is particularly interesting in that it is part of a relict fauna isolated in remaining waters of a larger Pleistocene lake that previously covered much of the basin floor (Hubbs and Miller, 1948). Early residents of the area recalled when the suckers and other fishes were very abundant and would ascend the creeks in the spring for spawning (Coombs *et al.*, 1979). Cope (1883) also noted the great abundance of fishes and fish-eating birds in Warner Basin. The Warner sucker is presently known to occur in portions of Crump and Hart Lakes, the spillway canal north of Hart Lake, and portions of Snyder, Honey, Twentymile, and Twelvemile Creeks (Andreasen, 1975; Coombs *et al.*, 1979; Swenson, 1978) Land on the valley floor is primarily in private ownership, although the Hart Mountain National Wildlife Refuge includes portions of Crump and Hart Lakes. Away from the valley floor, much of the stream habitat is within Bureau of Land Management holdings.

Habitats of Warner sucker include large natural lakes and associated marshes. Although primarily lacustrine, this species spawns in headwaters of streams, tributary to lakes. Warner suckers mature at 3–4 years of age at approximately 5–6 inches (130–160 mm) in length (Coombs *et al.*, 1979). The species is a moderate-sized member of the family Catostomidae and reaches a maximum length of about 20 inches (510 mm). Lateral-line scales average 76 and scales around the caudal peduncle vary from 18–22 (Andreasen, 1975; Bond, 1973). A bright orange lateral stripe is present on adults during spawning runs.

The introduction of exotic fish species and the modification of stream flows into lakes of the Warner Valley by diversion structures have modified the Warner sucker's habitat. Predation on juvenile Warner suckers by large numbers of exotic centrarchid and ictalurid fishes may be particularly significant. All these actions have contributed to the decline in Warner sucker populations (Bond, 1974; Coombs and Bond, 1980; Coombs *et al.*, 1979; Kobetich, 1977). The water diversion structures are especially significant in that they prevent this obligatory stream-spawning sucker from reaching its spawning and rearing areas. Water pollution and siltation of gravel beds needed by the fish for spawning are also adversely affecting the lake and stream habitats. This species spawns in silt-free, gravel-bottomed flowing sections of creeks.

The depleted status of the Warner sucker has been recognized by the scientific community. The Warner sucker was listed as endangered in Bond's 1974 publication, "Endangered Plants and Animals of Oregon: I. Fishes." The species is also listed as endangered by Deacon *et al.*, (1979). However, recent work of Coombs and Bond (1980) and Coombs *et al.*, (1979) documented continued, although reduced, spawning of this species and recommended a threatened classification.

The Warner sucker was included in the Service's Vertebrate Notice of Review published December 30, 1982 (47 FR 58454). On April 12, 1983, the Desert Fishes Council petitioned the Service to add the Warner sucker to the List of Endangered and Threatened Wildlife. After evaluation of the petition, the Service published a notice on June 14, 1983 (48 FR 27273), which found that substantial information was presented in the petition to indicate that action may be warranted to list the species. The proposed rule to list the Warner sucker as a threatened species and to designate critical habitat was published by the Service on May 21, 1984 (49 FR 21383), in accordance with section 4(b)(3)(B)(ii) of the Endangered Species Act of 1973, as amended.

Summary of Comments and Recommendations

In the May 21, 1984, proposed rule (49 FR 21383) and associated notifications, all interested parties were requested to submit factual reports or information that might contribute to the development of a final rule. Appropriate State agencies, county governments, Federal agencies, scientific organizations, and other interested parties were contacted and requested to comment. Newspaper notices were published in the *Lake County Examiner, Medford Mail Tribune*, and *Eugene Register-Guard*, which invited general public comment. These notices were published on June 20 and 21, 1984. A public hearing concerning the proposed listing and critical habitat determination was held in Lakeview, Oregon, on August 29, 1984. The period for accepting written comments was extended for 60 days following the hearing and closed on October 29, 1984 (49 FR 36667).

Seventeen written comments were received in response to the proposed rule. Seven of these comments were not substantive as they offered no information regarding the status of the Warner sucker, nor did they offer an opinion on whether the species should be protected under the Endangered Species Act of 1973, as amended. Of the ten respondents providing an opinion on the listing, five were opposed and five were in favor of the proposed action. Five individuals presented testimony at the August 29, 1984, public hearing. Four individuals presented testimony on behalf of the Warner Valley Association in opposition to the listing. Written comments were received from two of the four individuals and are included in the tally of written letters referenced above. The fifth individual provided no formal statement, but later submitted one of the five letters referenced above in opposition to the listing. The comments of all individuals and agencies are discussed below.

Six individuals, all landowners in Warner Valley, mentioned that Hart Lake and a portion of Crump Lake were dry in the early 1930's and early 1960's. They questioned how the Warner sucker survived then, and, because it had survived, they felt the species must be adaptable and not in need of Federal protection. The periodic fluctuation in lake levels appears to be a natural feature of the Valley hydrology. The Warner sucker is able to survive such periods by seeking refuge in streams tributary to the lake. However, natural population numbers decrease dramatically during such periods. Also, increased irrigation demands during such periods may aggravate the natural drought conditions and cause the lakes to remain dry for longer periods than would otherwise naturally occur. The presence of drought cycles argues for proper management of the stream resources, which serve as the only refugium for the Warner sucker at these times.

One local individual questioned why the U.S. Fish and Wildlife Service introduced crappies into the Warner Valley lakes. To the Service's knowledge, all introductions of exotic fishes have been made by State agencies. The consequences of these introductions are addressed in the next response.

Four individuals (all landowners or their representatives) stated that the introductions of crappie and other exotic fishes are a much greater threat then any other factor (e.g., irrigation practices) and that there is no use in listing the Warner sucker until everyone agrees to remove the exotic fishes. The Service agrees that interactions (primarily in the form of predation) between exotic fishes and the Warner sucker are a serious problem (see "Summary of Factors Affecting the Species" section), but the Service does not see the exotic fish problem as a reason for postponing listing. Listing the fish will likely bring additional attention to the fish and increase the likelihood of addressing the exotic fish problem. The Service will work closely with the State of Oregon to determine the severity of the problem. Courses of action ranging from no further introductions of exotic fishes to partial or total control of the exotics will be investigated.

One individual argued that there have been no new dams or weirs in the Valley in the past 30 years. Therefore, agricultural practices should not be criticized as causing a decline of the species. The Service agrees that irrigation has been practiced by Warner Valley landowners over the past 100 years. Many dams and other diversion structures were in place prior to 1950. However, the Service believes that the cumulative impact of past agricultural irrigation practices, more recent additions and modifications to these practices, and the effects of introduced fishes have combined to warrant threatened status for the Warner sucker.

One individual argued that the Valley has not changed for hundreds of years and therefore the sucker should be able to continue to survive. The Service agrees that in several respects the Valley has changed little over the past two hundred years. However, the stream and lake habitats of the Warner sucker have sustained a large number of changes over this time. All agricultural and water diversion practices have occurred within the past 150 years. Establishment of exotic fishes has occurred within the past 30 years. The life cycle of the Warner sucker requires movement from lakes to headwaters of tributary streams for spawning and then back to the lakes. Dams, water diversions, and other modifications have made such movement difficult or impossible.

Two individuals suggested that the Warner sucker population level may be more closely correlated to climatic cycles than to human-induced habitat changes. The Service responds that historically such was indeed the case. Drought conditions greatly reduce the population of Warner suckers. In more recent times, climatic cycles still undoubtedly affect population numbers, but, other factors have become more important. For example, with large numbers of predatory exotic fishes in the Warner Valley lakes, these waters provide poor conditions for the Warner sucker even in years of substantial precipitation. The effects of human habitat changes are even more serious during drought years, when habitat is already reduced. Therefore, conservation measures are needed so that natural climatic cycles and habitat alterations do not combine to eliminate the species.

One individual described the present high-water conditions that exist in the Valley and stated that the Warner sucker is in fine condition. The Service agrees that weather cycles do influence the Warner sucker population. However, past climatic data indicate that annual precipitation is cyclic in amount and that a series of wet years is usually followed by a series of dry years. Thus, present climatic conditions should have little influence on the decision to list the species.

Two individuals argued that there is no justification for spending Federal dollars for this species when our national budget is so far in debt. The Department of the Interior has a legal responsibility to carry out provisions of the Act. Decisions regarding the addition of species to the List of Endangered and Threatened Wildlife are to be made on the best available scientific and commercial data.

One individual landowner argued that threatened status and critical habitat designations would service no useful purpose. The Service responds that such designations would afford the Warner sucker protection under the Act. Various provisions of the Act are discussed elsewhere and will not be repeated here. It is the Service's belief that threatened status and critical habitat designation will result in improved conditions for the Warner sucker and could eventually lead to its recovery.

Two individuals questioned whether such designation would jeopardize their agricultural-based livelihood. The Service firmly believes that existing agricultural practices and enhanced conservation of the species are compatible. Modifications to existing diversion structures could be incorporated to enhance movement and survival of the species without changing the purpose or function of the structures. For example, fish screens could prevent diversion of adult and juvenile suckers into agricultural fields. Fish ladders or other passage structures could facilitate movement of the species within streams. The Service will work with the landowners on conservation and recovery of the Warner sucker.

Three individuals questioned the Service's conclusion that the Warner sucker had declined. They also suggested that the Service had not presented any proof to indicate that the Warner sucker was ever abundant. Conversely, one individual suggested that the sucker population may be increasing. The Service must base its decisions on the best available scientific and commercial data. Prior to 1900, many scientists were impressed by the large numbers of fishes in the Valley (Cope, 1883; Gilbert, 1897; Snyder, 1908). The Warner sucker, as such, was not mentioned by many of the early scientists because it was not recognized as distinct from other species of *Catostomus* until 1908. However, only two species of native fish, the Warner sucker and redband trout (*Salmo* sp.), are large enough to be readily noticed. Both species would have been particularly evident during their spring spawning runs. The implication that the Warner sucker was abundant historically is supported by claims of local residents (Coombs *et al.*, 1979). Although there are no historic population estimates to compare with recent data, the best scientific data available indicate that suckers were abundant, and only one species of sucker occurs in Warner Valley. Also, it should be noted that Snyder (1908) described Warner suckers collected from Warner Creek, a tributary of Deep Creek near Adel, Oregon. Recent surveys have failed to find this species near Adel or in any portion of the Deep Creek drainage.

One representative of the Warner Valley Association claimed that the Warner sucker is found in the Lahontan Basin of Nevada as well as in Oregon. The Service believes that this comment resulted from confusion in understanding some of the early ichthyological literature. Prior to 1908 when the species was described as *Catostomus warnerensis*, the sucker in Warner Valley was assumed to be the same as *C. tahoensis* of the Lahontan Basin. However, when the morphology of the Warner Valley form was closely examined by Snyder (1908), the Warner sucker was found to be unique and easily separable from *C. tahoensis* of the Lahontan Basin. The taxonomy of the Warner sucker has not been questioned since that time, and no individuals of *C. warnerensis* have ever been collected from outside the Warner Basin.

One individual questioned whether the recent studies (particularly Coombs *et al.*, 1979) were conducted under optimal conditions. If conditions were not optimal, the commenter felt that the studies were flawed. The Service responds that this comment is referencing conditions of high stream flow, poor roads, and cold weather encountered by recent investigators. Although conditions are not always ideal, valuable scientific data can be collected nonetheless. For example, Coombs *et al.* (1979) collected sufficient scientific data to determine the spawning sites, age and condition of spawners, and distribution of the species, despite less than perfect weather conditions during the study. Less than optimal weather did not seem to hamper adequate data collection by most recent investigators.

Four individuals, representing Oregon State University, the Oregon Cooperative Fishery Unit, the International Union for Conservation of Nature and Natural Resources, and the Oregon Department of Fish and Wildlife, and a private scientist, supported the proposed threatened status and critical habitat designation. Opinions were presented that agreed with the Service's analysis that the Warner sucker has become depleted since historic times. Other statements are similar to those presented in the "Summary of Factors Affecting the Species" section and will not be repeated here.

The Oregon Department of Fish and Wildlife recommended adding the upstream spring-source area of Snyder Creek to the critical habitat designation. Although this area is important for providing downstream habitat on Snyder Creek, the Service believes that flows from the spring-source area will be protected by downstream critical habitat designations. That is, a significant upstream water diversion would adversely modify critical habitat downstream on Snyder Creek, and thus could be dealt with in the manner described below under "Available Conservation Measures."

The Bureau of Land Management questioned why the proposed critical habitat on Twelvemile Creek stopped at the Oregon-Nevada border. The Service concurs that habitat in the Nevada portion appears as capable of supporting Warner suckers as does the Oregon portion. However, Warner suckers have not been collected from the Nevada portion of the creek, and the Service therefore extended critical habitat designation only to the border. The Bureau also questioned why the upper portion of Twentymile Creek was included as critical habitat. In question is the species' ability to ascend the steep canyon area in Twentymile Creek. The Service acknowledges that the pricise upstream distribution limit within Twentymile Creek is uncertain. Adult and juvenile Warner suckers have been collected in Twentymile Creek near its confluence with Twentymile Creek (Coombs *et al.* 1979). Based on our knowledge of the species' life history, it is likely that adult Warner suckers move upstream in Twentymile Creek during their spring spawning run. The Service has no evidence to indicate that the canyon area addressed in BLM's comment is a limiting factor in movement of adults. Further, information in our files indicates that some of the upstream area may provide habitat for the Warner sucker. Therefore, lacking scientific data to the contrary, we have adopted the proposed critical habitat designation for 9 miles of Twentymile Creek upstream of its confluence with Twentymile Creek.

Comments were received from the Director of the Bureau of Land Management, Supervisor of the Fremont National Forest, and the Oregon State Forester that offered guidance in management of the Warner sucker but offered no opinion as to whether the species should be listed or not. An error in the "Critical Habitat" section of the proposed rule was discovered during the comment period. In that section, the description of the proposed critical habitat did not agree with the actual metes and bounds as given in the "Proposed Regulations Promulgation" section of the rule. Service personnel prepared a handout for the public hearing that clarified the proposed critical habitat, and the critical habitat description is corrected in this final rule.

Summary of Factors Affecting the Species

After a thorough review and consideration of all information available, the Service has determined that the Warner sucker (*Catostomus warnerensis)* should be classified as a threatened species. Procedures found at section 4(a)(1) of the Endangered Species Act (16 U.S.C. 1531 *et seq.*) and regulations promulgated to implement the listing provisions of the Act (codified at 50 CFR Part 424) were followed. A species may be determined to be an endangered or threatened species due to one or more of the five factors described in section 4(a)(1). These factors and their application to the Warner sucker (*Catostomus warneresis)* are as follows:

A. *The present or threatened destruction, modification, or curtailment of its habitat or range.* The Warner sucker is endemic to the Warner Valley in south-central Oregon. It inhabits portions of Crump and Hart Lakes, the spillway north of Hart Lake, and some sections of Snyder, Honey, Twentymile, and Twelvemile Creeks (Andreasen, 1975; Coombs *et al.*, 1979; Swenson, 1978). The species typically ascends streams tributary to lakes in the Warner Basin to spawn. However, instream barriers and diversion structures have often prohibited the movement of suckers into spawning streams during recent years. During years with high precipitation, enough water flows by the diversion structures so that the suckers can utilize limited reaches of the streams for spawning. However, during periods of low flows, all water is often diverted, thereby eliminating any change for the fish to spawn. If suckers are successful in ascending the barriers, spawned-out fish and progeny are likely to be restricted to small areas of streams because of instream barriers, or sometimes diverted into agricultural fields where they die. Water diversion, used to promote farming activites, exists on all streams occupied by this species. Such water barriers and diversions are particulary detrimental to this obligatory stream-spawning species. Spawning habitat consists of silt-free gravel areas with moderate flows. Postlarval and young-of-the-year Warner suckers utilize shallow backwater pools and stream margins where current is slight or nonexistent.

In addition to water diversions, channelization of streams and overgrazing have disturbed soils in the watershed and degraded streams even further by allowing siltation of gravel beds normally used for spawning. Runoff and leachates containing fertilizers and pesticides from certain agricultural and ranching activities in the Warner Valley watershed further degrade water quality of the lakes and streams.

B. *Overutilization of commercial, recreational, scientific, or educational purposes.* There is no evidence to suggest overutilization of the Warner sucker for any of these purposes.

C. *Disease or predation.* Exotic centrarchid (sunfishes and freshwater basses) and ictalurid (catfishes) fishes have been stocked into lakes in the Warner Basin. Large centrarchids and ictalurids are capable of preying on Warner suckers. Of particular concern are large numbers of crappie (*Pomoxis* spp.) in Hart and Crump Lakes. Exotic fishes also may introduce new parasites and disease organisms to which the sucker might be susceptible. Exotic salmonid fishes (trouts) introduced into the steams may also exert predation pressures.

D. *The inadequacy of existing regulatory mechanisms.* Oregon State law provides protection against taking of the Warner sucker by requiring a collecting permit, but the State has no provisions for the protection of habitat.

E. *Other natural or manmade factors affecting its continued existence.* Any prolonged drought will hasten the demise of the Warner sucker if all or most of the water in the streams is diverted. During droughts of the 1930's and early 1960's, Hart and Crump Lakes were almost dry. During such times, maintenance of adequate stream habitat is critical to survival of the species and any diversion of stream flow would be particularly deterimental. The reduced numbers of populations and individuals make this species especially susceptible to any natural or manmade factors that adversely affect it.

The Service has carefully assessed the best scientific information available regarding the past, present, and future threats faced by this species in deciding to make this rule final. Based on the evaluation, the preferred action is to list the Warner sucker as threatened. The range and number of the species have been reduced substantially and alteration of habitat continues. Proper and adequate management could prevent the species from becoming endangered. Recent status information has provided essential habitat data and indicates that overcollecting is not a major threat. It appears prudent to propose critical habitat for the Warner sucker.

Critical Habitat

Critical habitat, as defined by section 3 of the Act, means: (i) The specific areas within the geographical area occupied by a species, at the time it is listed in accordance with the Act, on which are found those physical or biological features (I) essential to the conservation of the species and (II) that may require special management considerations or protection, and (ii) specific areas outside the geographical area occupied by a species at the time it is listed, upon a determination that such areas are essential for the conservation of the species.

Section 4(a)(3) of the Act requires that critical habitat be designated to the maximum extent prudent and determinable concurrently with the determination that a species is endangered or threatened. Critical habitat is being designated for the Warner sucker to include the following streams in Lake County, Oregon, and 50 feet on either side of the stream banks: 4 stream miles of Twelvemile Creek, 18 stream miles of Twentymile Creek, 2 stream miles of the spillway canal north of Hart Lake, 3 stream miles of Snyder Creek, and 16 stream miles of Honey Creek. The 50-foot riparian zone on each side of the streams is included to protect the integrity of the stream ecosystem. The Service determines that the maintenance of this riparian zone is essential to the conservation of the Warner sucker. Riparian vegetation helps prevent siltation and run-off of other pollutants. Shading from small trees and shrubs in the riparian zone helps maintain suitable water temperature and dissolved oxygen levels in the streams. These stream areas include spawing and rearing habitat for the species. The areas proposed did not include the entire historic or present habitat of the fish and modifications to critical habitat descriptions may be proposed in the future. No data were received during the comment period or from the public hearing that resulted in changes to the critical habitat as proposed on May 21, 1984 (49 FR 21383).

Section 4(b)(8) requires, for any proposed or final regulation that designates critical habitat, a brief description and evaluation of those activities (public or private) which may adversely modify such habitat or may be affected by such designation. These activities are: (1) Overgrazing by livestock, which would eliminate riparian vegetation and lead to streambank erosion and subsequent siltation of the stream and lake environment; (2) introduction of exotic fishes into streams or lakes of the Warner Valley, which might compete with or prey on Warner suckers; (3) construction of additional diversion dams, that do not have adequate fish-passage facilities, on streams inhabited by the Warner sucker; (4) channelization or diversion of streams inhabited by the Warner sucker; (5) application of herbicide or insecticide along stream courses or lakes inhabited by the Warner sucker, which could be toxic to the species or food; (6) pollution of stream or lake habitat by silt or other pollutants; and (7) removal of natural vegetation within or along streams.

Consultations with the U.S. Bureau of Land Management may be necessary for actions involving grazing leases along streams designated as critical habitat. Consultations with the U.S. Forest Service will be unlikely as no Warner sucker habitat occurs on National Forest lands. However, much of the watershed for streams designated as critical habitat is within Forest Service jurisdiction. Substantial increases in timber harvest and/or road construction in the Honey Creek drainage may require section 7 consultation. In addition to grazing leases and timber sales, habitat of forest management plans of the Bureau of Land Management of Forest Service will require consultations if their implementation may affect the Warner sucker. Also, consultation with the U.S. Army Corps of Engineers (Corps) may be necessary for any stream bank work under permits pursuant to section 404 of the Clean Water Act or permits pursuant to section 10 of the Rivers and Harbors Act on streams designated as critical habitat.

Section 4(b)(2) of the Act requires the Service to consider economic and other impacts of designating a particular area as critical habitat. The Service has considered the critical habitat designation in light of relevant additional information obtained and concludes that no significant economic or other impacts are expected to result from the designation. The Bureau of Land Management has already reduced or eliminated cattle grazing along portions of some streams herein designated as critical habitat. Such action was taken to protect riparian habitats rather than to exclusively conserve Warner suckers. Both the Forest Service and the Corps have indicated that they do not expect their activities to affect or be affected by the critical habitat designation. In addition, there is no known involvement of Federal funds or permits for the private land within the critical habitat. For these reasons, no adjustments to the boundaries of the proposed critical habitat were warranted.

Available Conservation Measures

Conservation measures provided to species listed as endangered or threatened under the Endangered Species Act include recognition, recovery actions, requirements for Federal protection, and prohibitions against certain practices. Recognition through listing encourages and results in conservation actions by Federal, State, and private agencies, groups, and individuals. The Endangered Species Act provides for possible land acquisition and cooperation with the States and requires that recovery actions be carried out for all listed species. Such actions are initiated by the Service following listing. The protection required of Federal agencies and the prohibitions against taking and harm are discussed, in part, below.

Section 7(a) of the Act, as amended, requires Federal agencies to evaluate their actions with respect to any species that is proposed or listed as endangered or threatened and with respect to its critical habitat, if any is being designated. Regulations implementing this interagency cooperation provision of the Act are codified at 50 CFR Part 402 and are now under revision (see proposal at 48 FR 29990; June 29, 1983). Section 7(a)(2) requires Federal agencies to ensure that activities they authorize, fund, or carry out are not likely to jeopardize the continued existence of a listed species or to destroy or adversely modify its critical habitat. If a Federal action may affect a listed species or its critical habitat, the responsible Federal agency must enter into formal consultation with the Service. Federal activities which may be affected by the determination of critical habitat for the Warner Sucker were discussed above in the "Critical Habitat" section of this rule.

The Act and implementing regulations found at 50 CFR 17.21 and 17.31 set forth a series of prohibitions and exceptions that generally apply to all threatened wildlife. These prohibitions, in part, make it illegal for any person subject to the jurisdiction of the United States to take, import or export, ship in interstate commerce in the course of a commercial activity, or sell or offer for sale in interstate of foreign commerce any listed species. It also is illegal to possess, sell, deliver, carry, transport, or ship any such wildlife that had been taken illegally. Certain exceptions would apply to agents of the Service and State conservation agencies. General regulations governing the issuance of permits to carry out otherwise prohibited activities involving threatened wildlife species under certain circumstances are set out at 50 CFR 17.32.

The Secretary has discretion under section 4(d) of the Act to issue such special regulations as are necessary and advisable for the conservation of a threatened species. The Warner sucker is threatened primarily by habitat disturbance or alterations, not by intentional, direct taking of the species or by commercialization. Given this fact, and the fact that the State of Oregon regulates direct taking of the species through the requirement of State collecting permits, the Service has concluded that the State's collection permit system is adequate to protect the species from excessive taking, so long as such takes are limited to: educational purposes, scientific purposes, the enhancement of propagation or survival of the species, zoological exhibition, and other conservation purposes consistent with the Endangered Species Act.

Therefore, the special rule adopted herein allows take of the Warner sucker for the above-stated purposes without the need for a Federal permit if a State collection permit is obtained and all other State wildlife conservation laws and regulations are satisfied. Rules are also promulgated to allow incidential take of the species during recreational fishing activities if the fishing is conducted in accordance with State law and if the Warner suckers are returned immediately into their habitat. The Service acknowledges that incidental take of the species by State-licensed recreational fishermen is not a significant threat to the Warner sucker. It should be recognized that any activities involving the taking of this species not otherwise enumerated in the special rule are prohibited. Without this special rule, all of the prohibitions under 50 CFR 17.31 would apply. The Service believes that this special rule will allow for more efficient management of this species, thereby facilitating its conservation. For these reasons, the Service has concluded that this regulatory action is necessary and advisable for the conservation of the Warner sucker.

National Environmental Policy Act

The Fish and Wildlife Service has determined that an Environmental Assessment, as defined under authority of the National Environmental Policy Act of 1969, need not be prepared in connection with regulations adopted pursuant to section 4(a) of the Endangered Species Act of 1973, as amended. A notice outlining the Service's reasons for this determination was published in the **Federal Register** on October 25, 1983 (48 FR 49244).

Regulatory Flexibility Act and Executive Order 12291

The Department of the Interior has determined that designation of critical habitat for this species will not constitute a major action under Executive Order 12291 and certifies that this designation will not have a significant economic effect on a substantial number of small entities under the Regulatory Flexibility Act (5 U.S.C. 601 *et seq.*). The critical habitat is found in the Warner Valley, Oregon, and is composed of approximately 43 stream miles and 50 feet on either side of the stream channel. The lands are federally and privately owned and are used for cattle grazing and crop production. No Federal involvement is expected to affect or be affected by this designation. Therefore, no significant economic or other impacts are anticipated to result from the critical habitat designation. In addition, no direct costs, enforcement costs, or information collection or recordkeeping requirements are imposed on small entities by this designation. These determinations are based on a Determination of Effects that is available from the U.S. Fish and Wildlife Service, at the address in the "ADDRESSES" section.

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Author

The primary author of this final rule is Dr. Jack E. Williams, U.S. Fish and Wildlife Service, Sacramento Endangered Species Office, 2800 Cottage Way, Room E–1823, Sacramento, California 95825 (916/978–4866 or FTS 460–4866).

List of Subjects in 50 CFR Part 17

Endangered and threatened wildlife, Fish, Marine mammals, Plants (agriculture).

Regulations Promulgation

PART 17—[AMENDED]

Accordingly, Part 17, Subchapter B of Chapter I, Title 50 of the Code of Federal Regulations, is amended as set forth below:

1. The authority citation for Part 17 continues to read as follows:

Authority: Pub. L. 93–205, 87 Stat. 884; Pub. L. 94–359, 90 Stat. 911; Pub. L. 95–632, 92 Stat. 3751; Pub. L. 96–159, 93 Stat. 1225; Pub. L. 97–304, 96 Stat. 1411 (16 U.S.C. 1531 *et seq.*).

2. Amend § 17.11(h) by adding the following, in alphabetical order under "Fishes," to the List of Endangered and Threatened Wildlife:

§ 17.11 Endangered and threatened wildlife.

* * * * *

(h) * * *

Species		Historic range	Vertebrate population where endangered or threatened	Status	When listed	Critical habitat	Special rules
Common name	Scientific name						
Fishes							
*	*	*	*	*	*	*	
Sucker, Warner	*Catostomus warnerensis*	U.S.A. (OR)	Entire	T	205	17.95(e)	17.44(l)
*	*	*	*	*	*	*	

3. Add the following as special rules to Section 17.44:

§ 17.44 Special rules—fishes.

* * * * *

(l) Warner sucker (*Catostomus warnerensis*)

(1) No person shall take the species, except in accordance with applicable State fish and wildlife conservation laws and regulations in the following instances: (i) For educational purposes, scientific purposes, the enhancement of propagation or survival of the species, zoological exhibition, and other conservation purposes consistent with the Act; (ii) incidental to State-permitted recreational fishing activities, provided that the individual fish taken is immediately returned to its habitat.

(2) Any violation of applicable State fish and wildlife conservation laws or regulations with respect to the taking of this species will also be a violation of the Endangered Species Act.

(3) No person shall possess, sell, deliver, carry, transport, ship, import, or export, by any means whatsoever, any such species taken in violation of these regulations or in violation of applicable State fish and wildlife laws or regulations.

(4) It is unlawful for any person to attempt to commit, solicit another to commit, or cause to be committed, any offense defined in paragraphs (l) (1) through (3) of this section.

* * * * *

4. Amend § 17.95(e) by adding critical habitat of the Warner sucker as follows: (The position of this entry under § 17.95(e) will follow the same sequence as the species occurs in § 17.11.)

§ 17.95 Critical habitat—fish and wildlife.

(e) * * *

* * * * *

Warner Sucker (Catostomus warnerensis)

Oregon: Lake County.

1. *Twelvemile Creek*—Approximately 4 stream miles and 50 feet on either side of the stream commencing at the confluence of Twelvemile Creek and Twentymile Creek and extending upstream, and including those portions of Twelvemile Creek in T40S, R23E, Section 35; and T41S, R23E, Sections 1, 2, 12, 13, 23, and 24.

2. *Twentymile Creek*—Approximately 18 stream miles and 50 feet on either side of the stream commencing about 9 miles upstream of the junction of Twelvemile and Twentymile Creeks and extending to a point about 9 miles downstream of the junction, and including those portions of Twentymile Creek in T40S, R22E, Sections 25, 35, and 36; T40S, R23E, Sections 19, 20, 24, 25, 28, 29, 30, 33, 34, 35 and 36; T40S, R24E, Sections 15, 16, 19, 20, 21, 22, 28, 29, 30; and T41S, R23E, Sections 2 and 3.

3. *Spillway Canal north of Hart Lake*—Approximately 2 stream miles and 50 feet on either side of the waterway commencing at its confluence with Hart Lake and extending to a point about 2 miles downstream, and including those portions of the waterway in T36S, R24E, Sections 7, 18, and 19.

4. *Snyder Creek*—Approximately 3 stream miles and 50 feet on either side of the stream commencing at the confluence of Snyder Creek and Honey Creek and extending to a point about 3 miles upstream on Snyder Creek, and including those portions of Snyder Creek in T36S, R22E, Sections 1 and 12; and T36S, R23E, Sections 7, 17, and 18.

5. *Honey Creek*—Approximately 16 stream miles and 50 feet on either side of the stream commencing at the confluence of Honey Creek with Hart Lake and extending to a point about 16 miles upstream on Honey Creek, and including those portions of Honey Creek in T36S, R24E, Sections 19, 20, 27, 28, 29, 30, 33, 34, and 35; T36S, R23E, Sections 17, 18, 20, 21, 22, 23, 24, 26, 27, and 28; and T36S, R22E, Sections 13, 14, 22, and 23.

Constituent elements of all areas proposed as critical habitat include streams 15 feet to 60 feet wide with gravel-bottom shoal and riffle areas with intervening pools. Streams should have clean, unpolluted flowing water and a stable riparian zone. The streams should support a variety of aquatic insects, crustaceans, and other small invertebrates for food.

* * * * *

Dated: September 3, 1985.

P. Daniel Smith,

Acting Deputy Assistant Secretary for Fish and Wildlife and Parks.

[FR Doc. 85-23075 Filed 9-26-85; 8:45 am]

BILLING CODE 4310-55-M

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50 CFR Part 17

Endangered and Threatened Wildlife and Plants; Final Rule To Determine Endangered Status and Critical Habitat for the White River Springfish and the Hiko White River Springfish

AGENCY: Fish and Wildlife Service, Interior.

ACTION: Final rule.

SUMMARY: The Service determines endangered status and critical habitat for the White River springfish (*Crenichthys baileyi baileyi*) and Hiko White River springfish (*Crenichthys baileyi grandis*). This action is being taken because the one known population of the White River springfish and the single remaining population of the Hiko White River springfish are threatened by habitat alteration and the presence of exotic species, which compete with and prey upon the springfishes. These springfishes occur in remnant waters of the Pluvial White River system in eastern Nevada. The White River springfish is presently known to occur only in Ash Springs while the Hiko White River springfish, extirpated from Hiko Spring, now exists as a single, small population in Crystal Springs. These spring areas are located in the Pahranagat Valley of Lincoln County, Nevada. This final rule implements Federal protection provided by the Endangered Species Act of 1973, as amended.

DATES: The effective date of this rule is October 28, 1985.

ADDRESSES: The complete file for this rule is available for inspection, by appointment, during normal business hours at the U.S. Fish and Wildlife Service, Suite 1692, Lloyd 500 Building, 500 NE. Multnomah Street, Portland, Oregon 97232.

FOR FURTHER INFORMATION CONTACT: Mr. Wayne S. White, Chief, Division of Endangered Species, at the above address (503/231-6131 or FTS 429-6131).

SUPPLEMENTARY INFORMATION:

Background

Crenichthys baileyi is one of the two species within the genus *Crenichthys*. Hubbs (1932) recognized the distinct qualities of these fishes when he described *Crenichthys* and *C. nevadae* from Railroad Valley in central Nevada. Distinctive characteristics of the genus include a lack of pelvic fins, uniserial bifid teeth, a long and coiled intestine, and restricted range. Fishes of the genus *Crenichthys* have been of particular scientific interest because of their adaptation to extermely high temperatures and low dissolved oxygen (Hubbs and Hettler 1964, Hubbs *et al.* 1967, Sumner and Sargent 1940).

Crenichthys baileyi is endemic to the remnant waters of the White River system in eastern Nevada. During pluvial times, 10,000 to 40,000 years ago, the White River was a much larger river that flowed into the Colorado River by way of the Virgin River (Hubbs and Miller 1948). As the White River desiccated in response to the more xeric Recent climate, the springfishes were restricted to remaining permanent waters, such as springs.

The White River springfish (*C. b. baileyi*) and Hiko White River springfish (*C. b. grandis*) were described by Williams and Wilde (1981) as two of five subspecies of *C. baileyi* restricted to the Pahranagat Valley of Lincoln County. Both of these subspecies are threatened by habitat alteration, as well as the presence of exotic species, that are detrimental to the springfishes because of increased competition, predation, and parasitism (Hubbs and Deacon 1964, Wilson *et al.* 1966 Deacon 1979).

Habitats occupied by these taxa are extremely localized and vulnerable to alteration. During the past 20 years these habitats have been impounded to facilitate agricultural diversion and create recreational swimming facilities. Whereas historic records document the subspecies' presence in several areas (Gilbert 1893), recent investigations (Courtenay *et al.* ms.) indicate the current absence from formerly occupied habitats and/or a severe reduction in numbers. The White River springfish is presently found in a single, small locality (surface area less than 2 acres) used by the public as a swimming facility and principally occupied by exotic fishes.

The Hiko White River springfish was extirpated from one of its two known habitats in 1967 when exotic game fishes gained entrance resulting from upstream migration. Efforts to restock the springfish in Hiko Spring have occurred in recent years; the long-term viability of this population is, however, questionable. The remaining population is extremely small (less than 100 individuals) and threatened by the presence of exotic fishes, such as the convict cichlid (*Cichlasoma nigrofasciatum*) and mosquitofish (*Gambusia affinis*).

On December 30, 1982, the Service published a Review of Vertebrate Wildlife (47 FR 58454) and included the White River springfish and the Hiko White River springfish as category 1 species. Category 1 indicates that the Service has substantial information to support the biological appropriateness of listing the species as threatened or endangered.

On April 12, 1983, the Service received a petition from the Desert Fishes Council requesting that the White River springfish and the Hiko White River springfish, along with 15 other fish species, be added to the List of Endangered and Threatened Wildlife. The Service published in the **Federal Register** (48 FR 27273) on June 14, 1983, a finding that the petition presented substantial information and that the petitioned action may be warranted. The

Service proposal to list these two springfishes as endangered species with critical habitat on May 7, 1984 (49 FR 19360), constituted the required 1-year finding in accordance with Section 4(b)(3)(B)(ii) of the Act.

Summary of Comments and Recommendations

In the May 7, 1984, proposed rule (49 FR 19360) and associated notifications, all interested parties were requested to submit factual reports or information that might contribute to the development of a final rule. Appropriate State agencies, county governments, Federal agencies, scientific organizations, and other interested parties were contacted and requested to comment. Newspaper notices of the proposal were published in *The Lincoln County Record* on June 14, 1984, the *Ely Daily Times* on June 12, 1984, and the *Las Vegas Review-Journal* on June 13, 1984.

A total of 14 written comments were received and are discussed below. Local interest in the proposal by Pahranagat Valley landowners led the Service to hold a public meeting in Alamo, Nevada during the comment period. Shortly after this meeting was scheduled, Mr. Kay Wright, Chairman of the Hiko Spring Water Board, also requested a public hearing. Mr. Wright was informed of the public meeting and later decided to withdraw his request.

Comments about the proposal were basically split into two areas: the listing of these springfishes as endangered species and the designation of critical habitat. Five comments supported the listing as well as the designation of critical habitat, three comments opposed only the designation of critical habitat, and six were noncommittal, but voiced concerns about the impact such designation may have on private activities on private lands.

Mr. William A. Molini, Director, Nevada Department of Wildlife; Mr. Keith Whipple, Chairman, Lincoln County Conservation District; and Mr. E.P. Pister, Executive Secretary, Desert Fishes Council, opposed designation of critical habitat. These negative comments were not reflected, however, in recommendations for listing the two species as endangered. Director Molini concurred with the proposal to list the springfishes as endangered, but stated concern that a critical habitat designation on private lands would arouse animosity and direct unfavorable attention to the fishes. Mr. Pister also concurred with the listing, but commented that designating critical habitat for these springfishes may adversely effect the springfishes because they occupy extremely restricted habitats where extirpation could occur from a single malicious act. Mr. Whipple expressed concern that Federal designation of private lands as critical habitat acts to identify parcels where private activities cannot occur and sites that will be future acquisitions, possibly by condemnation, by the Federal Government. The Service responds that critical habitat is designated to advise Federal agencies of the need for special care in particular areas that are essential to the conservation of listed species. Designation of critical habitat does not affect State, local government, or individual actions unless an activity is funded or permitted by the Federal Government. The Service has no intention of condemning land or waters for these springfishes. Should any acquisitions occur, they will proceed with full consent of the involved parties. The Service believes that the potential for adverse effects resulting from critical habitat designations does not outweigh the potential benefits, or protections, that arise from the designations.

Comments that stated concerns, but neither objections to, nor concurrence with, the proposal, were received from Congresswoman Barbara Vucanovich, Bank of America, Mr. Mitchell Hunt, Nevada State Division of Historical Preservation and Archaeology, and Mr. Kay Wright. Congresswoman Vucanovich requested that local economic opportunities be given adequate consideration when species are listed as threatened or endangered and critical habitats are designated, and that local interests also be given adequate time to comment on Service proposals. The 1982 Amendments to the Act require that determinations to list a species as threatened or endangered be based solely on the best available scientific and commercial information about the species. Economic impacts are not allowed to be considered in making a listing determination. The Act specifies, however, that the economic impact of designating a particular area as critical habitat must be considered. An economic analysis for the designation of critical habitat has been prepared with this rule and concludes that designation of critical habitat will not affect or be affected by small entities. A copy of this document is available from the Office of Endangered Species, Fish and Wildlife Service, U.S. Department of the Interior, Washington, D.C. 20240. The Service has actively sought public comment regarding the subject proposal. Letters and copies of the proposal were sent to each individual owning land within the proposed critical habitat; notification of the proposal was published in local and regional newspapers. Shortly following the **Federal Register** publication of the proposal, the Service voluntarily organized a public meeting and presented the proposal to interested citizens while the formal comment period was open.

The Bank of America asked what intentions the Service has for the Burns Ranch, and made notification that it holds the subject property in trust and must, therefore, approve any action that adversely affects this trust. Service plans for the Burns Ranch presently include only an interest in being provided access onto the land to monitor springfish populations. The aquatic habitat occurring on this ranch is currently habitat for the listed endangered Pahranagat roundtail chub (*Gila robusta jordani*); anticipated activities for springfish are not different than those anticipated for this chub. The Service recognizes the responsibility the Bank of America has to the Burns Ranch and will respect this during future programs.

Mr. Hunt expressed concern that finalization of the proposal would prohibit recreational activities in and around the Ash Springs Resort. The Service does not believe these activities presently conflict with programs required to conserve the species. Therefore, no change is believed necessary.

The Nevada State Division of Historical Preservation and Archaeology requested that it be permitted to comment on any management activities that might disturb land surrounding spring habitats. The Service has planned no management activities that might disturb land surrounding spring habitats. Should any such activities be planned in the future, the Service will make the proper notifications.

The Nevada Department of Wildlife Director, William Molini, stated concerns about taxonomy of the two springfishes by noting that a difference in head length of 0.1 mm is possibly not significant enough to warrant subspecific distinction between the two Pahranagat Valley springfishes. The Service responds that taxonomic distinction of these two subspecies is not based solely on the differences in head size; Williams and Wilde (1981) also noted statistically significant differences between the two forms in least bony interorbital length and caudal peduncle length, and differences in the number of dorsal and anal fin rays. The differences they recorded are within the range of difference accepted by taxonomists to distinguish unique subspecies of fish (Hubbs and Hubbs 1953).

The proposed listing action was supported by the Nevada Department of Wildlife, Nevada Division of State Parks, Defenders of Wildlife, American Society of Ichthyologists and Herpetologists, and Desert Fishes Council. Additional information regarding the proposal was presented by Mr. Edwin Higbee, lifetime resident of the Pahranagat Valley; Mr. Thomas Baugh, Research Associate, Endangered Fishes Research Center, University of Nevada at Las Vegas; Dr. Walter R. Courtenay, Jr., Chairman, American Society of Ichthyologists and Herpetologists, Environmental Quality Committee; and Mr. Edwin P. Pister, Executive Secretary, Desert Fishes Council.

Mr. Higbee stated that during the past 15 years he has observed a decreased number of springfish and increasing adundance of introduced fishes in the Pahranagat River through the Burns Ranch. Mr. Baugh enclosed data collected from Crystal Spring during 1983 and 1984 showing that the Hiko White River springfish occurs in exceeding low numbers; 21 were captured during 65 hours of trapping effort within an area less than one-half acre. Dr. Courtenay submitted a manuscript, recently accepted for publication by the *Southwestern Naturalist*, entitled "Comparative Status of Fishes Along the Course of the Pluvial White River, Nevada." Data summarized in this paper show the absence of springfish in Hiko Spring, and the comparatively small extant populations in Crystal and Ash Springs. Mr. Pister submitted the same manuscript submitted by Dr. Courtenay.

Summary of Factors Affecting the Species

After a thorugh review and consideration of all information available, the Service has determined that the White River springfish and Hiko White River springfish should be classified as endangered species. Procedures found at Section 4 of the Endangered Species Act (16 U.S.C. 1531 *et seq.*) and regulations promulgated to implement the listing provisions of the Act (codified at 50 CFR Part 424) were followed. A species may be determined to be an endangered or threatened species due to one or more of the five factors described in Section 4(a)(1). These factors and their application to the White River springfish (*Crenichthys baileyi baileyi*) and Hiko White River springfish (*Crenichthys baileyi grandis*) are as follows:

A. *The present or threatened destruction, modification, or curtailment of their habitat or range.* Habitats occupied by these two species have been extensively altered to enhance irrigation practices and provide for public recreation. These activities have changed the character of aquatic environments by eliminating bordering and aquatic vegetation, eliminating aquatic habitat by diverting the entire flow of some streams into pipes or cement canals, and seasonally manipulating water within stream channels to facilitate irrigation. These activities effectively reduce the amount of available habitat as well as reduce invertebrate populations utilized for food by the two fishes.

Exotic species introduced into the Pahranagat Valley during the past 50 years have effectively reduced populations of the springfishes through competition for food and space, and by predation (Courtenay *et al.* ms). All of these factors have combined to eliminate one Hiko White River springfish population and reduce the only remaining population to dangerously low numbers. The only population of White River springfish declined between 1965 and 1980, but has slightly rebounded since this time (Courtenay *et al.* ms). This population, however, remains small and its occupied habitat is estimated as covering less than two acres.

B. *Overutilization for commercial, recreational, scientific, or educational purposes.* None apparent.

C. *Disease or predation.* Wilson *et al.* (1966) identified diseases affecting native springfish (*Crenichthys baileyi moapae*) and Moapa dace (*Moapa coriacea*) within the Moapa Valley of southern Nevada. These diseases are not naturally known within populations of native fishes, but are common among fishes frequently utilized by aquarists and introduced into Pahranagat Valley aquatic habitats.These diseases may be reducing viability and/or causing mortality within White River springfish and Hiko White River springfish populations.

Predation has effected the demise of one Hiko White River springfish population. Williams and Wilde (1981) correlated the disappearance of this population with introduction of largemouth bass (*Micropterus salmoides*).

D. *The inadequacy of existing regulatory mechanisms.* The State of Nevada lists the entire White River springfish species (*Crenichthys baileyi*) as rare. However, this action does not provide habitat protection to the species on Federal land, or from federally funded or approved projects on private land.

E. *Other natural or manmade factors affecting their continued existence.* The introduction of exotic organisms, particularly fishes, into springfish habitats has reduced or eliminated populations through competition for food and/or space, and by direct predation (Deacon 1979, Courtenay *et al.* ms).

The Service has carefully assessed the best scientific and commercial information available regarding the past, present, and future threats faced by these species in determining to make this rule final. Based on this evaluation, the preferred action is to list the White River springfish and Hiko White River springfish as endangered, each with critical habitat. Endangered status is appropriate because of the restricted and reduced range of these species. If not listed, the threats to these fishes and their remaining habitat could cause the extinction of both species. Thus, endangered status is appropriate at this time. An explanation of the critical habitat designation is presented in the "Critical Habitat" section of this rule.

Critical Habitat

Critical habitat, as defined by Section 3 of the Act means: (i) The specific areas within the geographical area occupied by a species, at the time it is listed in accordance with the Act, on which are found those physical or biological features (I) essential to the conservation of the species and (II) that may require special management considerations or protection, and (ii) specific areas outside the geographical area occupied by a species at the time it is listed, upon a determination that such areas are essential for the conservation of the species.

Section 4(a)(3) of the Act requires that critical habitat be designated to the maximum extent prudent and determinable concurrently with the determination that a species is endangered or threatened. Critical habitat being designated for the White River springfish includes Ash Springs and its associated outflow in Pahranagat Valley, Lincoln County, Nevada. Critical habitat being designated for the Hiko White River springfish includes Crystal and Hiko Springs and their associated open outflows in Pahranagat Valley, Lincoln County, Nevada. Technical corrections have been made in the final rule to define accurately the location of Crystal Springs and associated outflows.

The areas designated as critical habitat for these two species satisfy all known criteria for their ecological, behavioral, and physiological requirements. The White River springfish still reproduces successfully in the source spring area of Ash Springs. The Hiko White River springfish, now extirpated from Hiko Spring, is known to occur only in Crystal Springs although its numbers there are reduced.

The most critical elements to survival of the springfishes are the consistent quality and quantity of springflows. The critical habitats include the springs and associated outflows that are the only known habitats for these fishes. The critical habitats also include land areas immediately surrounding these aquatic habitats. These land areas provide vegetative cover that contributes to the uniform water conditions preferred by the springfishes and provides habitat for insects and other invertebrates which constitute a substantial portion of their diet. The maintenance of these riparian areas is essential to the conservation of the species.

Section 4(b)(8) requires, for any proposed or final regulation that designates critical habitat, a brief description and evaluation of those activities (public or private) which may adversely modify such habitat or may be affected by such designation. Activities that may adversely affect the critical habitats of the White River springfish and Hiko White River springfish include pollution of the springwater, introduction of exotic species, excessive mining of water from nearby aquifers, and further physical modifications of Ash, Hiko, or Crystal Springs, such as channelization and diversion of springflows or clearing of the surrounding vegetation.

Approximately 0.1 acre of proposed critical habitat for the White River springfish is located on land administered by the Bureau of Land Management (BLM). This area is within the Pahranagat East Grazing Allotment. If BLM determined that fencing was required to protect these springs and their flows, approximately 200–300 feet of fence would be required at a cost of $0.43 per linear foot, and would result in a decrease of 0.002 Animal Unit Months (AUMs). The cost of fencing would be approximately $129, and the loss of annual grazing fees from the reduction in AUMs would be less than $0.03. BLM is presently involved in minimal activity within the proposed critical habitat. BLM's planning process identifies that a Habitat Management Plan (HMP) and Recreational Management Plan (RMP) will be prepared for this area in the future. These management plans are expected to be compatible with the critical habitat designation.

The remaining 11.9 acres of proposed critical habitat for the White River springfish consists of private land. Uses within this area include recreational swimming and grazing cattle on pastureland. There is no known involvement of Federal funds or permits for activities within this area. Present uses are considered suitable for conservation of this species. In addition, any conservation efforts by the private landowners would be voluntary.

Approximately 7 acres of privately owned land are proposed as critical habitat for the Hiko White River springfish. There is no known involvement of Federal funds or permits for these private lands. Any conservation efforts by the private landowners would be voluntary.

The Soil Conservation Service (SCS) of the U.S. Department of Agriculture has funded some irrigation projects in the Pahranagat Valley in the past, but there is little opportunity for additional SCS projects in the area. SCS has informed the Service that it does not anticipate any SCS projects that might affect or be affected by the critical habitat designation in the foreseeable future.

Environmental and other benefits may accrue from the designations of critical habitat for the Hiko White River and White River springfishes. No quantifiable estimate of the magnitude of the environmental or other benefits that may accrue from the critical habitat designations, however, can be developed at this time. Difficulties in estimating these benefits stem from: (1) Uncertainties about the nature and extent of the possible additional protection for the two springfishes that might result from the critical habitat designations; and (2) difficulties inherent in developing units of measure that adequately represent the social value of identifying, protecting, and conserving critical habitat for these fish species. No estimate of the number of persons, entities, species, or ecosystems that will be spared adverse effects by these designations of critical habitat can be developed at this time. In addition, no measure of the reduction in risk of ecosystem and species loss could be developed, although such benefits may result from the critical habitat designations and may be substantial.

Section 4(b)(2) of the Act requires the Service to consider economic and other impacts of designating a particular area as critical habitat. The Service has considered these critical habitat designations in light of the economic and other information obtained through the comment process and concludes that no adjustment of the proposed critical habitat is warranted.

Available Conservation Measures

Conservation measures provided to species listed as endangered or threatened under the Endangered Species Act include recognition, recovery actions, requirements for Federal protection, and prohibitions against certain practices. Recognition through listing encourages and results in conservation actions by Federal, State, and private agencies, groups and individuals. The Endangered Species Act provides for possible land acquisition and cooperation with the States and requires that recovery actions be carried out for all listed species. Such actions are initiated by the Service following listing. The protection required of Federal agencies and the prohibitions against taking and harm are discussed, in part, below.

Section 7(a) of the act, as amended, requires Federal agencies to evaluate their actions with respect to any species that is proposed or listed as endangered or threatened and with respect to its critical habitat, if any is designated. Regulations implementing this interagency cooperation provision of the Act are codified at 50 CFR Part 402 and are now under revision (see proposal at 48 FR 29990; June 29, 1983). Section 7(a)(2) requires Federal agencies to ensure that activities they authorize, fund, or carry out are not likely to jeopardize the continued existence of a listed species or to destroy or adversely modify its critical habitat. If a Federal action may affect a listed species or its critical habitat, the responsible Federal agency must enter into formal consultation with the Service. With respect to the White River and Hiko White River springfishes, there are currently no known Federal activities believed to be affected by the listing or designation of critical habitat. However, actions that may occur in the future have been outlined above in the "Critical Habitat" section of this rule.

The Act and implementing regulations found at 50 CFR 17.21 set forth a series of general prohibitions and exceptions that apply to all endangered wildlife. These prohibitions, in part, make it illegal for any person subject to the jurisdiction of the United States to take, import or export, ship in interstate commerce in the course of a commercial activity, or sell or offer for sale in interstate or foreign commerce any listed species. It also is illegal to possess, sell, deliver, carry, transport, or ship any such wildlife that had been taken illegally. Certain exceptions apply to agents of the Service and State conservation agencies.

Permits may be issued to carry out otherwise prohibited activities involving endangered wildlife species under certain circumstances. Regulations governing permits are at 50 CFR 17.22. Such permits are available for scientific purposes, to enhance the propagation or survival of the species, and/or for incidental take in connection which otherwise lawful activities.

National Environmental Policy Act

The Fish and Wildlife Service has determined that an Environmental Assessment, as defined under authority of the National Environmental Policy Act of 1969, need not be prepared in connection with regulations adopted pursuant to section 4(a) of the Endangered Species Act of 1973, as amended. A notice outlining the Service's reasons for this determination was published in the **Federal Register** on October 25, 1983 (48 FR 49244).

Regulatory Flexibility Act and Executive Order 12291

The Department of the Interior has determined that designation of critical habitat for these species will not constitute a major action under Executive Order 12291 and certifies that this designation will not have a significant economic effect on a substantial number of small entities under the Regulatory Flexibility Act (5 U.S.C. 601 *et seq.*).

Based on BLM's current management and proposed HMP and RMP, the absence of current or planned SCS projects, and the unquantifiable benefits that may result from the critical habitat designations, it is not expected that any significant economic or other impacts will result from the critical habitat designations on Federal land. In addition, there is no known involvement of Federal funds or permits for the private land proposed as critical habitat. Therefore, no significant economic impacts are expected to result from the designation of critical habitat on either Federal or private lands. This determination is based on a Determination of Effects that is available from the Regional Director, U.S. Fish and Wildlife Service, Suite 1692, Lloyd 500 Building, 500 NE Multnomah Street, Portland, Oregon 97232.

Literature Cited

Courtenay, W.R., Jr., J.E. Deacon, D.W. Sada, R.C. Allan, and G.L. Vinyard. Ms. Comparative status of fishes along the course of the pluvial White River, Nevada.

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Author

The primary author of this final rule is Donald W. Sada, U.S. Fish and Wildlife Service, Great Basin Complex, Reno, Nevada 89502 (702/784–5227 or FTS 470–5227).

List of Subjects in 50 CFR Part 17

Endangered and threatened wildlife, Fish, Marine mammals, Plants (agriculture).

Regulations Promulgation

PART 17—[AMENDED]

Accordingly, Part 17, Subchapter B of Chapter I, Title 50 of the Code of Federal Regulations, is amended as set forth below:

1. The authority citation for Part 17 continues to read as follows:

Authority: Pub. L. 93–205, 87 Stat. 884; Pub. L. 94–359, 90 Stat. 911; Pub. L. 95–632, 92 Stat. 3751; Pub. L. 96–159, 93 Stat. 1225; Pub. L. 97–304, 96 Stat. 1411 (16 U.S.C. 1531 *et seq.*).

2. Amend § 17.11(h) by adding the following, in alphabetical order under "Fishes," to the List of Endangered and Threatened Wildlife:

§ 17.11 Endangered and threatened wildlife.

* * * * *

(h) * * *

Species		Historic range	Vertebrate population where endangered or threatened	Status	When listed	Critical habitat	Special rules
Common name	Scientific name						
Fishes							
*	*	*	*	*	*	*	
Springfish, Hiko White River	*Crenichthys baileyi grandis*	U.S.A. (NV)	Entire	E	206	17.95(e)	NA
Springfish, White River	*Crenichthys baileyi baileyi*	U.S.A. (NV)	Entire	E	206	17.95(e)	NA
*	*	*	*	*	*	*	

3. Amend § 17.95(e) by adding critical habitat for the Hiko White River springfish as follows: (The position of this entry under § 17.95(e) will follow the same alphabetical sequence as the species occurs in § 17.11.)

§ 17.95 Critical habitat—fish and wildlife.

(e) * * *

* * * * *

Hiko White River Springfish (Crenichthys baileyi grandis).

Nevada, Lincoln County. Each of the following springs and outflows plus surrounding land areas for a distance of 50 feet from these springs and outflows:

Hiko Spring and associated outflows within T4S, R60E, SW¼ of NE¼ Sec. 14 and NW¼ of SE¼ Sec. 14.

Crystal Springs and associated outflows within T5S, R60E, all of NE¼ of Sec. 10 and NE¼ of SE¼ Sec. 10, SW¼ of NW¼ Sec. 11 and NW¼ of SW¼ Sec. 11.

Known constituent elements include warmwater springs and their outflows and surrounding land areas that provide vegetation for cover and habitat for insects and other invertebrates on which the species feeds.

* * * * *

4. Amend § 17.95(e) by adding critical habitat for the White River springfish as follows: (The position of this entry under § 17.95(e) will follow the same sequence as the species occurs in § 17.11.)

§ 17.95 Critical habitat—fish and wildlife.

(e) * * *

* * * * *

White River Springfish (Crenichthys baileyi baileyi).

Nevada, Lincoln County. Ash Springs and associated outflows plus surrounding land areas for a distance of 50 feet from the springs and outflows within the following areas: T6S, R60E, E½ of E½ Sec. 1 and T6S, R61E, NW¼ of NW¼ Sec. 6.

Known constituent elements include warmwater springs and their outflows and surrounding land areas that provide vegetation for cover and habitat or insects and other invertebrates on which the species feeds.

* * * * *

Dated: August 22, 1985.

P. Daniel Smith,

Acting Deputy Assistant Secretary for Fish and Wildlife and Parks.

[FR Doc. 85–23074 Filed 9–26–85; 8:45 am]

BILLING CODE 4310–55–M

Proposed Rules

Federal Register
Vol. 50, No. 188
Friday, September 27, 1985

This section of the FEDERAL REGISTER contains notices to the public of the proposed issuance of rules and regulations. The purpose of these notices is to give interested persons an opportunity to participate in the rule making prior to the adoption of the final rules.

DEPARTMENT OF AGRICULTURE

Agricultural Stabilization and Conservation Service

7 CFR Part 735

[Amdt. No. 1]

Cotton Warehouses; Inspection Fees

AGENCY: Agricultural Stabilization and Conservation Service, USDA.

ACTION: Proposed rule.

SUMMARY: This proposed rule would amend the regulations at 7 CFR Part 735 relating to cotton warehouses licensed under the United States Warehouse Act.

The intended effect of this rule is to: (1) Establish an annual warehouse fee to be paid by each cotton warehouse licensed under the United States Warehouse Act, (2) allow for examination of a warehouse upon the request of a license holder and provide a fee for that examination; (3) provide for an examination after license suspension and provide a fee therefor; and (4) provide for a charge for a tag check of the warehouse inventory whenever the Department determines such tag check is required to satisfactorily complete an examination of the warehouse. This rule is promulgated under the authority of the United States Warehouse Act, as amended.

DATE: Written comments should be received on or before 30 days after publication in the **Federal Register** to assure consideration.

ADDRESS: Written comments on this proposed rule should be sent to Paul W. King, Director, Warehouse Division, Room 5968-South Agriculture Building, Agricultural Stabilization and Conservation Service, P.O. Box 2415, Department of Agriculture, Washington, D.C. 20013.

FOR FURTHER INFORMATION CONTACT: Harry J. Wishmire, 202-475-4028.

SUPPLEMENTARY INFORMATION:

Rulemaking Matters

This proposed rule has been reviewed in conformity with Executive Order 12291 and Departmental Regulation 1512-1 and has been classified as "not major." This action does not constitute a review as to the need, currency, clarity, and effectiveness of these regulations under those procedures. The sunset review date established for these regulations is February 17, 1986."

Everett Rank, Administrator, ASCS, has determined that this action is "not major" since implementation of the proposed rule will not result in: (a) An annual effect on the economy of $100 million or more; (b) major increases in costs or prices for consumers, individual industries, federal, State or local government, or a geographic region; or (c) significant adverse effects on competition, employment, investment, productivity, innovation, or the ability of U.S. based enterprises to compete with foreign-based enterprises in domestic or export markets.

The information collection requirements proposed by this rule will not become effective until they have been approved by the Office of Management and Budget in accordance with the Paperwork Reduction Act of 1980. Such approval has been requested and is under consideration. Comments concerning the information collection requirements contained in these proposed rules may be addressed to the Office of Information and Regulatory Affairs of OMB, Attention: Desk Officer, ASCS/USDA, Washington, D.C. 20503, Telephone (202) 395-7340.

Everett Rank, Administrator, ASCS, has certified that this action will not have a significant economic impact on a substantial number of small entities as defined in the Regulatory Flexibility Act (5 U.S.C. 601 *et seq.*) because (i) the proposed fees will represent a minimal part of total operating expenses, (ii) the proposed fees will be proportionate to volume of business, and (iii) application for a license and use of the service is voluntary. Consequently, no regulatory flexibility analysis is required.

It has been determined by an environmental evaluation that this action will have no significant adverse impact on the quality of the human environment, health, and safety. In addition, it will not adversely affect environmental factors such as wildlife habitat, water quality, or land use and appearance. Therefore, neither an Environmental Assessment nor an Environmental Impact Statement is needed.

This action will not have a significant impact specifically upon area and community development. Therefore, review as established by Executive Order 12372 was not used to assure that units of local government are informed of this action.

Background

The U.S. Warehouse Act (7 U.S.C. 241 *et seq.*) (the "Act") provides for the licensing of warehousemen who apply to the Secretary of Agriculture and meet certain statutory and regulatory standards. The primary objectives of the Act are to: (1) Protect producers and others who store their property in public warehouses; (2) assure the integrity of warehouse receipts as documents of title, thereby facilitating trading of agricultural commodities in interstate commerce; and (3) set and maintain a standard for sound warehouse operations.

These objectives have been attained by research and development of basic standards for good warehousing paractices; original and continuing examinations of applicants and licensees; financial and bonding requirements; adn licensing and regulatory requirements.

The Department's supervision of licensees has focused on examinations of subject warehouses. The Department conducts an examination of a warehouseman applying for a license in order to determine whether the warehouseman and the warehouse which is the subject of the license application meet the standards for licensing under the Act. The Department also conducts unannounced examinations of licensed warehouses to determine whether the warehouse and the warehouseman continue to meet these standards and whether the warehouseman is capable of fulfilling all of the obligations that may have been assumed as a licensee.

The Omnibus Budget Reconciliation Act of 1981, Pub. L. 97-35, amended section 10 of the Act (7 U.S.C. 251) to provide that "The Secretary of Agriculture, or the Secretary's designated representative, shall charge, assess, and cause to be collected a reasonable fee for (1) each examination or inspection of a warehouse (including the physical facilities and records thereof and the agricultural products therein) under this Act; (2) each license issued to any person to classify, inspect, grade, sample, or weigh agricultural products stored or to be stored under provisions of this Act; (3) each annual warehouse license issued to a warehouseman to conduct a warehouse under this Act; and (4) each warehouse license amended, modified, extended, or reinstated under this Act. Such fees shall cover, as nearly as practicable, the costs of providing such services and licenses, including administrative and supervisory costs."

In addition, section 156(d) of the Omnibus Budget Reconciliation Act of 1981 (95 Stat. 374), stated that "Notwithstanding any other provision of law, the Secretary shall take such action as may be necessary to insure that the . . . licensing and inspection procedures for cotton warehouses are preserved . . . ."

Accordingly, it has been determined that cotton warehousemen should be charged reasonable fees covering, as nearly as practicable, the costs to the Department of providing licenses and services in accordance with the Act, including applicable administrative and supervisory costs related to maintaining an effective program. After taking into account all available information, including comments received concerning fee proposals issued by the Department in 1981 and 1983, discussions with the cotton trade, and the fiscal situation expected to exist in fiscal year 1986, it has been determined that the imposition of reasonable charges and fees will not endanger the preservation of the licensing and inspection procedures for cotton warehouses.

It has also been determined, based on discussions with the cotton trade and other information available to the Department, that the most acceptable and practicable method of assessing annual fees is the assessment of a fixed fee for all warehouses, together with a variable fee based on the number of receipted bales handled and the average volume of bales stored.

Annual Fee

Under the proposed rule, a warehouseman would be charged an annual fee for those warehouses for which the warehouseman has a license and for those warehouses for which the warehousemen has requested a license. The annual fee will be calculated to take into account the amount of storage activity at each warehouse. The proposed fee will equal the total of the following:

(1) A fee of $200 for each warehouse to help offset certain fixed costs associated with maintaining licenses which do not tend to vary according to storage activity;

(2) For each bale handled at the warehouse during the twelve months of the calendar year preceding assessment of the fee, a charge of 2¢ for each bale for which card type receipts have been issued or a charge of 5¢ for each bale for which paper type receipts have been issued, to cover the costs associated with tracking and accounting for each bale handled at the warehouse; and

(3) A charge of 3¢ times the average of the number of bales in storage in the warehouse at the end of each month during the 12 months of the calendar year preceding assessment of the fee to offset the costs associated with the counting of bales that actually are in the warehouse at the time of the examination.

The total proposed annual fee reflects several cost factors, including salaries, rents, miscellaneous overhead, and includes applicable administrative and supervisory costs. Approximately 75 percent of the annual fee is directly related to the costs of conducting an examination of the warehouse. The remaining 25 percent reflects the costs of financial review and analysis, licensing and bonding, research and development, and other services.

Examination Fees

In addition, it has been determined that the fees for examinations currently provided for in 7 CFR 735.51 should be amended to more closely reflect actual costs incurred by the Department in connection with warehouse examinations, including examinations requested by the warehouseman and examinations conducted to determine whether a warehouseman's suspended license may be reinstated.

Under the proposed rule the present fee for a reexamination of a warehouse for the purpose of amending an existing license will be maintained. Such an examination consists largely of examining warehouse facilities. Consequently, the fee will remain tied to the capacity of the warehouse.

A warehouseman might request a warehouse examination in order to: (1) Meet requests or requirements of depositors or lending agencies, (2) determine the quantity or condition of cotton in store, (3) determine whether the quantity and quality of cotton in storage is sufficient to satisfy outstanding storage obligations, or (4) have an independent physical inventory coinciding with an end of fiscal year audit.

Under the proposed rule a warehouseman would submit a written request for such an examination stating the purposes of the examination and agreeing to pay the prescribed fee. The Department would conduct the examination if it did not adversely affect its ability to meet program commitments.

Since a requested examination will consist of only an onsite examination of the facility, the costs of providing such an examination will equal approximately 75 percent of the annual warehouse fee. Therefore, the fee proposed for this service is 75 percent of the annual fee.

If the Department has suspended a warehouseman's license because of deficiencies in operation and other violations of the applicable regulations by the warehouseman, the warehouseman may attain reinstatement of the warehouseman's license only if a reexamination of the warehouse has been made and the Department is assured that the deficiencies and violations have been corrected.

Costs of an examination for reinstatement would be much the same as those for a requested examination, except that additional time must be devoted to verify and report on the correction of the deficiencies which resulted in the suspension, thus an increased fee would be required to cover all of the costs incurred. Therefore, the proposed fee for a reinstatement examination is equal to 100 percent of the annual fee. The fee for a reinstatement examination will not be assessed the warehouseman if the Department determines, after an opportunity for hearing, that the license suspension was unjustified.

Tag Check Fee

Cotton is usually stored on an identity preserved basis, a warehouse receipt being issued for each individual bale received into storage, each bale having a visible and accessible tag, and arranged in storage so as to permit an accurate inventory check. The Department has had a policy of locating and verifying against the warehouse receipt each bale of cotton in the warehouse (commonly called a tag check).

Since costs for tag checks are very high, the Department proposes to accept a bale count as evidence that the warehouseman is meeting inventory requirements, provided that the bale count meets acceptable tolerances and does not otherwise indicate stock deficiencies or other adverse storage conditions. A tolerance of ½ of 1 percent of the total number of bales which should be in the warehouse to support all storage and non-storage obligations, according to the examiner's determination, has been determined to be acceptable. If a bale count is not within this tolerance or an examiner discovers stock discrepancies or other adverse conditions, the Department will conduct a complete tag check.

The cost of the tag check will be assessed to the warehouseman at the rate of 10 cents per bale checked. This represents the additional costs to the Department for a tag check examination as opposed to a bale count.

There will be no charge to a warehouseman when the Department makes a tag check for purposes of losses or damage or potential losses or damage from fire, floods or other situations where the Department considers the examination information necessary to protect the integrity of the program.

Payments of Commodity Credit Corporation.

The Commodity Credit Corporation (CCC) owns or has an interest in considerable quantities of cotton stored in federally licensed warehouses. The examination of such warehouses protects the interest of CCC and makes CCC a major beneficiary of the program. For this reason it is proposed, as is done presently with other agricultural commodities, that if CCC shares in the costs of the examination program at a warehouse, the applicable fees to be charged will be reduced to that warehouseman by the amount CCC pays. CCC will share the cost of the annual fee but CCC will not share in the cost of any examination for a license amendment, requested examination, reinstatement examination, or tag check.

Fee Payment

It is also proposed to amend § 735.52 to require that a warehouseman pay the annual fee on or before October 1 each year and that fees for other types of examinations be paid before the examination is made. Fees for tag checks must be paid within 15 days of the tag check. Failure to pay any prescribed fees shall be grounds for revoking a warehouseman's license.

Recordkeeping Requirements

A warehouseman's records should include information as to (1) how many and what bales of cotton are in the warehouse (i.e., in licensed space) at any given time, and (2) how many and what bales should be in the warehouse to support outstanding warehouse receipts and all other storage or non-storage obligations. Presently, pursuant to 7 CFR 735.34, each licensed warehouseman is required to make reports as requested by the Administrator. Pursuant to that section, each warehouseman will be asked to provide annually the number of bales handled during the year and the number of bales in storage at the end of each month during the preceding calendar year. This information will serve as the basis for determining the annual fee. Although warehousemen will be affected by the proposed changes in terms of recordkeeping, no amendment is necessary to 7 CFR 735.34.

List of Subjects in 7 CFR Part 735

Administrative practice and procedure, Cotton, Reporting and recordkeeping requirements, Fees, Warehouses.

Accordingly, it is proposed to amend 7 CFR Part 735 as follows:

PART 735—COTTON WAREHOUSES

1. The authority citation for 7 CFR Part 735 continues to read as follows:

Authority: Sec. 28, 39 Stat. 490 (7 U.S.C. 268).

2. Section 735.51 is revised to read as follows:

§ 735.51 Warehouse Inspection Fees.

(a)(1) There shall be charged and collected from each warehouseman, for each warehouse licensed under the Act or for each warehouse for which the warehouseman is applying for a license under the Act, an annual fee equal to the total of:

(i) A fixed charge of $200;

(ii) A charge of 2¢ a bale for each bale handled at the warehouse during the preceding calendar year and for which card type warehouse receipts have been issued or are subject to issue, or a charge of 5¢ a bale for each bale handled at the warehouse during the preceding calendar year and for which paper warehouse receipts have been issued or are subject to issue; and

(iii) A charge of 3¢ per bale for the average of the number of bales in store at the warehouse at the end of each month during the preceding calendar year;

(2) There shall be charged and collected from each warehouseman, for an amendment to an existing license under the Act, an examination fee computed at the rate of $50 for each 1,000 bales of added storage capacity, or fraction thereof, as determined by the Secretary, but not less than $100 nor more than $1,000;

(3) There shall be charged and collected from each warehouseman, for any examination of a licensed warehouse conducted by the Department at the request of the warehouseman, an examination fee equal to 75% of the fee provided for in subsection (a)(1). A request for such examination must be made in writing to the Department by the warehouseman and must state the purposes of the examination.

(4) There shall be charged and collected from each warehouseman, for each warehouse for which the license has been suspended by the Department, a fee equal to 100% of the fee provided for in subsection (a)(1) for any examination of such warehouse conducted by the Department for the purpose of determining whether the warehouseman's license should be reinstated. The charge for the examination will be made unless the Department determines after an opportunity for hearing that the suspension was unjustified.

(b) A tag check fee of 10¢ per bale checked will be charged by the Department for any warehouse for which the warehouseman is applying for a license under the Act and for any licensed warehouse whenever the Department determined that a tag check of the bales stored in a licensed warehouse is warranted. A tag check of the licensed warehouse shall be deemed to be warranted whenever the examiner's bale count is not within ½ of one percent of the total number of bales which should be in the warehouse as determined from the warehouseman's total of receipted and not receipted obligations.

(c) Any fes provided for by this section will be reduced by the amount paid by the Commodity Credit Corporation with respect to such fee.

3. Section 735.52 is revised to read as follows:

§ 735.52 Payment of Fees.

(a) Fees for each original warehouseman's license and for each amended, modified, extended, reinstated, or duplicate warehouseman's license and for each license or amendment issued to any person to classify, sample, or weigh cotton must be paid upon application for such license.

(b)(1) A warehouseman who has a license under the Act must pay the annual fee for each licensed warehouse on or before October 1 of each year.

(2) A warehouseman applying for a license under the Act must pay the annual fee upon application for the license.

(3) A warehouseman must pay any examination fees in advance of the date such examination is scheduled.

(4) Charges for a tag check conducted by the Department shall be due and payable upon completion of the tag check and must be paid no later than fifteen days following completion of the tag check.

(c) Failure of a warehouseman to pay any fees provided for in this part shall be a basis for suspension and revocation of such warehouseman's license.

(d) All fees and charges provided for by this part shall be paid to the "Agricultural Stabilization and Conservation Service, USDA".

Signed at Washington, D.C. on September 23, 1985.

Everett Rank,

Administrator, Agricultural Stabilization and Conservation Service.

[FR Doc. 85-23182 Filed 9-26-85; 8:45 am]

BILLING CODE 3410-05-M

Agricultural Marketing Service

7 CFR Part 907

Navel Oranges Grown in Arizona and Designated Part of California

AGENCY: Agricultural Marketing Service, USDA.

ACTION: Notice of Marketing Policy.

SUMMARY: This notice sets forth a summary of the 1985-86 marketing policy for navel oranges grown in Arizona and designated part of California. The marketing policy was submitted by the Navel Orange Administrative Committee which functions under the marketing order covering California-Arizona navel oranges. The marketing policy contains information on crop and market prospects for the 1985-86 season.

DATE: Written suggestions, views, or pertinent information relating to the marketing of the 1985-86 California-Arizona navel orange crop will be considered if received by October 11, 1985.

ADDRESS: Interested persons are invited to submit written statements in duplicate to: Docket Clerk, Room 2069-S, F&V, AMS, U.S. Department of Agriculture, Washington, DC 20250. Such submissions should reference the date and page number of the **Federal Register** and will be made available for public inspection in the office of the Docket Clerk during regular business hours.

FOR FURTHER INFORMATION CONTACT: William J. Doyle, Chief, Fruit Branch, F&V, AMS, USDA, Washington, DC 20250, telephone (202)447-5975. Growers and handlers of navel oranges may obtain a copy of the marketing policy directly from the Navel Orange Administrative Committee. Copies of the marketing policy are also available from Mr. Doyle.

SUPPLEMENTARY INFORMATION: Pursuant to § 907.50 of the marketing order covering navel oranges grown in Arizona and designated part of California the Navel Orange Administrative Committee, hereinafter referred to as the "committee", is required to submit a marketing policy to the Secretary prior to recommending regulations for the ensuing season. The order authorizes volume and size regulations applicable to fresh shipments of navel oranges to domestic markets including Canada. Export shipments of oranges and oranges utilized in the production of processed orange products are not regulated under the order.

The committee has adopted a marketing policy for the 1985-86 marketing season. The marketing policy is intended to inform the Secretary and persons in the industry of the committees's plans for recommending regulation of shipments during the marketing season and the basis therefor. The committee evaluates market conditions and makes recommendations to the Secretary as to the quantity of navel oranges that can be shipped each week to domestic outlets without disrupting markets. Under certain conditions, the committee may recommend size regulations applicable to fresh domestic shipments.

In its 1985-86 marketing policy, the committee projected the California-Arizona navel orange crop at 57,700 cars (1,000 cartons at 37½ pounds net weight each). Last year's production was slightly less at 54,232 cars. In District 1, Central California, the committee has estimated the crop at 49,300 cars, compared to 44,432 cars produced a year ago. In District 2, Southern California, the crop is expected to be 7,300 cars, compared to 8,514 cars produced in 1984-85. In District 3, Arizona-California desert valley, the revised crop estimate is 500 cars compared to 840 cars in 1984-85. In District 4, Northern California, a 400 car crop is projected compared to 446 cars last year.

It is expected that orange sizes will be larger than last year on the average. Fruit quality shipped to the domestic market is expected to be good to excellent.

The committee estimates that shipments to domestic fresh market outlets, including Canada, will account for 42,000 cars. Last year a total of 41,319 cars were shipped to domestic markets. Fresh export shipments are expected to total 5,500 cars compared to 5,337 cars last year. Processing and other disposition is forecast at 10,200 cars compared to 7,576 cars last year.

Based on current projections, shipments are expected to begin in late-October and finish in June. The committee has adopted a schedule of estimated weekly shipments during the 1985-86 season.

The committee reports that the Florida round orange production will be 212,000 cars, about two percent greater than last year. In Texas, following severe freeze damage in 1983, orange production for 1985-86 season is expected to be 1040 cars. Production of apples is estimated at 197.3 million bushels in 1985-86 compared to 192.2 million bushels in 1984-85. Winter pear production is estimated at 7.4 million bushels in 1985-86 compared to 8.1 million bushels last year. General economic conditions are expected to continue to be favorable during 1985-86.

In addition, the committee proposes to promote flexibility in marketing order operations by: (1) Recommending weekly volume regulations to cover two consecutive one-week periods and (2) recommending open movement for a prorate district when 85 percent of the crop in that district has been shipped. Both of those actions were initiated during the 1983-84 season.

Based upon information now available, the committee reports that the season f.o.b. equivalent parity price for fresh California-Arizona navel orange (under Marketing Order No. 907) is projected at $8.49 per carton as compared to $7.60 for the 1984-85 season.

As additional information on this price relationship becomes available, it will be reviewed by the Department of Agriculture in the light of program requirements and the provisions of the Agricultural Marketing Agreement Act of 1937, as amended.

In order to provide for public input, the Department will accept written views and information pertinent to the proposed marketing policy and the need for, or level of, regulation for the 1985-86 season.

Publication of this summary of the marketing policy does not create any legal obligations or rights, either substantive or procedural.

List of Subjects in 7 CFR Part 907

Marketing agreements and orders, California, Arizona, Oranges (Navel).

1. The Authority citation for 7 CFR Part 907 continues to read:

Authority: Secs. 1–19, 48 Stat. 31, as amended; 7 U.S.C. 601–674.)

Dated: September 25, 1985.

Thomas R. Clark,

Deputy Director, Fruit and Vegetable Division, Agricultural Marketing Service.

[FR Doc. 85-23275 Filed 9-26-85; 8:45 am]

BILLING CODE 3410-02-M

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7 CFR Parts 1006, 1007, 1011, 1012, 1013, 1046, 1093, 1094, 1096, 1097, 1098 and 1099

[Docket Nos. AO-366-A25-R01, et al.]

Milk in the Georgia and Certain Other Marketing Areas; Reopened Hearing on Proposed Amendments to Tentative Marketing Agreements and Orders and Termination of Proposed Termination of Proceeding on Proposed Amendments to Tentative Marketing Agreements and Orders

7 CFR Parts	Marketing area	Docket Nos.
1007	Georgia	AO-366-A25-R01
1006	Upper Florida	AO-56-A23-R01
1011	Tennessee Valley	AO-251-A28-R01
1012	Tampa Bay	AO-347-A26-R01
1013	Southeastern Florida	AO-286-A33-R01
1046	Louisville-Lexington-Evansville	AO-123-A54-R01
1093	Alabama-West Florida	AO-386-A4-R01
1094	New Orleans-Mississippi	AO-103-A46-R01
1096	Greater Louisiana	AO-257-A33-R01
1097	Memphis, Tennessee	AO-219-A41-R01
1098	Nashville, Tennessee	AO-184-A48-R01
1099	Paducah, Kentucky	AO-183-A40-R01

AGENCY: Agricultural Marketing Service, USDA.

ACTION: Notice of reopened public hearing on proposed rulemaking and termination of proposed termination.

SUMMARY: This action reopens a hearing on proposals to increase Class I milk prices under 12 southeastern Federal milk marketing orders and terminates a proposed termination of proceeding regarding such proposals. At the request of Dairymen, Inc., a public hearing was held June 25–28, 1985, to consider the proposals by Dairymen, Inc., to increase Class I price differentials. The transcript of the hearing in its current state is missing approximately two-thirds of the third day's testimony and one-third of the testimony on the fourth day of the hearing. The hearing is being reopened to obtain a transcript that can be certified as a complete record of the testimony concerning the economic and marketing conditions that relate to any of the proposals set forth in the original notice of hearing that was issued May 24, 1985 (50 FR 23021).

DATE: The reopened hearing will convene at 9:30 a.m., local time, on October 2, 1985.

ADDRESS: The reopened hearing will be held at the Ramada Hotel, Capital Plaza, 450 Capitol Avenue, SE., Atlanta, Georgia 30312.

FOR FURTHER INFORMATION CONTACT: Robert F. Groene, Marketing Specialist, Dairy Division, Agricultural Marketing Service, U.S. Department of Agriculture, Washington, D.C. 20250, (202) 447-2089.

SUPPLEMENTARY INFORMATION: This administrative action is governed by the provisions of sections 556 and 557 of Title 5 of the United States Code and, therefore, is excluded from the requirements of Executive Order 12291.

A notice was issued on May 24, 1985 (50 FR 23021), giving notice of a public hearing to be held at the Ramada Hotel, Capital Plaza, 450 Capitol Avenue, SE., Atlanta, Georgia 30312, beginning at 9:30 a.m., local time, on June 25, 1985, with respect to proposed amendments to 12 southeastern Federal milk marketing orders. The transcript of the hearing was incomplete in that portions of the third and fourth day's testimony were missing. As a result of the delay in receipt of the complete transcript, the Department was able to take notice of more current market information and, on August 16, 1985 (50 FR 33761), sought comments on a proposal to terminate the rulemaking. Upon consideration of the comments received it is determined that the hearing should be reopened.

Notice is hereby given, pursuant to the rules of practice applicable to such proceedings (7 CFR Part 900), that the said hearing is being reopened at the Ramada Hotel, Capital Plaza, 450 Capitol Avenue, SE., Atlanta, Georgia 30312 (404) 688-1900, beginning at 9:30 a.m., local time, on October 2, 1985. The purpose of the reopened hearing is to obtain a transcript that can be certified as a complete record of the initial hearing. Also, additional evidence will be received concerning economic and marketing conditions that have developed subsequent to the close of the formal hearing and that relate to any of the proposals set forth in the original notice of hearing issued May 24, 1985 (50 FR 23021).

This action also terminates a proposed termination of proceeding that was issued by the Department on August 16, 1985 (50 FR 33761). On the basis of comments received in response to the proposed termination, it is hereby determined that the hearing should be reopened. Dairymen, Inc., stated that "an incomplete record, due to court report error or some other reason, is no grounds for termination of the proceedings. The Department *should* reopen the hearing at the earliest possible date to retake missing testimony to obtain a transcript that can be certified as a complete record of the hearing testimony. An incomplete record, due to court reporter error, is no basis for determining that the proposed amendments to the orders are not justified." In addition, the Department has concluded that the reopened hearing will allow a full discussion of economic and marketing conditions relative to the proposals under consideration which have come to the attention of the Department since the hearing closed. Accordingly, pursuant to the provisions of the Agricultural Marketing Agreement Act of 1937, as amended (7 U.S.C. 601 et seq.), the proposed termination of proceeding should be and is hereby terminated.

Prior documents in this proceeding:

Notice of Hearing: Issued May 24, 1985, published May 30, 1985 (50 FR 23021).

Extensions of Time for Filing Briefs: Issued July 18, 1985; Issued August 1, 1985.

Proposed Termination of Proceeding: Issued August 16, 1985; published August 21, 1985 (50 FR 33761).

List of Subjects in 7 CFR Parts 1006, 1007, 1011, 1012, 1013, 1046, 1093, 1094, 1096, 1097, 1098, and 1099

Milk marketing orders, Milk, Dairy products.

Authority: Secs. 1–19, 48 Stat. 31, as amended (7 U.S.C. 601–674).

Signed at Washington, D.C., on: September 25, 1985.

William T. Manley,

Deputy Administrator, Marketing Programs.

[FR Doc. 85-23252 Filed 9-26-85; 8:45 am]

BILLING CODE 3410-02-M

COMMODITY FUTURES TRADING COMMISSION

17 CFR Parts 1, 33 and 190

Amendments to Minimum Financial and Related Requirements for Futures Commission Merchants and Introducing Brokers, Contract Markets and Clearing Associations; Default; Bankruptcy, and Commodity Options; Margin

AGENCY: Commodity Futures Trading Commission.

ACTION: Extension of comment period.

SUMMARY: On August 5, 1985, the Commodity Futures Trading Commission ("Commission") published in the **Federal Register** (1) proposed amendments to the minimum financial and related requirements for futures commission merchants and introducing brokers (50 FR 31612); (2) proposed options margin guidelines (50 FR 31625); and (3) an advance notice of proposed rulemaking regarding the transfer of liquidation of open commodity contracts carried by a clearing member futures commission merchant which has defaulted on a margin obligation. (50 FR 31623). By letters dated August 28, and August 29, 1985, two exchanges requested a sixty-day extension of the comment period on certain of the matters published for comment. As one reason for the extension, both exchanges stated that they and other exchanges, among others, were analyzing a proposal for a futures-style margining system for commodity options and that a final recommendation would not be forthcoming until after the expiration of the present comment period for the proposed options margin guidelines.

In this regard, the Commission notes that a proposal on margin rule generally for exchange-traded options has been open since March 9, 1985, and that such a proposal deals with significant issues which are not presented by the proposed guidelines. The Commission, of course, will receive any pertinent comments in this regard, but may choose to treat these matters separately and to take final action on the proposed guidelines prior to addressing any recommendation made by commenters on a futures-style margining system for commodity options. Similarly, commenters should address specifically the financial rules proposed. Although general comments on the Commission's existing capital requirements will of course be considered, they also may be considered separately at a later date.

Nonetheless, in order to ensure that all affected parties have an opportunity to comment on the Commission's proposals, the Commission has concluded to grant an extension of the comment period, although for a shorter period of time. Accordingly, the Commission has determined to grant a thirty day extension of the comment period on each of the above-referenced matters. For the reasons set forth above, the Commission would not expect to grant any further requests for extension of the comment period on any of the foregoing matters.

DATES: Notice is hereby given that all comments on the proposed options margin guidelines (50 FR 31625, August 5, 1985) must be submitted by October 4, 1985 and all comments on the proposed amendments to minimum financial and related requirements for futures commission merchants and introducing brokers (50 FR 31612, August 5, 1985) and the advance notice of proposed rulemaking regarding the liquidation or transfer of open commodity positions carried by a defaulting clearing member futures commission merchant (50 FR 31623, August 5, 1985) must be submitted by November 4, 1985.

FOR FURTHER INFORMATION CONTACT: Kevin M. Foley, Chief Counsel, or Lawrence B. Patent, Associate Chief Counsel, Division of trading and Markets, Commodity Futures Trading Commission, 2033 K Street, NW., Washington DC 20581. Telephone: (202) 254-8955

Issued in Washington, D.C. on September 24, 1985, by the Commission.

Jean A. Webb,

Secretary to the Commission.

[FR Doc. 85-23087 Filed 9-26-85; 8:45 am]

BILLING CODE 6351-01-M

DEPARTMENT OF ENERGY

Federal Energy Regulatory Commission

18 CFR Part 116

[Docket No. RM83-39-000]

Electric Utilities (Federal Power Act); List of Property for Use in the Accounting for the Addition and Retirement of Reactor Plant Equipment

September 24, 1985.

AGENCY: Federal Energy Regulatory Commission, Energy.

ACTION: Notice of Proposed Rulemaking.

SUMMARY: The Federal Energy Regulatory Commission (Commission) proposes to revise its regulations at 18 CFR Part 116, "Units of Property for Use in Accounting for Additions and Retirements of Electric Plant," by codifying a list of retirement units of property in Account 322, "Reactor Plant Equipment." The Commission is establishing the list to promote proper and uniform recordkeeping by utilities to account for the addition and retirement of reactor plant equipment. The Commission is also proposing to make certain minor, technical amendments to Instruction 6 of Part 116.

DATE: Written comments must be received by the Commission by November 26, 1985.

ADDRESS: Federal Energy Regulatory Commission, 825 North Capitol Street NE., Washington, DC 20426.

FOR FURTHER INFORMATION CONTACT: Kasha Ciaglo, Office of the General Counsel, Federal Energy Regulatory Commission, 825 North Capitol Street, NE., Washington, DC 20426; (202) 357-8465.

I. Introduction

The Federal Energy Regulatory Commission (Commission) is proposing to amend its regulations at 18 CFR Part 116, "Units of Property for Use in Accounting for Additions and Retirements of Electric Plant" by codifying a list of retirement units of property in Account 322, "Reactor Plant Equipment."[1] The list would be required to be used by utilities owning nuclear facilities to classify certain reactor plant equipment as "retirement units" for accounting purposes. The list would replace the current guidelines in Account 322 which allow utilities to submit their own list of retirement units to the Commission.[2] The Commission has found that the lists developed by some utilities under the current guidelines are inconsistent with other classifications of the same items of property or are overly general designations of property. Therefore, the Commission proposes a specific list of retirement units to provide a minimum standard for utilities to use when they record the addition and retirement of reactor plant equipment.

II. Background

The Commission defines "retirement units" as "those items of electric plant which, when retired, with or without replacement, are accounted for by crediting the book cost thereof to the electric plant account in which included." (18 CFR Part 101, Definition 32.) In contrast, "minor items of property" are "the associated parts or items of which retirement units are

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[1] The Commission may require a list of units of property under its accounting authority in section 301 of the Federal Power Act, 16 U.S.C. 825 (1982). Since 1937, the Commission, or its predecessor, the Federal Power Commission, has required a list of units of property under the Uniform System of Accounts. *See* Order No. 45, 2 FD 171, January 26, 1937. Since 1961, a list of retirement units for reactor plant equipment has been required. *See* Order No. 235, 26 FR 9,887, October 21, 1961.

[2] Account 322 provides that a "[u]tility shall adopt such list of retirement units deemed appropriate for reactor plant equipment in harmony with prescribed retirement units for other accounts, and file a copy of such a retirement units list with the Commission."

composed." (18 CFR Part 101, Definition 18.) The distinction between retirement units and the minor items of property of which retirement units are composed is necessary for determining how the cost of these properties is recorded on the books and records of the company. Ultimately, this distinction is useful in considering the appropriate cost of service treatment for particular property or expenses.

The Commission has found that the current general guidelines in Account 322 are not adequate for Commission accounting purposes. For example, certain utilities have classified a particular item of property as a retirement unit in one Commission filing and as a minor item of property in another filing. Certain utilities have also filed lists of retirement units that are too general for Commission accounting needs.

III. Discussion

To promote greater consistency and specificity in accounting for this equipment, the Commission has developed the proposed list as a minimum requirement for utilities to use in classifying reactor plant equipment as retirement units. The list was developed based on the Commission's experience in collecting and analyzing such data and is consistent with the types of lists that the Commission has developed for other accounts. Consistent with current practice under existing regulations, utilities can continue to add items to the list of retirement units in Account 322, if such items are both relatively costly and not an integral part of a larger retirement unit.[3] In addition, utilities may continue to account for new or replaced minor items of property in the same manner as retirement units if these items result in a substantial addition to or betterment of the plant.[4]

The Commission notes that most utilities already provide information that generally satisfies the requirements of the proposed rule, and submit lists of retirement units that are more detailed than the one proposed here. However, a few utilities may have to revise parts of the lists that they currently use in order to conform to the Commission's proposal. Any such revision should not result in a significant burden to the affected utilities, either in time or expense. Moreover, the Commission believes that any burden would be outweighed by the benefits to the Commission and to the utilities of having a standardized minimum list that applies to all nuclear reactor plants. In addition, because the rule is merely a codification of existing, accepted accounting practice, it should have no direct effect on utility rates.

IV. Summary of Proposed Changes

This notice proposes to replace the general guidelines in Account 322 with a definitive list of retirement units for reactor plant equipment. The list is divided under three headings: boiling water reactor, pressurized water reactor, and high temperature gas reactor. As with lists of retirement units in other accounts, and subject to the same limitations, the list is a minimum requirement upon which the utility may expand by including new items or items that it currently classifies as retirement units under its own system.[5]

The proposed list of reactor plant equipment corresponds to the format of the retirement units and instructions prescribed for other accounts in Part 116. Upon adoption of this proposed list, utilities would no longer be required to develop and file with the Commission their own list of retirement units for Account 322.

The Commission also proposes two non-substantive amendments to Instruction 6 in Part 116: (1) Redesignating items (a) through (q) as items (1) through (17) to be consistent with the corresponding list of general retirement units for gas plants,[6] and (2) revising the newly designated item (11) by deleting the term "(non-nuclear)" from the description of plant piping, because this provision applies to both nuclear and non-nuclear equipment.

V. Paperwork Reduction Act Statement

The Paperwork Reduction Act (PRA) (44 U.S.C. 3501–3520 (1982)) and the Office of Management and Budget's (OMB) regulations at 5 CFR 1320.12 (1984) require that OMB approve certain information collection and recordkeeping requirements imposed by an agency. The list of retirement units proposed for Account 322 is being submitted to OMB for its review. Interested persons can obtain information on the recordkeeping requirements by contacting the Federal Energy Regulatory Commission, 825 North Capitol Street NE., Washington, DC 20426 (Attention: Kasha Ciaglo (202) 357–8465). Comments on the recordkeeping requirements can be sent to the Office of Information and Regulatory Affairs of OMB (Attention: Desk Officer for the Federal Energy Regulatory Commission).

VI. Regulatory Flexibility Act Certification

The Regulatory Flexibility Act (RFA) 5 U.S.C. 601–612 (1982) requires agencies to prepare certain statements, descriptions, and analyses of proposed rules that will have a "significant economic impact on a substantial number of small entities." The Commission is not required to make such analyses if a rule would not have such an impact.

The Commission does not believe that this rule will have such an impact on small entities. The proposed rule establishes a standard list of retirement units of nuclear reactor equipment owned by utilities. Most utilities do not fall within the RFA's definition of small entity.[7] In addition, those utilities that independently own nuclear facilities are all classified as "major utilities" under the Commission's uniform System of Accounts.[8] Moreover, the level of investment necessary for nuclear generating facilities generally limits their ownership to other than small entities. Although small or municipal utilities may purchase shares in these

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[3] 18 CFR Part 116, Instruction 4.

[4] In 18 CFR Part 101, "Uniform System of Accounts Prescribed for Public Utilities and Licensees Subject to the Provisions of the Federal Power Act," Instruction 10.C. of "Electric Plant Instructions" provides that: "The addition and retirement of minor items of property shall be accounted for as follows:

(1) When a minor item of property which did not previoulsy exist is added to plant, the cost thereof shall be accounted for in the same manner as for the addition of a retirement unit. . . . if a substantial addition results, otherwise the charge shall be to the appropriate maintenance expense account.

* * * * *

(3) When a minor item of depreciable property is replaced independently of the retirement unit of which it is a part, the cost of replacement shall be charged to the maintenance account appropriate for the item, except that if the replacement effects a substantial betterment (the primary aim of which is to make the property affected more useful, more efficient, of greater durability, or of greater capacity), the excess cost of the replacement over the estimated cost at current prices of replacing without betterment shall be charged to the appropriate electric plant account.

[5] *See supra*, notes 3 and 4 and accompanying text.

[6] 18 CFR Part 216, Instruction 6.

[7] 5 U.S.C. 601(3), citing to section 3 of the Small Business Act, 15 U.S.C. 632 (1982). Section 3 of the Small Business Act defines a "small-business concern" as a business which is independently owned and operated and which is not dominant in its field of operation. *See also* SBA's revised Small Business Size Standards, 49 FR 5024 (Feb. 9, 1984) (to be codified at 13 CFR Part 121).

[8] 18 CFR Part 101, "General Instructions"

1. *Classification of Utilities*

A. * * *

(1) Major. Utilities and licensees that had, in each of the last three consecutive years, sales or transmission service that exceeded any one or more of the following:

(1) One million megawatt-hours of total sales;

(2) 100 megawatt-hours of sales for resale;

(3) 500 megawatt-hours of gross interchange out; or

(4) 500 megawatt-hours of wheeling for others (deliveries plus losses).

facilities, such utilities are not subject to the Uniform System of Accounts and the provisions of this rule.

This proposed rulemaking will not impose any regulatory or administrative burden upon small entities because it merely standardizes an accepted utility accounting practice. Therefore, it does not require any expenditures of resources by such entities. For these reasons, the Commission certifies that the rulemaking, if promulgated, will not have a significant enconomic impact upon a substantial number of small entities.

VII. Written Comment Procedures

The Commission invites interested persons to submit written comments on this proposal. An original and 14 copies of such comments should be filed with the Commission by November 26, 1985. Comments should be submitted to the Office of the Secretary, Federal Energy Regulatory Commission, 825 North Capitol Street NE., Washington, DC 20426 and should refer to Docket No. RM83-39-000.

All written submissions will be placed in the public file which has been established in this docket and which is available for public inspection at the Commission's Division of Public Information, Room 1000, 825 North Capitol Street NE., Washington, DC 20426, during regular business hours.

List of Subjects in 18 CFR Part 116

Electric power plants, Electric utilities, Reporting and recordkeeping requirements, Uniform system of accounts.

In consideration of the foregoing, the Commission proposes to amend Part 116 of Chapter I, Title 18, *Code of Federal Regulations*, as set forth below.

By direction of the Commission.

Kenneth F. Plumb,
Secretary

PART 116—[AMENDED]

1. The authority citation for Part 116 continues to read as follows:

Authority: Department of Energy Organization Act, 42 U.S.C. 7102–7352 (1982); Executive Order 12,009, 3 CFR 142 (1978); Federal Power Act, 16 U.S.C. 791a–828c (1982), Public Utility Regulatory Policies Act, 16 U.S.C. 7601–7645 (1982).

2. In Instruction 6 of Part 116, items (a) through (q) are redesignated as items (1) through (17), respectively.

3. In Instruction 6 in Part 116, newly designated item (11) is amended by deleting the term "(non-nuclear)".

4. Account 322 in Part 116 is revised to read as follows:

322 Reactor Plant Equipment

Boiling Water Reactor

A. Reactor:

1. Reactor vessel internals including core.

2. Reactivity control systems.

3. Reactor vessels and appurtenances.

4. Reactor trip systems.

B. Reactor Coolant System and Connected Systems:

1. Coolant recirculation systems and controls.

2. Main steam systems and controls.

3. Main steam isolation systems and controls.

4. Reactor core isolation cooling systems and controls.

5. Residual heat removal systems and controls.

6. Feedwater systems and controls.

7. Reactor coolant pressure boundary leakage detection systems.

8. Other coolant subsystems and controls.

9. Engineered safety feature instrument systems.

10. Systems required for safe shutdown.

11. Safety related display instrumentation.

12. Coolant injection systems.

13. Other instrument systems.

C. Containment System:

1. Reactor containment.

2. Containment heat removal systems and controls.

3. Containment air purification and cleanup systems and controls.

4. Containment isolation systems and controls.

5. Containment combustible gas control systems and controls.

6. Other containment systems and controls.

D. Fuel Storage and Handling Systems:

1. New fuel storage facilities.

2. Spent fuel storage facilities.

3. Spent fuel pool cooling and cleanup systems and controls.

4. Fuel handling systems.

E. Auxiliary Water Systems:

1. Cooling systems for reactor auxiliaries and controls.

F. Auxiliary Process Systems:

1. Process sampling system.

2. Failed fuel detection systems.

3. Reactor coolant cleanup systems and controls.

4. Liquid poison systems and controls.

G. Radioactive Waste Management Systems:

1. Liquid radioactive waste management systems.

2. Gaseous radioactive waste management systems.

3. Process and effluent radiological monitoring systems.

4. Solid radioactive waste management systems.

H. Radiation Protection Systems:

1. Area monitoring systems.

2. Airborne radioactivity monitoring systems.

3. Control room habitability system and controls.

I. Other Systems:

1. Auxiliary boiler systems.

2. Control air systems.

3. Service water systems.

4. Vent and drain systems.

5. Ventilating equipment.

6. Water supply and purification of cleanup system.

Note.—See list of general retirement units.

Pressurized Water Reactor

A. Reactor:

1. Reactor vessel internals including core.

2. Reactivity control systems.

3. Reactor vessels and appurtenances.

4. Reactor trip systems.

B. Reactor Coolant System and Connected Systems:

1. Coolant recirculation systems and controls.

2. Main steam systems and controls.

3. Main steam isolation systems and controls.

4. Emergency core cooling systems and controls.

5. Residual heat removal systems and controls.

6. Feedwater systems and controls.

7. Reactor coolant pressure boundary leakage detection systems.

8. Other coolant subsystems and controls.

9. Engineered safety feature instrument systems.

10. Systems required for safe shutdown.

11. Safety related display instrumentation.

12. Other instrument systems.

C. Containment Systems:

1. Reactor containment.

2. Containment heat removal systems anc controls.

3. Containment air purification and cleanup systems and controls.

4. Containment isolation systems and controls.

5. Containment combustible gas control systems and controls.

6. Other containment systems and controls.

D. Fuel Storage and Handling Systems:

1. New fuel storage facilities.

2. Spent fuel storage facilities.

3. Spent fuel cooling and cleanup systems and controls.

4. Fuel handling systems

E. Auxiliary Water Systems:

1. Cooling systems for reactor auxiliaries and controls.

F. Auxiliary Process Systems:

1. Process sampling system.

2. Failed fuel detection systems.

3. Chemical and volume control systems and controls.

G. Steam and Power Conversion Systems:

1. Steam generator blowdown systems and controls.

H. Radioactive Waste Management Systems:

1. Liquid radioactive waste management systems.

2. Gaseous radioactive waste management systems.

3. Process and effluent radiological monitoring systems.

4. Solid radioactive waste management systems.

I. Radiation Protection Systems:

1. Area monitoring systems.

2. Airborne radioactivity monitoring systems.

3. Control room habitability systems and controls.

J. Other Systems:

1. Auxiliary boiler systems.

2. Control air systems.

3. Service water system.

4. Vent and drain system.

5. Ventilating equipment.

6. Water supply and purification or cleanup system.

Note.—See list of general retirement units.

High Temperature Gas Reactor

A. Reactor:

1. Reactor.

2. Reactor reflector system.

B. Reactor Coolant System and Connected Systems:

1. Primary coolant systems and controls.

2. Secondary coolant systems and controls.

3. Feedwater and condensate system and controls.

4. Reactor plant piping.

5. Hydraulic power system and controls.

6. Moisture monitoring control system.

7. Linear neutron flux monitor and control rod calibration.

8. Analytical depressurization box controls.

9. Analytical liquid sampling control system.

10. Analytical gaseous sampling control system.

11. Tritium monitoring control system.

C. Fuel Storage and Handling System.

1. Fuel storage systems and controls.

2. Fuel handling systems and controls.

D. Radioactive Waste Management Systems:

1. Radioactive liquid waste systems management and controls.

2. Radioactive gaseous waste management systems and controls.

3. Decontamination system and controls.

E. Radiation Protection Systems:

1. Air monitor control systems and controls.

F. Auxiliary Boiler System.

G. Alternate Cooling Method.

Note.—See list of general retirement units.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

Food and Drug Administration

21 CFR Part 170

[Docket No. 84N-0080]

Eligibility for Classification of Food Substances as Generally Recognized as Safe; Extension of Comment Period

Correction

In FR Doc. 85-20937 beginning on page 35571 in the issue of Tuesday, September 3, 1985, make the following corrections on page 35571:

1. In the third column, in the summary, in the seventh line, "shwon" should read "shown".

2. In the third column, in the supplementary information, in the first paragraph, in the second to last line, "FRAS" should read "GRAS".

BILLING CODE 1505-01-M

DEPARTMENT OF TRANSPORTATION

Federal Highway Administration

23 CFR Part 635

[FHWA Docket No. 85-11]

Labor and Employment

AGENCY: Federal Highway Administration (FHWA), DOT.

ACTION: Notice of proposed rulemaking.

SUMMARY: The FHWA is requesting comments on a proposal to amend its regulation prescribing the inclusion of prevailing wage rates determined by the Secretary of Labor in advertisements and contracts for Federal-aid highway projects. The proposed amendment would preclude the payment of Federal-aid funds for excess costs due to State prevailing wage rates on such projects higher than that determined by the Secretary of Labor.

DATE: Written comments are due on or before November 12, 1985.

ADDRESS: Submit written comments, preferably in triplicate, to FHWA Docket No. 85-11, Federal Highway Administration, Room 4205, HCC-10, 400 Seventh Street, SW., Washington, DC 20590. All comments received will be available for examination at the above address between 8:30 a.m. and 3:30 p.m. ET., Monday through Friday. Those desiring notification of receipt of comment must include a self-addressed, stamped postcard.

FOR FURTHER INFORMATION CONTACT: Paul E. Cunningham, Office of Highway Operations, 202-426-0392, or Hugh T. O'Reilly, Office of the Chief Counsel, 202-426-0780, Federal Highway Administration, 400 Seventh Street, SW., Washington, DC 20590. Office hours are from 7:45 a.m. to 4:15 p.m. ET, Monday through Friday.

SUPPLEMENTARY INFORMATION: The Federal statute commonly known as the Davis-Bacon Act (40 U.S.C. 276a-276a-7) requires that laborers and mechanics employed on Federal construction work be paid not less than a "prevailing wage" to be determined by the Secretary of Labor. Such wage rates are extended to Federal-aid highway construction projects by 23 U.S.C. 113, which provides (in part) that:

> the Secretary shall take such action as may be necessary to insure that all laborers and mechanics employed by contractors or subcontractors on the construction work performed on highway projects on the Federal-aid systems, * * * shall be paid wages at rates not less than those prevailing on the same type of work on similar construction in the immediate locality as determined by the Secretary of Labor in accordance with the Act of August 30, 1935, known as the Davis-Bacon Act (40 U.S.C. 267a).

According to a recent survey, Thieblot, "Prevailing Wage Laws of the States," *Government Union Review* (Fall 1983) there were thirty seven States plus the District of Columbia which had effective prevailing wage laws for State and some local public works as of 1983. Approximately nineteen of these statutes are so drafted and applied as to commonly result in the payment of wage rates in Federal-aid highway projects which are higher than the Davis-Bacon rates determined by the Secretary of Labor. The States which appear to fall into this category are: Alaska, California, Connecticut, Delaware, Hawaii, Illinois, Kansas, Kentucky, Maryland, Massachusetts, Minnesota, Missouri, Nevada, New Jersey, New York, Washington, West Virginia, Wisconsin, and Wyoming.

The method used to calculate a prevailing wage varies considerably from State to State. Prevailing wage statutes in many States fail to specify how the pevailing wage is to be calculated. Legislative guidance regarding the selection of a prevailing wage rate is provided in 20 States but there is little consistency among States in the type of guidance provided. Statutes in two States specify that the majority rate shall be selected, but provide no guidance for proceeding when a majority of workers in a classification are not paid at the same rate. Some States specify the mean wage rate, some the median, and some require selection of the modal rate in the wage distribution. Use of the modal rate (the wage rate that appears most frequently in the wage distribution) is tantamount to adopting the union wage rate, since a union contract is the most likely reason that a significant number of workers in a given classification are paid at exactly the same rate. Five States simply adopt collectively bargained rates as prevailing.

An important part of the Federal prevailing wage law is that a wage determination can be appealed to the Administrator of DOL's Wage and Hour Division, or, if necessary, to the DOL's Wage Appeals Board, but in several States there is little or no appeal mechanism available to contractors or other interested parties to challenge arbitrary wage determinations. The result of this difference between Federal and State statutes is that a contractor who believes that he is being forced to pay a wage rate that is higher than what actually prevails in a local area has an avenue to appeal the wage determination decision to a higher level if the Federal law has established the rate. If the wage rate has been established under State law, however, the contractor would, in several cases, have no official appeal mechanism available.

In 1983, the Labor Department amended its procedures for predetermination of wage rates (29 CFR Part 1), which will result in slightly lower wage rates. The major part of these amendments was upheld against court challenge in the case of *Building Construction Trades Department, AFL–CIO* v. *Donovan*, 712 F.2d. 611, decided in early 1984. It is too early to tell whether, and to what extent, States with their own prevailing wage laws will follow suit. The result could be even more States than the 19 mentioned with labor provisions inconsistent with Federal requirements.

While it is true that the Davis-Bacon Act and 23 U.S.C. 113 provide for a minimum wage rate on Federal-aid projects, a floor not a ceiling, it is also evident that this is not solely a question of minimum wages as such. Rather, it is a question of both the Federal Government and the State Government determining the "prevailing wage rates in a locality" which must be paid as a minimum. There is nothing in 23 U.S.C. 113 which requires the Secretary to accede to higher (State) prevailing wage rate determinations than those established by the Secretary of Labor.

In addition, some of the State prevailing wage laws contain labor classification rules, employment preferences and other requirements more restrictive and costly than those provided under 23 U.S.C. 113 and the Labor Department regulations covering Davis-Bacon job classifications and wage rate determinations. These inconsistent requirements, which contribute to the problem of high labor costs, are only partially addressed by existing regulation, 23 CFR 635.124(b), which prohibits discrimination against labor from other States, and assures a contractor's free choice of his or her own labor. A rule denying Federal-aid participation in excessive labor costs due to State prevailing wage laws would remove much of the incentive for maintaining such inconsistent requirements or over-generous rate determinations.

Excess wage payments as outlined above are now seen as being an unwarranted drain on limited Federal-aid funds and counterproductive to full and open competition. Small and minority owned businesses are discouraged from bidding on Federal-aid projects with such high rates, since many are relatively new firms which could not practically jump their wage scale for one job, then attempt to return to a regular scale.

In those cases where the State-determined prevailing wage rate is higher than the wage determined to be prevailing rate under provisions of the Federal Davis-Bacon Act, the use of the wage rates established by State prevailing wage laws adds to the cost of Federal-aid highway construction in two ways. First, use of State-determined wage rates can add directly to project's wage costs by the amount of the difference between the higher prevailing wage established under State law and the Davis-Bacon wage (multiplied by the number of labor hours involved). Second, including the higher State-determined wage rate in Federal-aid contracts adds indirectly to future wage costs by ensuring that the higher wage rate will have greater influence in future wage surveys, and perpetuate higher rates regardless of true market conditions.

Since the Federal-aid highway projects are funded jointly by Federal and State governments with, in the instance of interstate construction and rehabilitation, the Federal government paying at least 90% of all costs, and States paying only 10% or less of those costs, the FHMA has an obligation to ensure that Federal dollars are not used to pay inflated wage rates imposed by State law. Stated another way, only 10 to 25 percent of project costs are paid from local or State funds. Thus, there is often only minimal incentive for those governments to avoid inflated wage levels, especially since high wages are politically attractive and the major portion of the cost is paid from Federal funds.

For example, for a recent project in one State, the Federal Davis-Bacon rate (combined wage/fringe benefit rate) for unskilled labor was $15.01, while the State's "little Davis-Bacon" rate was $19.08 (27 percent higher). For another project, the Federal Davis-Bacon rate for unskilled labor was $12.56, while the State-determined rate was $16.74 (33 percent difference). On still another project, the unskilled labor prevailing wage rate determined by the Federal Government was $13.74 while the State-determined rate was $14.89 (8 percent higher). State rates for unskilled labor have been set as high as $20.67 per hour, substantially higher than for comparable non-federally funded projects.

While its not possible to precisely reconstruct the amount of excess payments which have resulted from the application of these laws over the years, the payment of any amount in excess of that required by Federal law is not consistent with sound fiscal stewardship of Federal funds. All Federal agencies have a duty to analyze expenditures to determine if public money is being used and expended economically and efficiently. Certainly, it is unarguable that the payment of any element of excess cost will necessarily result in the building of fewer miles of highway or other necessary improvements within the States affected.

It thus becomes apparent that action to preclude Federal-aid in such excess costs will aid the program. Therefore, this proposal makes such excess costs ineligible for Federal-aid, without affecting the validity of any State law. No State's apportionment of funds is affected by this proposal.

The proposal, if promulgated as a final rule, would operate in the following manner. States would continue to publish Federal Davis-Bacon minimum wage rates in the advertisement or call for bids on any contract for a Federal-aid project, as now required by 23 U.S.C. 635.124(d). Likewise, those States with their own minimum wage rate laws may also continue to publish state rates in the advertisement or call, since the proposal does not prohibit their use. This proposal would not affect any contract already awarded. Upon award of new contracts and commencement of work, however, those States will be required to compute the difference between Federal and State wage rates actually paid out using the contractors' weekly payrolls, which are already required to be submitted by 40 U.S.C. 276c.

It is this difference which would be ineligible for Federal-aid participation. The amount of wages paid *above* the level of State-imposed minimums by contractors, whether due to market pressure or union agreements, would be unaffected by the proposal and would continue to be eligible. The burden on the affected States is expected to be in two parts. The first, a short-term task, consists of establishing a method of review of weekly payrolls to determine the ineligible differential. The second consist of long-term use of that review method as part of the already required State review of weekly payrolls for compliance with other labor laws. It is estimated that this computation would cost a State with an average size program about $50,000 per year. Comment is specifically requested on the amount of lead time the States might need to implement this provision.

Although neither the Davis-Bacon Act nor 23 U.S.C. 113 preempts State law in this regard, the Federal Highway Administrator has broad discretionary power under statute, 23 U.S.C. 106, to approve Federal-aid projects proposed by the States. This statutory power necessarily includes the power to disapprove projects which are too costly from whatever standpoint, whether in design, materials, or labor costs. This is demonstrated not only by the extensive legislative history of the provision in the Federal-aid Road Act of 1916, 39 Stat. 355, which became 23 U.S.C. 106, but by numerous court decisions such as *Mahler* v. *United States*, 306 F.2d. 713 (1962) which drew heavily on this legislative history in ruling that "The concern of Congress was to make sure that federal funds were effectively employed and not wasted."

The statutory discretionary power of the Administrator to disapprove projects for excessive cost is buttressed by other enactments. The first section of the Department of Transportation Act, codified as 49 U.S.C. 101, begins:

> (a) The national objective of general welfare, economic growth and stability, and security of the United States require the development of transportation policies and programs that contribute to providing fast, safe, efficient, and convenient transportation at the lowest cost consistent with those and other national objectives, including the efficient use and conservation of the resources of the United States.

Further, 23 U.S.C. 101(e), expressing a Congressional policy of preventing waste in the Federal-aid programs, states:

> It is the national policy that * * * the Secretary and all other affected heads of Federal departments * * * shall encourage * * * the best use of available manpower and funds.

The FHWA has determined that this document does not contain a major rule under Executive Order 12291. The proposed rulemaking is considered significant under the Department of Transportation's regulatory policies and procedures because of the public interest and controversy that the proposed rulemaking is likely to generate.

To the extent that the proposed regulation reduces wage costs on Federal-aid highway construction projects, an important impact of the proposed change will be to make funds available for additional highway construction purposes. The regulatory and economic impacts are addressed in more detail in a Draft Regulatory Evaluation/Initial Regulatory Flexibility Analysis which has been prepared and is available for inspection in the public docket and may be obtained by contacting Mr. Paul E. Cunningham at the address provided under the heading "For Further Information Contact."

The Regulatory Evaluation suggests, based on a number of assumptions, that this proposal may save up to $60,000,000 per year in Federal-aid funds. These assumptions may bear public examination and comment. Public comment is also requested regarding the size of and reasons for the differences in prevailing wage determinations as set by the 19 States and the DOL, and how this proposal is likely to affect those differences. The FHWA is also interested in how this proposal might practically affect the operation of highway construction programs in the States concerned, particularly with regard to whether any delays or hindrance might be involved.

With regard to the assessment of the impact this proposed rulemaking would have on small entities pursuant to the Regulatory Flexibility Act (Pub. L. 96–354), the reasons for, objectives, and legal basis for this action have been previously explained in this notice. This rulemaking would not impose any additional reporting, recordkeeping or other compliance requirements on small entities and does not duplicate, overlap, or conflict with any other Federal rules. The proposal is not expected to have a significant economic impact on a substantial number of small entities. In fact, the proposed regulation should promote a competitive environment in which small and economically disadvantaged businesses may have greater opportunity to compete for federally-funded highway contracts.

Because sufficient information was not available to allow more precise estimation of the impacts of the proposed change in those States where State-determined prevailing wage requirements have been applied to Federal-aid highway construction contracts, FHWA encourages all interested parties to comment on the preliminary assessment of impacts and to provide information that will assist in improving the initial assessment. Comments directed to the items listed below will be particularly helpful in preparation of a final assessment of impacts.

(1) Specific examples of Federal-aid highway projects where State level wage determinations resulted in the establishment of a prevailing wage requirement higher than that established under provision of the Federal Davis-Bacon Act (including project location, difference between Davis-Bacon minimum and State-determined minimum, job classifications affected).

(2) In which States do State prevailing wage determinations result in the establishment of higher prevailing wage rates on Federal-aid highway projects than those established according to the Davis-Bacon Act (the 19 listed above, or others)? In these States, by how much do the State determinations generally exceed the Federal determination, on a percentage basis? How often do State level wage determinations increase wage costs on Federal-aid projects (always, often, seldom, never)? Which job classifications are typically affected?

(3) To what extent do State level wage determinations applicable to Federal-aid projects limit competition from potential bidders? Will the proposed regulation improve the competitive environment

for small and economically disadvantaged contractors?

(Catalog of Federal Domestic Assistance Program Number 20.205, Highway Research, Planning, and Construction. The regulations implementing Executive Order 12372 regarding intergovernmental consultation on Federal programs and activities apply to this program)

In consideration of the foregoing and under the authority of 23 U.S.C. 113 and 315, and 49 CFR 1.48(b), the FHWA proposes to amend Part 635, Subpart A to Chapter 1 of Title 23, Code of Federal Regulations, as set forth below.

List of Subjects in 23 CFR Part 635

Government contracts, Grant programs—transportation, Highways and roads.

Issued on: September 24, 1985.

Ray Barnhart,

Federal Highway Administrator.

PART 635—[AMENDED]

The FHWA proposes to amend Part 635, Subpart A to Chapter 1 of Title 23, Code of Federal Regulations, as follows:

1. The authority citation for Part 635 continues to read as follows:

Authority: 23 U.S.C. 112, 113, 114, 117, 128 and 315; 42 U.S.C. 3334, 4231–4233, 4601 *et seq.*; 49 CFR 1.48(b).

2. In § 635.124, paragraph (d) is revised to read as follows:

§ 635.124 Labor and employment.

* * * * *

(d) The advertisement or call for bids on any contract for the construction of a project on the Federal-aid system either shall include the minimum wage rates determined therefore by the Secretary of Labor or shall provide that such rates are set out in the advertised specifications, proposal or other contract document, and shall further specify that such rates are the minimum rates that must be paid under the contract covering the project. If any provision of State law, regulations, specification, or policy may operate in any manner to require the establishment of prevailing wage rates higher than those determined by the Secretary of Labor under the Davis-Bacon Act, and applied to Federal-aid work by 23 U.S.C. 113, Federal-aid funds shall not participate in any excess costs due to such provisions.

[FR Doc. 85-23185 Filed 9-26-85; 8:45 am]

BILLING CODE 4910-22-M

National Highway Traffic Safety Administration

Federal Highway Administration

23 CFR Part 1208

[Docket 85-12; Notice 1]

National Minimum Drinking Age

AGENCIES: National Highway Traffic Safety Administration (NHTSA), Federal Highway Administration (FHWA), DOT.

ACTION: Notice of proposed rulemaking.

SUMMARY: The purpose of this notice is to solicit comments on a proposed regulation which would clarify the provisions which a State must incorporate or have incorporated into its laws in order to prevent the withholding of a portion of its Federal-aid highway funds for noncompliance with the Natural Minimum Drinking Age. The rulemaking is undertaken at this time to implement section 6 of Pub. L. 98–363.

DATES: All written comments must be received by October 28, 1985. The regulation will become effective as of the date of publication of the final rule.

ADDRESSES: Written comments should refer to the docket number and the number of this notice and ten copies should be submitted to: Docket Section, National Highway Traffic Safety Administration, Room 5109, Nassif Building, 400 Seventh Street SW, Washington, DC 20590. Docket hours are 8 a.m. to 4 p.m.

FOR FURTHER INFORMATION CONTACT:

NHTSA: Mr. George Reagle, Associate Administrator for Traffic Safety Programs, National Highway Traffic Safety Administration, 400 Seventh Street SW., Washington, DC 20590 (202–426–0837)

FHWA: Mr. R. Clarke Bennett, Director, Office of Highway Safety, Federal Highway Administration, 400 Seventh Street SW., Washington DC 20590 (202 426–1153) and Mr. David Oliver, Office of the Chief Counsel, Federal Highway Administration, 400 Seventh Street SW., Washington DC 20590 (202–426–0825)

SUPPLEMENTARY INFORMATION: There has been increasing concern over the incidence of alcohol-related crashes involving the nation's youth. Studies show a direct correlation between the minimum drinking age and alcohol-related crashes in the 18 to 21 age group. In 1981, 24,000 drinking drivers died in alcohol-related crashes. Approximately 16 percent of these drivers, 3,840, were between the ages of 18 and 20. This death toll of young Americans is grossly disproportionate to the population of this age group and can be accurately termed a national tragedy. In fact, fatal crashes for every 100 million vehicle miles traveled drops from 4.12 for 18 year olds down to 2.52 for those 22 to 24 years old.

As a result of this growing awareness, on July 17, 1984, the President signed legislation which strongly encourages States to have laws prohibiting the purchase and public possession of alcoholic beverages by anyone under 21 years of age by withholding a portion of Federal-aid highway funds from States without such laws.

In enacting this legislation, both Congress and the President recognized that raising the drinking age results in a decrease both in the number of traffic crashes and in the number of fatalities. For example, Michigan, which had a drinking age of 18 since January 1972, raised the age back to 21 in December 1978. In the first 12 months after the age limit was raised, a study showed a statistically significant reduction of 31 percent in alcohol-related crashes among drivers aged 18–20. Other studies have shown that increasing the drinking age has a positive effect on the number of single vehicle nighttime male driver crashes, most of which involve drinking drivers. For example, a 1980 study in Illinois showed a 8.8 percent decline in single vehicle nighttime male driver crashes involving drivers aged 19 to 20 after the drinking age was raised.

In addition to the increase in drinking age, Congress and the President expressed overwhelming concern for the continuing "blood border" problem that exists when young people can drive from one State to another and purchase alcoholic beverages which are otherwise prohibited to them. For example, a report released by the State or New York in 1981 indicates that 39 percent of the New Jersey drivers involved in alcohol-related crashes in New York border counties, where there is a lower drinking age, are under 21 years of age, and 49 percent of similarly involved Pennsylvania drivers are under 21. A uniform national drinking age will solve the problem that now occurs when teenagers in one State with a drinking age of 21 easily drive into another State with a lower age limit, consume alcohol, and drive home. Too often, these trips result in the needless killing or maiming of young people or others who become innocent victims.

Adoption of National Minimum Drinking Age

The legislation clearly states that the Secretary must withhold a portion of

Federal-aid highway funds from any State whose laws permit the purchase or public possession of any alcoholic beverage by a person who is less than 21 years of age. If any such State does not enact a new law or amend the existing laws to make age 21 the legal minimum drinking age by October 1, 1986 (fiscal year 1987), five percent of its Federal-aid highway apportionment under 23 U.S.C. 104(b)(1); 104(b)(2); 104(b)(5) and 104(b)(6), which are primary system, secondary system, interstate system (including resurfacing, restoring, rehabilitating and reconstructing funds) and urban system funds, shall be withheld. If by October 1, 1987 (fiscal year 1988) no such law is adopted or amendments made, an additional ten percent of its Federal-aid highway funds under these sections will be withheld.

Apportionment of Withheld Funds

Pub. L. 98–363 provides that funds which have been withheld from a State which does not have 21 as the minimum drinking age be apportioned to that State "if in any succeeding fiscal year such State makes unlawful [such] purchase or public possession."

Section 1208.5 of the proposed rule notes that the apportionment of withheld funds to states is subject to their availability under 23 U.S.C. section 118(b). In relevent part, section 118(b) provides that—

> (1) Sums apportioned to each Federal-aid System (other than the Interstate System) shall continue available for expenditure in that State for the appropriate Federal-aid System or part thereof (other than the Interstate System) for a period of three years after the close of the fiscal year for which such sums are authorized and any amounts for apportioned remaining unexpended at the end of such period shall lapse.
>
> (2) Expect as otherwise provided in this subsection, sums apportioned for the Interstate System in any State shall remain available for expenditure in that State for the Interstate System until the end of the fiscal year for which authorization * * *.

NHTSA sought the advice of the Office of Management and Budget (OMB), because of its expertise and coordinating role in budgeting matters, concerning the interaction of section 118(b) with 23 U.S.C. 158, which establishes the national minimum drinking age. Consistent with OMB's advice, § 1208.5 provides that highway funds withheld for noncompliance with national minimum drinking age requirements would be subject to the standard periods of availability for Federal-aid highways funds set forth in section 118(b). That is, funds withheld from States that do not pass laws establishing 21 as the minimum drinking age in a timely manner would lapse, as provided by section 118(b), if not expended within the periods designated by that statute. For example, primary system funds for fiscal year 1987, withheld from a State because of its failure to pass an appropriate minimum drinking age law by October 1, 1986, would lapse after September 30, 1990, if not obligated by that date, regardless of whether the State had, meanwhile enacted such a statute.

Compliance Criteria

"Alcoholic beverages" are clearly defined in the legislation by references to the Internal Revenue Code of 1954 and those definitions, along with the definition of wine prescribed in the statute, are incorporated into the text of the proposed rule.

The agencies are proposing to define "public possession" to mean the possession of an alcoholic beverage for any reason, including consumption, on any street or highway or in any public place or in any place open to the public. Congress made it clear in their discussions of this legislation that they did not intend for its provisions to apply to any person under the age of 21 when the selling, transporting, delivering, serving or other handling of an alcoholic beverage was in pursuance of that person's employment and that understanding has been incorporated into the proposed definition of "public possession."

Questions have been raised concerning other parameters of "public possession" and the agencies are requesting comments on these issues. For example, there are currently eight States that have provisions in their laws that allow underage persons to consume and/or possess alcoholic beverages as part of an established religious service. The agencies are, therefore, proposing within the definition of "public possession" language clarifying that alcohol consumed for religious purposes need not be denied to underage persons.

It is clear that "public possession" does not include consumption of alcoholic beverages by underage persons in individuals' homes. However, most States do have statutes that regulate private clubs and we are requesting comments on whether or not they should be included in the definition of "public". The issue was also raised as to whether underage persons can consume alcoholic beverages in public when accompanied by a parent, spouse or legal guardian who is age 21 or over. Some States currently have laws that permit underage individuals to possess, consume or purchase alcoholic beverages in such instances. However, in these States the parent, spouse or legal guardian may be civilly liable for any damage to property or injury of persons that is proximately caused by the underage person's drinking. The legislative history does not suggest that Congress intended to permit such an exception and, therefore, we do not propose to incorporate such a provision into the definition of "public possession."

Currently, three states exempt members of the Armed Forces of the United States from the provisions of their legal drinking age laws. The debate in both the House and the Senate included arguments that persons old enough to vote and to fight for their country should be old enough to drink. However, proponents of the legislation stressed that while there may be a relationship between voting and fighting, there is no such relationship between voting and drinking. The legislative history is clear that Congress sees the right to vote as a fundamental political right of all citizens while both drinking and driving are privileges and in Congress' view are subject to reasonable regulation in the interests of public health and safety.

Further, to permit a blanket exclusion within a State for members of the military would continue the problem of "blood borders" which Congress so clearly intended to eliminate. The legislative history supports the conclusion that the statute does not permit a blanket exclusion for the military from a State's minimum drinking age requirements. It is important to note, however, that State drinking age laws do not generally apply to alcohol consumed on premises controlled by the military, such as officers' clubs. While the Department of Defense and the military services have been cooperating with NHTSA to reduce alcohol-related crashes both on and off duty, the military services retain fully authority over their lands. The agencies applaud the military's efforts to reduce drunk driving and further encourage the military to incorporate State law into their regulations governing drinking while on the premises of a military establishment in order to decrease the number of "blood borders."

Pub. L. 98–363 prohibits the "purchase" of an alcoholic beverage by an underage person. It has come to our attention that some States include within their laws governing the minimum drinking age provisions that also prohibit the "attempt to purchase" and the "selling, giving or serving" of alcoholic beverages to underage individuals. The legislative history does not reflect any Congressional intention to extend the word "purchase" to encompass these other acts. We have, therefore, not included them within the definition.

Notification and Advance Notification of Compliance

NHTSA and FHWA will notify all States of initial determinations of compliance or non-compliance with Pub. L. 98–363 by March 1, 1986 and 1987, and each State initially found not to comply will have an opportunity to rebut this initial determination. By May 1, 1986 and 1987, the agencies will notify in writing all States of determinations of compliance or noncompliance with Pub. L. 98–363. If a State comes into compliance after May 1 but prior to the beginning of the fiscal year, any funds earmarked for withholding will be released.

NHTSA and FHWA recognize that States will want to know as soon as possible whether or not their laws satisfy the requirements of Pub. L. 98–363. Advance notification would allow States with nonconforming laws to take the necessary measures to enact conforming laws prior to the date for the withholding of funds. The agencies are, therefore, proposing to permit States to request advance notification by submiting a copy of all applicable State laws to the Director, Office of Alcohol Countermeasures, National Highway Traffic Safety Administration, 400 Seventh Street SW., Washington, DC 20590.

Procedures for Commenting on Proposal

Interested persons are invited to submit written comments on this proposal. It is requested but not required that 10 copies be submitted.

The comment period established for this notice is necessarily short in order to issue a final rule in time for States to prepare their legislative agendas for sessions that begin in January 1985.

Comments should not exceed 15 pages in length (*See* 49 CFR 553.21). Necessary attachments may be added to these submissions without regard to the 15 page limit. This limitation is intended to encourage commenters to detail their primary arguments in a concise manner.

All comments received before the comment closing date will be considered and will be available for examination in the docket at the above address before and after that date.

To the extent possible, comments filed after the closing date will also be considered. However, the rulemaking action may proceed at any time after that date. NHTSA will continue to file relevant material in the docket as it becomes available after the closing date, and it is recommended that interested persons continue to examine the docket for new material.

Those persons desiring to be notified upon receipt of their comments in the docket should enclose in the envelope with their comments, a self-addressed stamped postcard. Upon receiving the comments, the docket supervisor will return the postcard by mail.

Copies of all written comments will be placed in Docket 85–12; Notice 1 of the NHTSA Docket Section in Room 5109, Nassif Building, 400 Seventh Street SW., Washington, DC 20590.

Regulatory Evaluation

The agencies have determined that this rulemaking should be classified as significant under the Department's regulatory policies and procedures. The agencies have not prepared a regulatory evaluation because this is a ministerial regulation implementing an Act of Congress. The regulatory impact is not greater than $100 million. Any economic impact that may occur is not attributable to this regulation but will be the result of State decisions to enact statutes that conform with the Federal statute. Such decisions are not mandated by this regulation. The agencies have determined that since this rule not have an annual impact of $100 million on the economy, it is not a major rule within the meaning of Executive Order 12291.

Regulatory Flexibility Act

We hereby certify that the requirements that will be established by this rulemaking action will not have a significant economic impact on a substantial number of small entities. Any economic impact on liquor stores or other establishments will be the result of State decisions to enact statutes that conform with the Federal statute. Such decisions are not mandated by this regulation. Additionally, States are the subject of any funds which may be withheld and, therefore, preparation of an Initial Flexibility Analysis is not necessary.

List of Subjects in 23 CFR Part 1208

Alcohol, Highway safety.

In consideration of the foregoing, a new Part 1208 is added to Title 23 of the Code of Federal Regulations to read as follows:

PART 1208—NATIONAL MINIMUM DRINKING AGE

Sec.
1208.1 Scope.
1208.2 Purpose.
1208.3 Definitions.
1208.4 Adoption of National Minimum Drinking Age.
1208.5 Apportionment of withheld funds.
1208.6 Notification of compliance.
1208.7 Advance notification of compliance.

Authority: 23 U.S.C. 158.

§ 1208.1 Scope.

This part prescribes the requirements necessary to implement 23 U.S.C. 158, which establishes the National Minimum Drinking Age.

§ 1208.2 Purpose.

The purpose of this part is to clarify the provisions which a State must have incorporated into its laws in order to prevent the withholding of Federal-aid highway funds for noncompliance with the National Minimum Drinking Age.

§ 1208.3 Definitions.

As used in this part:

(a) "Alcoholic beverage" means beer, distilled spirits and wine containing one-half of one percent or more of alcohol by volume. Beer includes, but is not limited to, ale, lager, porter, stout, sake and other similar fermented beverages brewed or produced from malt, wholly or in part or from any substitute therefor. Distilled spirits include alcoholic spirits and spirits that contain that substance known as ethyl alcohol, ethanol or spirits of wine in any form, including all dilutions and mixtures thereof from whatever process produced.

(b) "Public possession" means the possession of any alcoholic beverage for any reason, including consumption, on any street or highway or in any public place or in any place open to the public. The term does not apply to the possession of alcohol for an established religious purpose or to the sale, handling, transport, delivery, service in dispensing of any alcoholic beverage pursuant to the lawful employment of a person under the age of twenty-one years by a duly licensed manufacturer, wholesaler, or retailer of alcoholic beverages.

(c) "Purchase" means the purchase by a person who is less than twenty-one years of age.

§ 1208.4 Adoption of National Minimum Drinking Age.

(a) The Secretary shall withhold five percent of the amount required to be apportioned to any State under each of sections 104(b)(1), 104(b)(2), 104(b)(5) and 104(b)(6) of title 23 of the United States Code on the first day of the fiscal year succeeding the fiscal year beginning after September 30, 1985, in which the purchase or public possession in such State of any alcoholic beverage by a person who is less than twenty-one years of age is lawful.

(b) The Secretary shall withhold ten percent of the amount required to be apportioned to any State under each of sections 104(b)(1), 104(b)(2), 104(b)(5) and 104(b)(6) of title 23 of the United States Code on the first day of the fiscal year succeeding the second fiscal year beginning after September 30, 1985, in which the purchase or public possession in such State of any alcoholic beverage by a person who is less than twenty-one years of age is lawful.

§ 1208.5 Apportionment of withheld funds.

Funds withheld pursuant to § 1208.4 shall be apportioned to a State, subject to the availability of such funds under 23 U.S.C. 118(b), if such State makes unlawful the purchase or public possession of any alcoholic beverage by a person who is less than twenty-one years of age.

§ 1208.6 Notification of compliance.

(a) Each State will be notified by certified mail of NHTSA's and FHWA's initial determination of compliance or non-compliance with Pub. L. 98–363 by March 1, 1986 and 1987.

(b) If NHTSA and FHWA initially find the State in non-compliance, the notice shall state the reasons for the initial determination and shall inform the State that it may, within 20 days of its receipt of the notification, submit documentation showing why it is in compliance.

(c) Each State will be notified by certified mail of its compliance or non-compliance with Pub. L. 98–363 by May 1, 1986 and 1987.

§ 1208.7 Advance notification of compliance.

Any State wanting notification prior to the dates set forth in § 1208.6 shall submit a copy of all applicable laws to the Director, Office of Alcohol Countermeasures, NHTSA, 400 Seventh Street SW., Washington, DC 20590.

(Sec. 6, Pub. L. 98–363 (23 U.S.C. 158); 98 Stat. 435; delegations of authority at 49 CFR 1.48 and 1.50)

Issued on: September 24, 1985.

Diane K. Steed,

National Highway Traffic Safety Administrator.

L.P. Lamm,

Deputy Administrator, Federal Highway Administration.

[FR Doc. 85–23078 Filed 9–24–85; 10:57 am]

BILLING CODE 4910-59-M; 4910-22-M

VETERANS ADMINISTRATION

DEPARTMENT OF DEFENSE

38 CFR Part 21

Veterans Education; Waiver of Right To Receive Benefits Under The G.I. Bill

Correction

In FR Doc. 85–22185 beginning on page 3770 in the issue of Tuesday, September 17, 1985, make the following correction: On page 37701, in the first column, in § 21.5040(g), in the sixth line, "39 U.S.C." should read "38 U.S.C.".

BILLING CODE 1505-01-M

FEDERAL COMMUNICATIONS COMMISSION

47 CFR Part 63

[CC Docket No. 83-1230]

International Communications Policies

AGENCY: Federal Communications Commission.

ACTION: Proposed Rule; extension of time.

SUMMARY: This order extends, at the request of the United States Telephone Association, the time for filing comments and reply comments in CC Docket No. 83-1230, the Commission's rulemaking looking toward development of policies governing the granting of Recognized Private Operating Agency (RPOA) status, the assignment of data network identification codes and determination of the eligibility of non-common carriers to acquire indefeasible rights of user in submarine telephone cables.

DATES: Comments are due on or before October 28, 1985, and reply comments are due on or before November 18, 1985.

ADDRESS: Federal Communications Commission Washington, D.C. 20554.

FOR FURTHER INFORMATION CONTACT: Stuart Chiron, Chief, International Policy Division, Common Carrier Bureau, Federal Communications Commission, Washington, D.C. 20554, (202) 632-4047.

Order

In the matter of International Communications Policies Governing Designation of Recognized Private Operating Agencies, Grants of IRUs in International Facilities and Assignment of Data Network Identification Codes; CC Docket No. 83-1230.

Adopted: September 20, 1985.

Released: September 24, 1985.

By the Chief, Common Carrier Bureau.

1. On August 19, 1985 (50 FR 34867, Aug. 28, 1985) the Commission released its Notice of Proposed Rulemaking in the above-captioned proceeding, FCC 85-368, calling for comments on the issues to be filed on or before September 27, 1985, and reply comments on or before October 18, 1985. On September 19, 1985, the United States Telephone Association (USTA) filed a request for extension of the time for filing comments until October 28, 1985, and of the time for filing reply comments until November 18, 1985. USTA states that it needs an extension because of the variety and complexity of issues in the proceeding and that its experts need more time properly to examine the technical issues the various Commission proposals raise.

2. We believe that USTA has shown good cause for a grant of its request. The requested extension will allow all parties better to study the issues and to prepare more detailed comments. The period of the request will not unduly delay the outcome of the proceeding or cause any interested person an undue hardship.

3. Accordingly, pursuant to § 0.291 of the Commission's Rules and Regulations, 47 CFR 0.291 (1984), IT IS ORDERED that the above-referenced request of the United States Telephone Association is GRANTED and that comments in this proceeding should be filed on or before October 28, 1985, and reply comments on or before November 18, 1985.

Federal Communications Commission.

Albert Halprin,

Chief, Common Carrier Bureau.

[FR Doc. 85-23109 Filed 9-26-85; 8:45 am]

BILLING CODE 6712-01-M

DEPARTMENT OF TRANSPORTATION

National Highway Traffic Safety Administration

49 CFR Part 571

[Docket No. 85-11; Notice 1]

Federal Motor Vehicle Safety Standards; Motorcycle Helmets

AGENCY: National Highway Traffic Safety Administration (NHTSA), DOT.

ACTION: Notice of proposed rulemaking.

SUMMARY: This notice proposes to amend Standard No. 218 *Motorcycle Helmets*, to extend the current minimum performance requirements of the standard to all helmet sizes and to improve test procedures and conditions.

Currently, the standard applies only to those helmets which can be "placed on" the size C headform for compliance testing. Until recently, that was the only available size of headform. Now there are two more. The specifications developed for the exterior geometry of a replacement (medium size) for the size C headform have been scaled down to produce a small headform and up to produce a large headform. Adherence to the specifications for exterior geometry would ensure that all headforms of a particular size are identical in shape. The addition of the small and large test headforms would assure that all sizes of helmets tested according to the requirements of Standard No. 218 provide a reasonable degree of protection.

The proposed improvements in the Standard No. 218 test procedures and conditions are based on the NHTSA's compliance testing experience under Standard No. 218, as well as statements of the helmet industry and various research organizations. These changes would improve the consistency of testing practices and test results.

DATES: Comments must be received on or before November 26, 1985. This proposal would become effective 180 days after publication of a final rule in the **Federal Register**.

ADDRESSES: Comments should refer to the docket and notice number stated above and be submitted to Docket Section, Room 5109, National Highway Traffic Safety Administration, 400 Seventh Street SW., Washington, D.C. 20590. The docket is open on weekdays from 8 a.m. to 4 p.m.

FOR FURTHER INFORMATION CONTACT: Mr. William J. J. Liu, Office of Vehicle Safety Standards, NRM-12, National Highway Traffic Safety Administration, 400 Seventh Street SW., Washington, DC 20590 (202-426-2264).

SUPPLEMENTARY INFORMATION:

Headforms

Standard No. 218, *Motorcycle Helmets*, specifies minimum performance requirements for helmets designed for use by motorcyclists and other motor vehicle users. Although the standard has been in effect since March 1, 1974, it has never covered all helmet sizes. The major reason has been that compliance testing involves the use of a headform and until now only one size of headform has been available. On the effective date, the standard specified four test headform sizes, namely, sizes A, B, C, and D, but only size C was available. The size C headform was adopted from the American National Standards Institute (ANSI) Z90.1-1971 standard which required only one headform for testing all helmet sizes. The other three headforms were specified in Standard No. 218 in response to public comments in 1972 and 1973 on the original proposed standard.

The NHTSA tried to fabricate a complete set of headforms for the standard by contract when the original standard was proposed. Unfortunately, the headforms fabricated did not meet dimensional requirements and other specifications. Therefore, the standard stated: "Helmets that do not fit headform size C will not be covered by this standard until it is extended to those sizes by further amendment." Consequently, manufacturers tested and labeled only a portion of their production.

The NHTSA did not expect the unavailability of other headform sizes to diminish the effectiveness of the original standard, because the ANSI standard stated that the size C headform "is considered suitable to allow for proper testing of protective headgear which will accurately fit approximately 95 percent of the population of all races." Yet, after Standard No. 218 became effective, the NHTSA discovered that only 40 to 45 percent of helmets available for sale in the United States were actually being certified by the helmet manufacturers. This conclusion was based on a NHTSA survey of manufacturers of approximately 80 percent of helmets sold in the United States at that time.

The NHTSA attempted to remedy the situation by providing a legal definition of the word "fit." The certification rate did not increase and the standard was considered incomplete by the industry. Finally, the Safety Helmet Council of America (SHCA) complained about unfair practices by some manufacturers, such as using the DOT label on untested helmets, and requested that more helmets be required to be certified on the size C headform. These practices led to uncertainty among retailers and consumers about the validity of the DOT label on some helmets.

In response to this need, the NHTSA issued a notice of proposed rulemaking in 1979 to extend application of the Standard's requirements to all helmets that could "be placed on", rather than "fit", the size C headform. The extension was adopted and became effective on May 1, 1980. This amendment to the standard was an interim rule requiring the certification of all large-size and many small-size helmets and was effective until headform sizes A (small) and D (large) could be developed and incorporated into the standard. The agency anticipated that certification of all adult helmets would end any confusion in the marketplace about the validity of the DOT label. According the SHCA, more than 90 percent of helmets currently manufactured by SHCA members are certified as complying with Standard No. 218. The size B headform contemplated in the original rule was no longer considered necessary to this effort, because sizes A, C, and D can be used to test all helmet sizes adequately. Under this interim amendment, extra-large helmets were required to be tested on the size C headform without the use of "shims" or other devices to obtain a secure fit of the helmet on the headform.

The development of the size A and D headforms began with the creation of more detailed specifications for the size C headform. The Appendix to Standard No. 218 currently includes simplified exterior dimensions for the headforms. Both the agency and the motocycle helmet companies have recognized the need for more detailed specifications. After measuring the exterior geometry of the size C headform, the agency developed a numerical table describing in detail this exterior geometry. This table was then used to manufacture the new medium headform. To produce the small and large headform sizes, scaling factors of 0.8941 and 1.069, respectively, were applied to this numerical table. The scaling factor of any headform is defined as the ratio of the radius of its equivalent sphere to the radius of the equivalent sphere of the medium headform. (The radius of the equivalent sphere of any headform is derived from the total drop weight of that headform and the circumference of the medium headform.) These specifications would be included in the proposed revision of the Appendix to the standard. The NHTSA believes the new test headforms, manufactured according to these specifications, would enhance the objectivity and repeatability of the tests.

The agency also developed the interior geometry for the small, medium and large headforms for the first time. However, these interior geometries would be added to the revised Appendix to the standard for reference purposes only. They would be used as guidelines, not as requirements of the standard, to give a manufacturer flexibility in the design of a headform interior. The flexibility is needed because Standard No. 218 currently limits the combined weight of the test headform and the supporting assembly for the drop test. If a slightly heavier supporting assembly is used by a manufacturer of drop test equipment, a manufacturer might have to take some weight out of the interior of the test headform in order to meet the combined weight limit. This would be permitted, as long as the center of gravity of the headform is not altered.

This proposal would require use of the new set of small, medium, and large test headforms for agency compliance testing. To ensure testing with the same medium size headform by the agency, the agency would discontinue the use of the size C headform to test medium size helmets after the proposed effective date. The new medium headform is considered to be a suitable replacement for several reasons. First, the validity of the new medium test headform has been demonstrated by the consistent, although not indentical, helmet test responses achieved when testing the new medium and the existing size C headforms. Second, the new medium headform would have better defined dimensions for its exterior geometry. Third, the center of gravity location would be fixed. Interior geometry specifications are referenced for the first time. As a result, the medium headform's center of gravity location would be constant at the center of the ball socket joint. Currently, the center of gravity location varies in the size C headform. Due to the constant center of gravity location, the new medium headform would give more uniform helmet test responses.

Test Procedures and Conditions

The agency contracted with Dayton T. Brown, Inc. to test all helmet sizes on the small, medium, and large headforms. The final report of this contract can be found in the docket under this notice. Dayton T. Brown's test results, as well as information available to the agency from compliance testing and other sources, resulted in the proposed changes in test procedures and conditions which are discussed below.

To achieve a more uniform helmet testing practice, the proposal states which helmets with a manufacturer's designated size range would be tested on the small, medium, and large headforms. If a manufacturer's designated helmet size range is 6⅝ (European size: 53) or smaller, the helmet would be tested only on the small headform. The medium headform would be used for testing helmets with a manufacturer's designated size range between 6¾ (European size: 54) and 7½ (European size: 60). The large headform would be used for testing helmets with a manufacturer's designated size range of 7⅝ (European size 61) or larger. However, if a helmet with an manufacturer's designated size range includes more than one of the three size ranges specified above, the helmet would be tested on more than one headform.

The need for this occasional multiple testing requirement stems from the differing practices of the manufacturers in assigning size ranges for small, medium, large, and extra-large helmets. The proposed change would eliminate the earlier problems with requiring testing of helmets that could "fit" or could "be placed on" a particular headform, because this approach would no longer be necessary. The NHTSA is aware that increased numbers of tests may be required for helmets with wide size ranges, but believes this is necessary and desirable to ensure the safety of helmets for all users.

Another proposed change concerns the helmet testing time limit after a helmet is removed from the conditioning environment. The standard currently allows five minutes after removal to complete two successive, identical impacts on a helmet test site during the impact attenuation test. After removal from the conditioning environment, the standard requires that a helmet be fixed and secured on a test headform and, then positioned for impact. To allow enough time to accomplish these tasks, and to provide a common starting time, the agency would require that the impact testing for each helmet start at two minutes after the time of removal from the conditioning environment. If the two successive impacts at one site are not completed within four minutes from the removal time, testing would stop and the helmet would be returned to the conditioning environment as currently specified in the standard. Once reconditioned, the test sequence could start again. Like the two-minute starting time, the four-minute stopping point is intended to reduce the effects of helmet temperature changes on test results. The proposed test time limit would reduce the temperature gradient across the helmet when the helmet is tested. The agency expects improved test objectivity and repeatability to result, particularly during the low temperature test.

Similarly, during the penetration test, the application of the two penetration blows would start at two minutes after removal from the conditioning chamber. The second blow would have to be completed within four minutes after removal. If this time limit is not met, the helmet would have to be reconditioned before resuming testing. The purpose of this change is to minimize possible helmet response variations due to helmet temperature changes during the testing period. The agency believes that the performance of conditioned helmets can change rapidly with time during both the impact attenuation and penetration tests. The goal of more repeatable, objective test results make this change desirable. The test compliance laboratories believe this change is reasonable and practicable.

The agency is not presently considering requiring a time limit for the retention test, because a helmet's performance during this test is not believed to be time-sensitive. If manufacturers or test laboratories think otherwise, comments and supporting data are requested.

The conditioning requirements for helmets being tested remain the same because the NHTSA believes these requirements reflect various environental conditions which a motorcycle helmet wearer may encounter. The low temperature test at 15 °F, for example, tests helmet material sensitivity to impacts in a cold temperature environment. Testing at this temperature is currently required by industry standards.

Another improvement would be provided by a proposed change in the resonant frequency limitation of a test headform. Helmet testing under the existing standard has shown that no current or past headforms satisfy the current requirement that test headforms "exhibit no resonant frequencies below 3,000 Hz." Results from NHTSA contract tests indicate that resonant frequencies of headforms usually range between 2,000 and 3,000 Hz. These headform frequencies do not influence helmet test results because they are sufficiently above the fundamental helmet frequencies (estimated to be far below 1,000 Hz.) to avoid significant distortion of helmet response measurements. Therefore, the proposed amendment would state that test headforms "exhibit no resonant frequency below 2,000 Hz." Moreover, the proposed 2,000 Hz. value would allow flexibility in the design of the interior of virtually all headforms. Standard No. 218 specifies the combined weight of the test headforms and the supporting assembly for the drop test. If a slightly heavier supporting assembly is used by a manufacturer of drop test equipment, this flexibility would permit taking some weight out of the test headform so that the combined weight limit would be met.

The use of the currently available monorail for the drop test is specified in the proposal for the impact attenuation test to achieve more consistent, objective results. Research testing results found that the twin-wire testing system for the drop test may not produce similar results, because the headform may rotate significantly during the impact. Monorail testing equipment is currently used by NHTSA contract laboratories and by most other helmet test laboratories. Further, this equipment is available to the industry. Therefore, the requirement to use monorail equipment should not pose a hardship.

Research results show that more frequent adjustment of the monorail equipment may be necessary when the large test headform is used. The agency requests comments on the industry's experience in using the large test headform with the monorail equipment.

A proposed technical change is to provide metric equivalents for all inch and pound measurements for the first time.

Other Issues

The time duration or "dwell time" aspect of the impact attenuation test has been controversial. The current requirement provides that accelerations in excess of 200g and 150g shall not exceed 2 milliseconds and 4 milliseconds (ms), respectively. Supporting data for these g levels and their time durations were found in the Japan Automobile Research Institute, Inc. (JARI) study, published in 1980. This is the most recent study available on the threshold for head injury in frontal and occipital impacts and gives experimental head injury data using subhuman primates and human cadaver skulls.

The JARI Human Head Impact Tolerance Curve (JHTC) was extrapolated from the curves for primates and cadaver skulls. The JHTC shows the threshold of human concussion at about 200g—2.3 ms. Because average individual tolerance to head injury varies and the JHTC was derived from many scattered data points for primates and cadavers, the agency believes that retaining the 200g—2 ms requirement provides the necessary margin of safety for a motorcycle helmet user.

Another controversial aspect of this test has been the second impact which is also the test point at which most failures occur. Paragraph S7.1.2 states: "Each helmet is impacted at four sites with two successive, identical impacts at each site." The purpose of requiring the second impact at each test site is to establish a minimum level of helmet residual impact absorbing capability, as described in the following paragraph.

All other known standards which have been established by private standard organizations or by foreign countries require higher second impact levels than Standard No. 218. The NHTSA notes that in real world accidents, a second impact may occur quickly after the first, perhaps within one or two seconds, and perhaps at a different place on the helmet surface. The human head's injury tolerance is known to be lower when subjected to repeated blows. However, there is no state-of-technology test method for conducting second impacts within such a short time frame. Moreover, changing the impact point after each drop would double the total test time, resulting in a test which would arguably be less related to an accident situation.

The minimum interval in testing laboratories between the first and second impacts is about 20 seconds. The crush liner in currently available motorcycle helmets should be able to recover most of its residual impact absorbing capability in 20 seconds after the first drop, but might not do so in one or two seconds. Accordingly, this test is not intended to reproduce potential multiple impacts in a single accident. However, the NHTSA believes that the second impact test is a valid test to establish that, at least, the material has sufficient ability to recover its protective capabilities in the particular location which has been impacted. This is important motorcycle helmets may be worn agains after one severe blow, despite the required manufacturer's warning not to do so in paragraph S5.6.1 of the existing standard.

Since the recovery performance of a helmet may vary with time, the agency proposes a prescribed test time of two minutes for both impacts at each site as discussed earlier. This prescribed test time would improve the repeatability and objectivity of this test. All helmets tested would have nearly the same time in which to recover from the first impact. Thus, retaining the second impact test ensures that all helmets would have a certain minimum second impact performance level.

The agency is not proposing any change in the time duration or second impact requirements, but invites public comment on these issues.

Safety Benefits

Current fatalities of motorcycle riders, both in absolute terms and as a percentage of total highway traffic fatalities, are roughly twice those in the early 1970's. Motorcycle fatalities have kept pace with motorcycle registrations. Motorcyclist deaths in 1982 were 4,453 compared to 2,280 in 1970, a 95 percent increase, while the increase in total motorcycle registrations during the same period was 104 percent. The number of fatalities to helmet users was considerably less, totaling 1,533. During this period, many States have repealed or relaxed laws requiring helmet use. At this time, the agency estimates that 43% of registered motorcyclists are subject to State helmet use laws.

Benefits from the proposed rule would accrue directly to future users of small or large size helmets. The effectiveness of motorcycle helmets in preventing death and injury has been well established in studies prepared for DOT.

The agency tentatively estimates that about ten percent of motorcycle helmets primarily in the small size range are not currently required to be tested for compliance with Standard No. 218, because these helmets cannot be "placed on" the size C test headform. The proposed extension of the standard to cover all sizes of helmets could result, therefore, in improved safety for about 10 percent, or 388,000, of the estimated 3,880,960 motorcycle riders who wear helmets.

Data limitations preclude precise estimates of the safety benefits of this rulemaking. One benefit of the rulemaking should be to increase the percentage of these small helmets which, if tested, would meet the standard. Based on agency compliance testing results between FY 1979 and FY 1981, the rate of noncompliance of certified helmets was 25 percent. The agency believes it reasonable to assume that uncertified helmets are not manufactured to the same level of performance as certified helmets and that therefore the noncompliance rate for currently uncertified helmets would be higher if they were tested now. Extension of the standard to those helmets should reduce their rate of noncompliance to that of the currently certified helmets.

Another benefit would be that the improvements in the specification of the test procedures and conditions may lead manufacturers of currently complying helmets to upgrade their helmets in some respects so as to ensure continued compliance with the standard.

Overall helmet usage may increase as a result of a standard that covers all helmet sizes. Extension of the standard to cover all helmet sizes should benefit NHTSA's efforts to encourage States to implement laws requiring helmet use by motorcyclists.

If additional helmet use laws are enacted, fatalities and injuries would be reduced. In 1982, in States without mandatory helmet use laws, 2,171 fatalities occurred to motorcycle riders not wearing helmets, and 524 fatalities to helmet users for a total of 2,695 fatalities. The extent of any reduction in fatalities and injuries would depend on the number of States which enact mandatory helmet use laws.

In addition to other issues raised in this notice, the agency requests comments on the following questions:

1. How many helmet manufacturers have, or do not have, their own testing equipment? Of the manufacturers with equipment, what percentage of helmet testing is done by outside laboratories?

2. How many test headforms would helmet manufacturers, who conduct their own testing, need to purchase to meet the requirements of the proposed rule? How many helmet manufacturers would do their own headform machining?

3. What are the testing costs for helmet manufacturers conducting their own testing?

4. What is the cost of redesigning a motorcycle helmet shell and its liner?

5. What percent of current helmet production can be placed on the size C headform?

6. What percent of helmet production would be tested on each of the small, medium, and large test headforms?

7. What percent of helmets would need to be tested on more than one size test headform?

8. Is there any data comparing effectiveness of complying versus non-complying helmets?

The agency also requests comments on the following issues which may be considered in future rulemaking proceedings and asks commenters to state the reasons for their responses, based on safety needs, and to include estimates of additionl testing costs for each change:

1. Should NHTSA consider adopting additional requirements, which are contained in other motorcycle helmet standards, for example, the Snell Foundation standard, the American National Standards Institute (ANSI) standard, or European standards, such as the ECE standard?

2. Should the geometric configuration of the pointed penetrated test striker be modified to resemble the narrow surface in the 1985 Snell standard?

3. Should the retention test be changed to require dynamic testing to prevent the helmet from rotating on the head and perhaps coming off the head in an accident situation?

4. Should the test line marking the limit of the test surface in Figure 2 of the standard be lowered or should the test be revised in other ways to provide more protection in accidents for the lower part of the back of the head or the front of the head in the forehead area, or to improve the performance of the helmet from the side? What requirements would represent the optimal trade-off between helmet weight, visibility, hearing, and other helmet design criteria? Do current requirements represent a reasonable trade-off?

5. Should a test procedure be developed for the chin guard area of full facial coverage helmets?

6. Should the low temperature conditioning requirement be changed so that the interior surface of the helmet, or the headform, is at body temperature for the impact attenuation and penetration tests?

Costs and Other Impacts

The agency has considered costs and other impacts of this proposal and has determined that the proposal would not be major within the meaning of Executive Order 12291 or significant within the meaning of the Department of Transportation's regulatory procedures. The agency has determined that this proposal would not result in a major increase in costs for the motorcycle helmet industry or consumers.

The agency estimates tentatively that recurring annual manufacturing costs resulting from helmet testing and labeling should not exceed $.10 per helmet. In addition, one-time costs associated with new testing procedures and helmet redesign should not exceed $250,000 for the motorcycle helmet industry. However, this estimate could increase if helmets must be redesigned to a greater extent than anticipated as a result of the introduction of the new small and large test headforms. The extent of the necessary redesign is not precisely known because of the new requirements in this proposal. Small or child-size helmets would be required to be tested for the first time. Large-size helmets would be required to be tested on the new, heavier large test headform. the testing performed for NHTSA by the contractor who developed the new headforms did not show a greater number of helmet failures. However, the results of industry-wide testing cannot be foreseen. Industry testing will involve many more helmet models, as well as greater numbers of helmets. If some helmets do not comply when tested on the new headforms, a manufacturer may decide to redesign them. Redesign of small or large helmets should improve their physical characteristics, thus increasing safety benefits to the user.

A 1980 cost benefit study prepared for the agency stated that the extent and cost of helmet redesign resulting from the introduction of small and large test headforms was uncertain. However, this study also concluded that increased consumer costs due to possible redesign should not exceed $1 per helmet. If there were a greater cost increase for some helmets, it would be due to the need for more extensive helmet redesign. That redesign should increase safety benefits as well as costs.

Based on this agency's review of this proposal under the Regulatory Flexibility Act, I certify that it would not have a significant eocnomic impact on a substantial number of small entities. Accordingly, no regulatory flexibility analysis has been prepared. There are approximately 11 domestic manufacturers of motorcycle helmets, all of whom are believed to have fewer than 500 employees. However, this proposal only extends an existing rule, and imposes estimated costs for helmet testing, labeling, and redesign of up to $250,000 on the motorcycle helmet industry. These costs would be spread over an annual production level of approximately 1.8 million helmets. While small organizations may purchase the small, medium, and large test headforms for testing motorcycle helmets produced by the manfacturers, their costs should be minimally affected by the impact of this proposal.

Finally, the NHTSA has considered the environmental implications of this rule in accordance with the National Environmental Policy Act and determined that the proposal would not significantly affect the human environment.

Interested persons are invited to submit comments on the proposal. It is requested but not required that 10 copies be submitted.

All comments must be limited not to exceed 15 pages in length. (49 CFR 553.21). Necessary attachments may be appended to these submissions without regard to the 15-page limit. This limitation is intended to encourage commenters to detail their primary arguments in a concise fashion.

If a commenter wishes to submit certain information under a claim of confidentiality, three copies of the complete submission, including purportedly confidential information, should be submitted to the Chief Counsel, NHTSA, at the street address given above, and seven copies from which the purportedly confidential information has been deleted should be submitted to the Docket Section. A request for confidentiality should be accompanied by a cover letter setting forth the information specified in the agency's confidential business information regulation (49 CFR Part 512).

All comments received before the close of business on the comment closing date indicated above will be considered, and will be available for examination in the docket at the above address both before and after that date. To the extent possible, comments filed after the closing date will also be considered. However, the rulemaking action may proceed at any time after that date, and comments received after the closing date and too late for consideration in regard to the action will be treated as suggestions for future rulemaking. The NHTSA will continue to file relevant material as it becomes available in the docket after the closing date, and it is recommended that interested persons continue to examine the docket for new material.

Those persons desiring to be notified upon receipt of their comments in the rules docket should enclose, in the envelope with their comments, a self-addressed stamped postcard. Upon receiving the comments, the docket supervisor will return the postcard by mail.

List of Subjects in 49 CFR Part 571

Consumer protection, Motor vehicle safety, Motorcycle helmets.

To accomplish the changes outlined above, the agency would amend Standard No. 218, *Motorcycle Helmets*, in Title 49 of the Code of Federal Regulations at Part 571, as follows:

PART 571—[AMENDED]

The authority citation for Part 571 would continue to read as follows:

Authority: 15 U.S.C. 1392, 1401, 1403, 1407; delegation of authority at 49 CFR 1.50.

§ 571.218 [Amended]

1. Paragraph S3 would be revised to read as follows:

S3. *Application*. This standard applies to helmets designed for use by motorcyclists and other motor vehicle users.

2. Paragraph S4 would be amended by revising the definitions for "Reference headform" and Test headform to read as follows:

S4. *Definitions*.

* * * * *

"Reference headform" means a measuring device contoured to the dimensions of one of the three headforms, designated small, medium, or large and described in the Appendix, with surface markings indicating the locations of the basic, mid-sagittal, and reference planes, and the centers of the external ear openings.

"Test headform" means a test device contoured to the dimensions of one of the three headforms described in the Appendix, with surface markings indicating the locations of the basic, mid-sagittal, and reference planes.

3. Section 5 would be revised to read as follows:

S.5 *Requirements*. Each helmet shall meet the requirements of S5.1 through S5.3 when subjected to any conditioning procedure specified in S6.3, and tested in accordance with S7.1, S7.2, and S7.3

4. Section 5.3.1(b) would be revised to read as follows:

(b) The adjustable portion of the retention system test device shall not move more than 1 inch (2.5 cm) measured between preliminary and test load positions.

5. Section S5.4 would be revised to read as follows:

S5.4 *Configuration*. Each helmet shall have a protective surface of continuous contour at all points on or above the test line described in S6.2.3. The helmet shall provide peripheral vision clearance of at least 105° to each side of the mid-sagittal plane, when the helmet is adjusted as specified in S6.3. The vertex of these angles, shown in Figure 3, shall be at the point on the anterior surface of the reference headform at the intersection of the mid-sagittal and basic planes. The brow opening of the helmet shall be at least 1 inch (2.5 cm) above all points in the basic plane that are within the angles of peripheral vision (see Figure 3).

6. Section S5.5 would be revised to read as follows:

S5.5 *Projections*. A helmet shall not have any rigid projections inside its shell. Rigid projections outside any helmet's shell shall be limited to those required for operation of essential accessories, and shall not protrude more than 0.20 inch (5 mm).

7. Section 5.6.1 introductory text and paragraph (5) would be revised to read as follows:

S5.6.1 Each helmet shall be permanently and legibly labeled, in a manner such that the label(s) can be easily read without removing padding or any other permanent part, with the following:

* * * * *

(5) The symbol DOT, constituting the manufacturer's certification that the helmet conforms to the applicable Federal Motor Vehicle Safety Standards. This symbol shall appear on the outer surface, in a color that contrasts with the background, in letters at least ⅜ inch (1 cm) high, centered laterally with the horizontal centerline of the symbol located a minimum of 1⅛ inches (2.9 cm) and a maximum of 1⅜ inches (3.5 cm) from the bottom edge of the posterior portion of the helmet.

8. Section S6 would be revised to read as follows:

S6. *Preliminary test procedures*. Before subjecting a helmet to the testing sequence specified in S7, prepare it according to the following procedures.

S6.1 *Selection of appropriate headform*.

S6.1.1 A helmet with a manufacturer's designated discrete size or size range of 6⅝ (European size: 53) or smaller is tested on the small headform. A helmet with a manufacturer's designated discrete size or size range from 6¾ (European size: 54) to 7½ (European size: 60) is tested on the medium headform. A helmet with a manufacturer's designated discrete size or size range of 7⅝ (European size: 61) or larger is tested on the large headform.

S6.1.2 A helmet with a manufacturer's designated size range which includes two or all three size ranges described in S6.1.1 is tested on each headform covered by the size range.

S6.2 *Reference marking*.

S6.2.1 Use a reference headform that is firmly seated with the basic and reference planes horizontal. Place the complete helmet to be tested on the appropriate reference headform, as specified in S6.1.1 and S6.1.2.

S6.2.2 Apply a 10-pound (4.5 kg) static load normal to the helmet's apex. Center the helmet laterally and seat it firmly on the reference headform according to its helmet positioning index.

S6.2.3 Maintaining the load and position described in S6.2.2, draw a line (hereinafter referred to as "test line") on the outer surface of the helmet coinciding with portions of the intersection of that surface with the following planes, as shown in Figure 2:

(a) A plane 1 inch (2.5 cm) above and parallel to the reference plane in the anterior portion of the reference headform;

(b) A vertical transverse plane 2.5 inches (6.4 cm) behind the point on the anterior surface of the reference headform at the intersection of the midsagittal and reference planes;

(c) The reference plane of the reference headform;

(d) A vertical transverse plane 2.5 inches (6.4 cm) behind the center of the external ear opening in a side view; and

(e) A plane 1 inch (2.5 cm) below and parallel to the reference plane in the posterior portion of the reference headform.

S6.3 *Helmet positioning.*

S6.3.1 Prior to each test, fix the helmet on a test headform in the position that conforms to its helmet positioning index. Secure the helmet so that it does not shift position prior to impact or to application of force during testing.

S6.3.2 In testing as specified in S7.1 and S7.2, place the retention system in a position such that it does not interfere with free fall, impact, or penetration.

S.6.4 *Conditioning.* Immediately prior to conducting the testing sequence specified in S7, condition each test helmet in accordance with any one of the following procedures:

(a) Ambient conditions. Expose to a temperature of 70° F (21° C) and a relative humidity of 50% for 12 hours.

(b) Low temperature.. Expose to a temperature of 14° F (−10° C) for 12 hours.

(c) High temperature. Expose to a temperature of 122° F (50° C) for 12 hours.

(d) Water immersion. Immerse in water at a temperature of 77° F (25° C) for 12 hours.

If during testing, as specified in S7.1.3 and S7.2.3, a helmet is returned to the conditioning environment before the time out of that environment exceeds 4 minutes, the helmet is kept in the environment for a minimum of 3 minutes prior to resumption of testing with that helmet. If the time out of the environment exceeds 4 minutes, the helmet is returned to the environment for a minimum of 3 minutes for each minute or portion of a minute that the helmet remained out of the environment in excess of 4 minutes or for a maximum of 12 hours, whichever is less, prior to the resumption of testing with that helmet.

9. Section 7 is revised to read as follows:

S7. *Test conditions.*

S7.1 *Impact attenuation test.*

S7.1.1 Impact attenuation is measured by determining acceleration imparted to an instrumented test headform on which a complete helmet is mounted as specified in S6.3, when it is dropped in guided free fall upon fixed hemispherical and flat steel anvils.

S7.1.2 Each helmet is impacted at four sites with two successive identical impacts at each site. Two of these sites are impacted upon a flat steel anvil and two upon a hemispherical steel anvil as specified in S7.1.10 and S7.1.11. The impact sites are at any point on the area above the test line described in S6.2.3, and separated by a distance not less than one-sixth of the maximum circumference of the helmet in the test area.

S7.1.3 Impact testing at each of the four sites, as specified in S7.1.2, shall start at two minutes, and be completed by four minutes, after removal of the helmet from the conditioning environment.

S7.1.4 The guided free fall drop heights for the helmet and test headform combination onto the hemispherical anvil and flat anvil shall be such that the minimum impact speeds are 17.1 feet/second (5.2m/sec) and 19.7 ft./sec (6.0 m/sec), respectively. The minimum drop heights are 54.5 inches (138.4 cm) and 72 inches (182.9 cm), respectively. The drop heights are adjusted upward from the minimum to the extent necessary to compensate for friction losses.

S7.1.5 Test headforms for impact attenuation testing are constructed of magnesium alloy (K–1A), and exhibit no resonant frequencies below 2,000 Hz.

S7.1.6 The monorail system is to be used for impact attenuation testing.

S7.1.7 The weight of the drop assembly, as specified in Table I, is the combined weight of the instrumented test headform and supporting assembly for the drop test. The weight of the supporting assembly is not less than 2.0 lbs. and not more than 2.4 lbs. (0.9 to 1.1 kg). The supporting assembly weight for the monorail system is the drop assembly weight minus the combined weight of the test headform, the headform's clamp down ring, and its tie down screws.

S7.1.8 The center of gravity of the test headform is located at the center of the mounting ball on the supporting assembly and lies within a cone with its axis vertical and forming a 10° included angle with the vertex at the point of impact. The center of gravity of the drop assembly lies within the rectangular volume bounded by $x=0.25$ inch (0.64 cm), $x=0.85$ inch (-2.16 cm), $y=0.25$ inch (0.64 cm), and $y=-0.25$ inch (-0.64 cm) with the origin located at the center of gravity of the test headform. The x-y-z axes are mutally perpendicular and have positive or negative designations in accordance with the right-hand rule. The x-axis is horizontal with its negative direction passing through the vertical centerline of the monorail from the origin. The y-axis is also horizontal and the positive z-axis is upward.

S7.1.9 The acceleration transducer is mounted at the center of gravity of the test headform with the sensitive axis aligned to within 5° of vertical when the test headform is in the impact position. The acceleration data channel complies with SAE Recommended Practice J211 requirements for channel class 1,000.

S7.1.10 The flat anvil is constructed of steel with a 5-inch (12.7 cm) minimum diameter impact face, and the hemispherical anvil is constructed of steel with a 1.9-inch (4.8 cm) radius impact face.

S7.1.11 The rigid mount for both of the anvils consists of a solid mass of a least 300 pounds (136.1 kg), the outer surface of which consists of a steel plate with minimum thickness of 1 inch (2.5 cm) and minimum surface area of 1 ft.^{2} (929 cm ^{2}).

S7.1.12 The drop system shall restrict side movement during the impact attenuation test so that the sum of the areas bounded by the acceleration-time response curves for both the x-and y-axes (horizontal axes) is less than five percent of the area bounded by the acceleration-time response curve for the vertical axis.

S7.2 *Penetration test.*

S7.2.1 The penetration test is conducted by dropping the penetration test striker in guided free fall, with its axis aligned vertically, onto the outer surface of the complete helmet, when mounted as specified in S6.3 at any point above the test line, described in S6.2.3, except on a fastener or other rigid projection.

S7.2.2 Two penetration blows are applied at least 3 inches (7.6 cm) apart, and at least 3 inches (7.6 cm) from the centers of any impacts applied during the impact attenuation test.

S7.2.3 The application of the two penetration blows, specified in S7.2.2, shall start at two minutes and be completed by four minutes, after removal of the helmet from the conditioning environment.

S7.2.4 The height of the guided free fall is 118.1 inches (3 m), as measured from the striker point to the impact point on the outer surface of the test helmet.

S7.2.5 The contactable surface of the penetration test headforms are constructed of a metal or metallic alloy having a Brinell hardness number no greater than 55, which will readily permit detection should contact by the striker occur. The surface is refinished if necessary prior to each penetration test blow to permit detection of contact by the striker.

S7.2.6 The weight of the penetration striker is 6 pounds, 10 ounces (3 kg).

S7.2.7 The point of the striker has an included angle of 60°, a cone height of 1.5 inches (3.8 cm), a tip radius of 0.02 inch (standard 0.5 millimeter radius) and a minimum hardness of 60 Rockwell, C-scale.

S7.2.8 The rigid mount for the penetration test headform is as described in S7.1.11.

S7.3 *Retention system test.*

S7.3.1 The retention system test is conducted by applying a static tensile load to the retention assembly of a complete helmet, which is mounted, as described in S6.3, on a stationary test headform as shown in Figure 4, and by measuring the movement of the adjustable portion of the retention system test device under tension.

S7.3.2 The retention system test device consists of both an adjustable loading mechanism by which a static tensile load is applied to the helmet retention assembly and a means for holding the test headform and helmet stationary. The retention assembly is fastened around two freely moving rollers, both of which have a 0.5 inch (1.3 cm) diameter and a 3-inch (7.6 cm) center-to-center separation, and which are mounted on the adjustable portion of the tensile loading device (Figure 4). The helmet is fixed on the test headform as necessary to ensure that it does not move during the application of the test loads to the retention assembly.

S7.3.3 A 50-pound (22.7 kg) preliminary test load is applied to the retention assembly, normal to the basic plane of the test headform and symmetrical with respect to the center of the retention assembly for 30 seconds, and the maximum distance from the extremity of the adjustable portion of the retention system test device to the apex of the helmet is measured.

S7.3.4 An additional 250-pound (113.4 kg) test load is applied to the retention assembly, in the same manner and at the same location as described in S7.3.3, for 120 seconds, and the maximum distance from the extremity of the adjustable portion of the retention system test device to the apex of the helmet is measured.

* * * * *

10. Figure 2 is revised as follows:

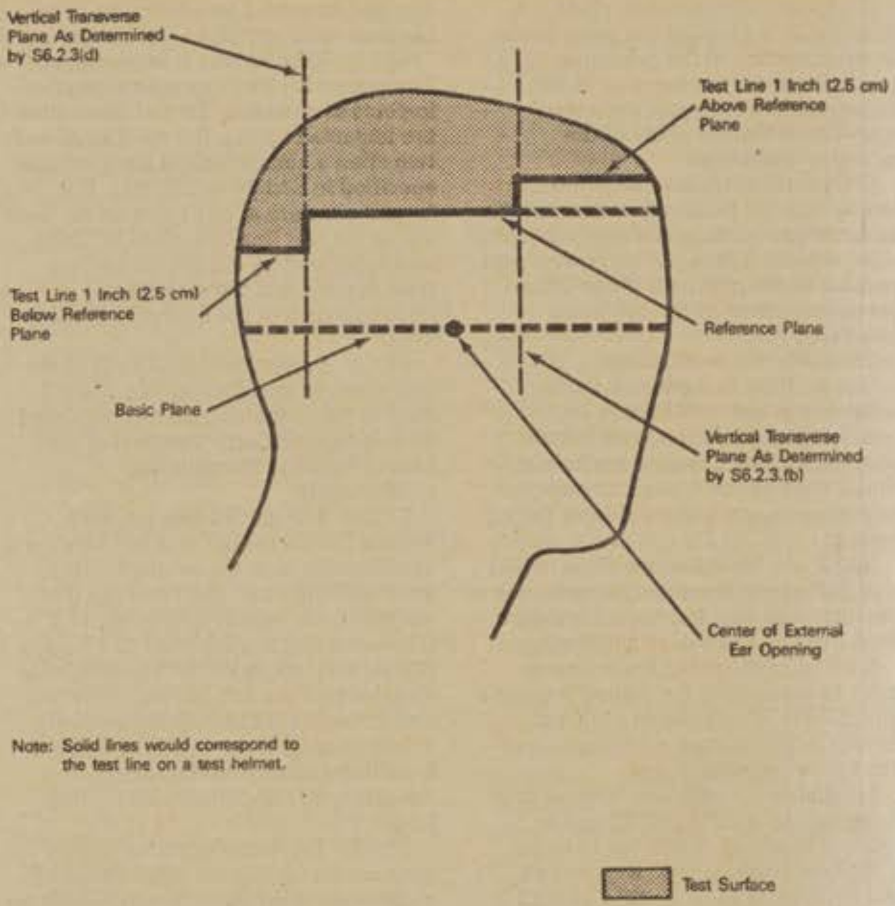


FIGURE 2

11. Table I is revised to read as follows:

TABLE I.—WEIGHTS FOR IMPACT ATTENUATION TEST DROP ASSEMBLY

Test headform size	Weight (lbs)[1]
Small	7.8 (3.5 kg)
Medium	11.0 (5.0 kg)
Large	13.4 (6.1 kg)

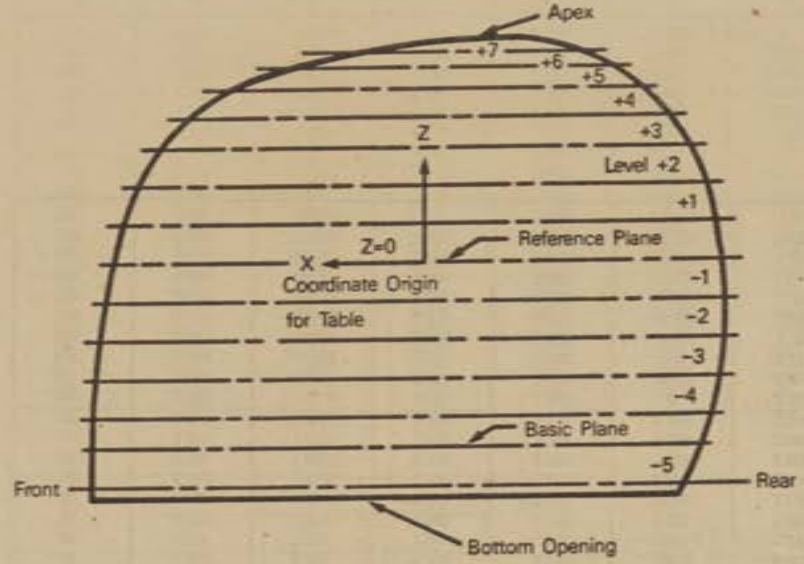
[1] Combined weight of instrumented test headform and supporting assembly for drop test.

12. The Appendix is revised as follows:

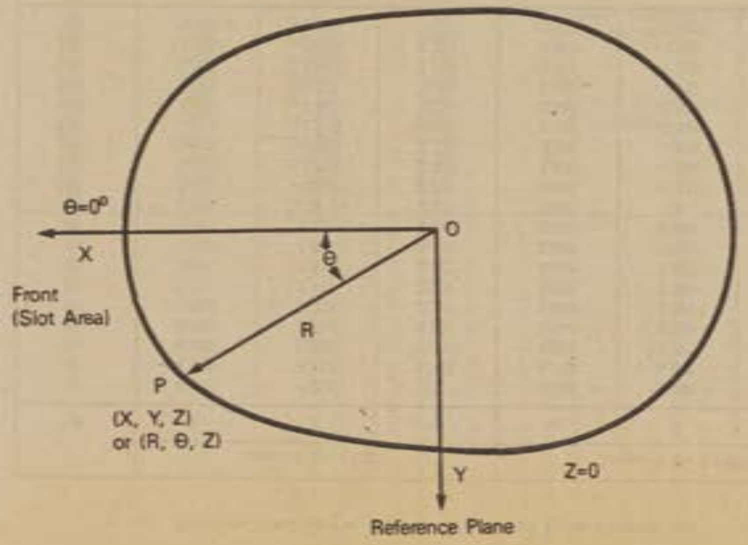
BILLING CODE 4910-59-M

APPENDIX
HEADFORM SECTIONS

Mid-Sagittal Plane (Symmetrical Plane)



Headform Coordinate Systems



Medium Headform — Exterior Dimension Table

	Bottom Opening Z= -3.02			Level—5 Z= -2.900		
θ	R	X	Y	R	X	Y
0	4.292	4.292	0	4.293	4.293	0
10	4.266	4.201	0.741	4.270	4.205	0.742
20	4.159	3.908	1.423	4.172	3.920	1.427
30	3.967	3.436	1.984	3.961	3.430	1.981
40	3.660	2.804	2.353	3.670	2.811	2.359
50	3.332	2.142	2.553	3.352	2.155	2.568
60	3.039	1.520	2.632	3.067	1.534	2.656
70	2.839	0.971	2.668	2.869	0.981	2.696
80	2.720	0.472	2.679	2.772	0.481	2.730
90	2.675	0	2.675	2.709	0	2.709
100	2.703	-0.469	2.662	2.724	-0.473	2.683
110	2.764	-0.945	2.597	2.794	-0.956	2.626
120	2.888	-1.444	2.501	2.917	-1.459	2.526
130	2.985	-1.919	2.287	3.040	-1.954	2.329
140	3.100	-2.375	1.993	3.175	-2.432	2.041
150	3.175	-2.750	1.588	3.232	-2.799	1.616
160	3.186	-2.994	1.090	3.246	-3.050	1.110
170	3.177	-3.129	0.552	3.237	-3.188	0.562
180	3.187	-3.187	0	3.246	-3.246	0

	Basic Plane Z= -2.360			Level—4 Z= -2.000		
θ	R	X	Y	R	X	Y
0	4.272	4.272	0	4.247	4.247	0
10	4.248	4.184	0.738	4.223	4.159	0.733
20	4.147	3.897	1.418	4.120	3.872	1.409
30	3.961	3.430	1.981	3.940	3.412	1.970
40	3.687	2.824	2.370	3.683	2.821	2.367
50	3.384	2.175	2.592	3.392	2.180	2.598
60	3.111	1.556	2.694	3.132	1.566	2.712
70	2.927	1.001	2.751	2.960	1.012	2.782
80	2.815	0.489	2.772	2.860	0.497	2.817
90	2.779	0	2.779	2.838	0	2.838
100	2.802	-0.487	2.759	2.861	-0.497	2.818
110	2.887	-0.987	2.713	2.958	-1.012	2.780
120	3.019	-1.510	2.615	3.098	-1.549	2.683
130	3.180	-2.044	2.436	3.260	-2.096	2.497
140	3.306	-2.533	2.125	3.405	-2.608	2.189
150	3.398	-2.943	1.699	3.516	-3.045	1.758
160	3.458	-3.250	1.183	3.585	-3.369	1.226
170	3.475	-3.422	0.603	3.612	-3.557	0.627
180	3.472	-3.472	0	3.609	-3.609	0

Medium Headform — Exterior Dimension Table (Continued)

θ	Level–3 Z= –1.500			Level–2 Z= –1.000		
	R	X	Y	R	X	Y
0	4.208	4.208	0	4.148	4.148	0
10	4.179	4.116	0.726	4.112	4.050	0.714
20	4.075	3.829	1.394	4.013	3.771	1.373
30	3.902	3.379	1.951	3.844	3.329	1.922
40	3.654	2.799	2.349	3.609	2.765	2.320
50	3.377	2.171	2.587	3.352	2.155	2.568
60	3.094	1.547	2.680	3.137	1.569	2.717
70	2.982	1.020	2.802	2.989	1.022	2.809
80	2.891	0.502	2.847	2.902	0.504	2.858
90	2.876	0	2.876	2.884	0	2.884
100	2.918	–0.507	2.874	2.943	–0.511	2.898
110	3.021	–1.033	2.839	3.052	–1.044	2.868
120	3.170	–1.585	2.745	3.225	–1.613	2.793
130	3.337	–2.145	2.556	3.397	–2.184	2.602
140	3.483	–2.668	2.239	3.536	–2.708	2.273
150	3.604	–3.121	1.802	3.657	–3.167	1.829
160	3.682	–3.460	1.259	3.751	–3.525	1.283
170	3.725	–3.668	0.647	3.807	–3.749	0.661
180	3.741	–3.741	0	3.822	–3.822	0

θ	Level–1 Z= –0.500			Reference Plane Z=0.0		
	R	X	Y	R	X	Y
0	4.067	4.067	0	3.971	3.971	0
10	4.033	3.972	0.700	3.935	3.875	0.683
20	3.944	3.706	1.349	3.853	3.621	1.318
30	3.777	3.271	1.889	3.701	3.205	1.851
40	3.552	2.721	2.283	3.491	2.674	2.244
50	3.323	2.136	2.546	3.279	2.108	2.512
60	3.125	1.563	2.707	3.101	1.551	2.686
70	2.987	1.022	2.807	2.979	1.019	2.799
80	2.912	0.506	2.868	2.910	0.505	2.866
90	2.893	0	2.893	2.890	0	2.890
100	2.895	–0.503	2.851	2.945	–0.511	2.900
110	3.064	–1.048	2.879	3.062	–1.047	2.877
120	3.231	–1.616	2.798	3.228	–1.614	2.796
130	3.411	–2.193	2.613	3.413	–2.194	2.615
140	3.560	–2.727	2.288	3.563	–2.729	2.290
150	3.682	–3.189	1.841	3.681	–3.188	1.841
160	3.783	–3.555	1.294	3.773	–3.546	1.290
170	3.865	–3.826	0.675	3.832	–3.774	0.665
180	3.857	–3.857	0	3.844	–3.844	0

Medium Headform — Exterior Dimension Table (Continued)

θ	Level+1 Z=0.500			Level +2 Z=1.000		
	R	X	Y	R	X	Y
0	3.830	3.830	0	3.665	3.665	0
10	3.801	3.743	0.660	3.613	3.558	0.627
20	3.725	3.500	1.274	3.554	3.340	1.216
30	3.587	3.106	1.794	3.436	2.976	1.718
40	3.399	2.604	2.185	3.271	2.506	2.103
50	3.206	2.060	2.456	3.102	1.994	2.376
60	3.044	1.522	2.636	2.969	1.480	2.563
70	2.927	1.001	2.751	2.854	0.976	2.682
80	2.861	0.497	2.818	2.792	0.485	2.750
90	2.855	0	2.855	2.783	0	2.783
100	2.897	–0.503	2.853	2.832	–0.492	2.789
110	3.007	–1.029	2.826	2.938	–1.005	2.761
120	3.176	–1.588	2.751	3.102	–1.551	2.686
130	3.372	–2.168	2.583	3.294	–2.117	2.523
140	3.520	–2.697	2.263	3.450	–2.643	2.218
150	3.643	–3.155	1.822	3.564	–3.087	1.782
160	3.728	–3.503	1.275	3.637	–3.418	1.244
170	3.777	–3.720	0.656	3.675	–3.619	0.638
180	3.782	–3.782	0	3.670	–3.670	0

θ	Level +3 Z=1.450			Level +4 Z=1.860		
	R	X	Y	R	X	Y
0	3.419	3.419	0	3.061	3.061	0
10	3.382	3.331	0.587	3.035	2.989	0.527
20	3.299	3.100	1.128	2.966	2.787	1.014
30	3.197	2.769	1.599	2.872	2.487	1.436
40	3.052	2.338	1.962	2.754	2.110	1.770
50	2.911	1.871	2.230	2.642	1.698	2.024
60	2.786	1.393	2.413	2.522	1.261	2.184
70	2.700	0.924	2.537	2.477	0.847	2.328
80	2.647	0.460	2.607	2.442	0.424	2.405
90	2.636	0	2.636	2.442	0	2.442
100	2.691	–0.467	2.650	2.492	–0.473	2.454
110	2.796	–0.956	2.627	2.599	–0.899	2.442
120	2.961	–1.481	2.564	2.758	–1.379	2.389
130	3.147	–2.023	2.411	2.936	–1.887	2.249
140	3.301	–2.529	2.122	3.081	–2.361	1.980
150	3.408	–2.951	1.704	3.176	–2.751	1.588
160	3.479	–3.269	1.190	3.230	–3.035	1.105
170	3.514	–3.461	0.610	3.270	–3.220	0.568
180	3.502	–3.502	0	3.271	–3.271	0

Medium Headform — Exterior Dimension Table (Continued)

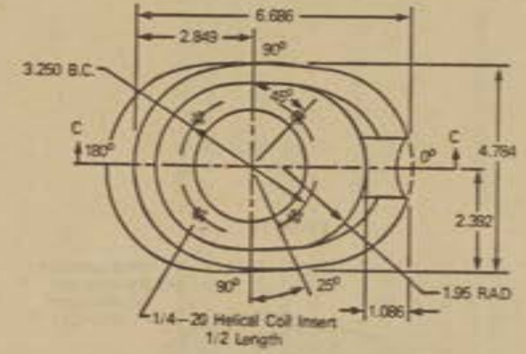
	Level +5 Z=2.250			Level +6 Z=2.560		
θ	R	X	Y	R	X	Y
0	2.526	2.526	0	1.798	1.798	0
10	2.521	2.483	0.483	1.798	1.771	0.312
20	2.464	2.315	0.843	1.757	1.651	0.601
30	2.387	2.067	1.194	1.719	1.489	0.860
40	2.305	1.766	1.482	1.678	1.285	1.079
50	2.232	1.435	1.710	1.652	1.062	1.266
60	2.174	1.087	1.883	1.641	0.821	1.421
70	2.144	0.733	2.015	1.646	0.563	1.546
80	2.132	0.370	2.100	1.673	0.291	1.648
90	2.147	0	2.147	1.712	0	1.712
100	2.213	-0.384	2.179	1.809	-0.314	1.782
110	2.316	-0.792	2.176	1.925	-0.658	1.809
120	2.463	-1.232	2.133	2.066	-1.033	1.789
130	2.624	-1.687	2.010	2.213	-1.423	1.695
140	2.763	-2.117	1.776	2.356	-1.806	1.516
150	2.863	-2.479	1.432	2.469	-2.138	1.235
160	2.919	-2.743	0.988	2.536	-2.383	0.867
170	2.954	-2.909	0.513	2.561	-2.522	0.445
180	2.958	-2.958	0	2.556	-2.556	0

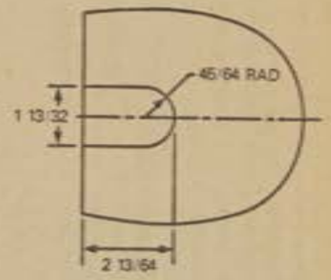
	Level +7 Z=2.750		
θ	R	X	Y
0	1.081	1.081	0
10	1.088	1.072	0.189
20	1.055	0.991	0.361
30	1.039	0.900	0.520
40	1.039	0.796	0.668
50	1.052	0.676	0.806
60	1.068	0.534	0.925
70	1.106	0.378	1.039
80	1.171	0.203	1.153
90	1.242	0	1.242
100	1.422	-0.247	1.400
110	1.489	-0.509	1.399
120	1.683	-0.842	1.458
130	1.801	-1.158	1.380
140	1.954	-1.497	1.256
150	2.083	-1.804	1.042
160	2.138	-2.009	0.731
170	2.175	-2.142	0.378
180	2.175	-2.175	0

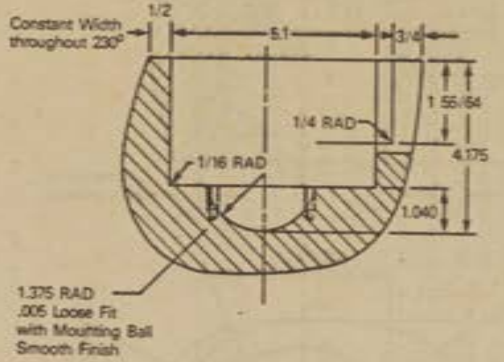
Notes:

1. Apex is located at (-0.75, 0, 3.02) for (X, Y, Z) or (0.75, 180, 3.02) for (R, θ, Z)
2. Scale all dimensions by 0.8941 for small headform.
3. Scale all dimensions by 1.069 for large headform.
4. Headform is symmetrical about the mid-sagital plane.
5. Units:
   R, X, Y, Z — inches.
   θ — degrees.

Small Headform — Interior Design

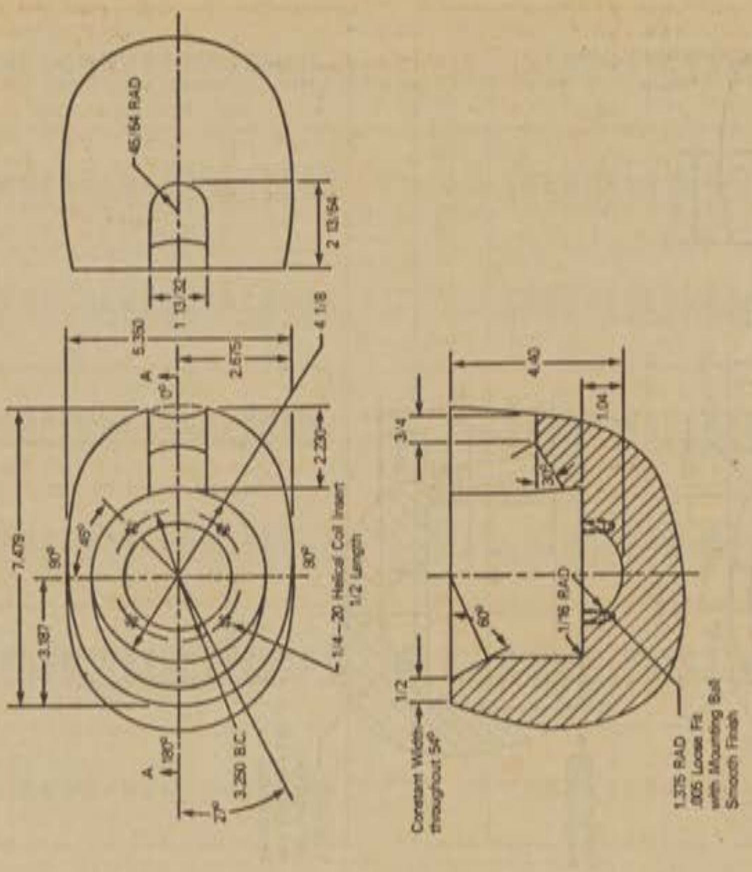




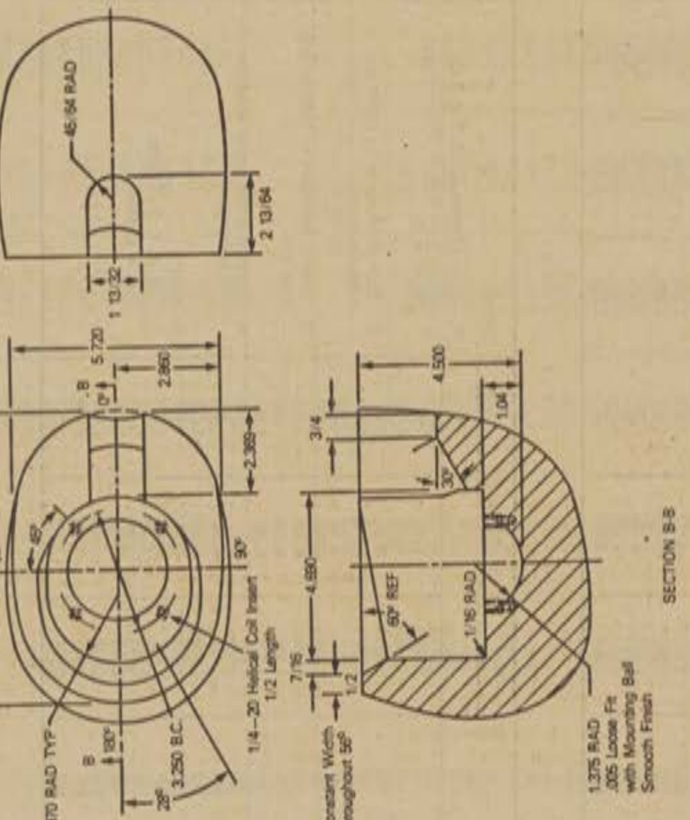


Section C-C

Large Headform — Interior Design



Medium Headform — Interior Design



BILLING CODE 4910-59-C

Issued: September 23, 1985.

Barry Felrice,

Associate Administrator for Rulemaking.

[FR Doc. 85-23082 Filed 9-26-85; 8:45 am]

BILLING CODE 4910-59-M

DEPARTMENT OF COMMERCE

National Oceanic and Atmospheric Administration

50 CFR Part 642

King Mackerel and Spanish Mackerel; Public Hearings

AGENCY: National Marine Fisheries Service (NMFS), NOAA, Commerce.

ACTION: Notice of public hearings and request for comments.

SUMMARY: The Gulf of Mexico Fishery Management Council will convene Mackerel Public Hearings to review reduced bag limits for recreational fishermen and reduced harvest quotas for commercial fishermen, and other measures to manage the stock of king mackerel. This reduction could be of sufficient magnitude to close the fishery. An increase in the size limit for Spanish mackerel will also be discussed.

DATES: Written comments should be received by October 30, 1985. All hearings will be convened at 7:00 p.m., and will adjourn at approximately 10:00 p.m. The hearings are scheduled as follows:

1. October 7, 1985, Largo, Florida
2. October 8, 1985, Corpus Christi, Texas
3. October 9, 1985, Houma, Louisiana
4. October 10, 1985, Pensacola, Florida.

ADDRESSES: Written comments should be sent to Wayne Swingle, Executive Director, Gulf of Mexico Fishery Management Council, Lincoln Center, Suite 881, 5401 West Kennedy Boulevard, Tampa, Florida 33609; or Jack Brawner, Director, Southeast Region, National Marine Fisheries Service, Duval Building, 9450 Koger Boulevard, St. Petersburg, Florida 33702. The hearings will be held at the following locations:

1. October 7, 1985—Pinellas County Cooperative Extension Service Auditorium, 12175 125th Street, North, Largo, Florida
2. October 8, 1985—Texas A&M Research and Extension Center, Highway 44, Corpus Christi, Texas
3. October 9, 1985—East Park Recreation Center, 2007 East Park Street, Houma, Louisiana
4. October 10, 1985—Pensacola Junior College Fine Arts Auditorium, 1000 College Boulevard, Pensacola, Florida.

FOR FURTHER INFORMATION CONTACT: Wayne Swingle, Executive Director, Gulf of Mexico Fishery Management Council, 813-228-2815.

Dated: September 24, 1985.

Richard B. Roe,

Director, Office of Protected Species and Habitat Conservation, National Marine Fisheries Service.

[FR Doc. 85-23192 Filed 9-26-85; 8:45 am]

BILLING CODE 3510-22-M

Notices

Federal Register
Vol. 50, No. 188
Friday, September 27, 1985

This section of the FEDERAL REGISTER contains documents other than rules or proposed rules that are applicable to the public. Notices of hearings and investigations, committee meetings, agency decisions and rulings, delegations of authority, filing of petitions and applications and agency statements of organization and functions are examples of documents appearing in this section.

DEPARTMENT OF AGRICULTURE

Forest Service

Leola-Sullivan Timber Sale, Colville National Forest, Pend Oreille County, WA; Intent To Prepare an Environmental Impact Statement

The U.S. Department of Agriculture, Forest Service, will prepare an environmental impact statement for the development of the Leola-Sullivan timber sale on the Sullivan Lake Ranger District. The environmental impact statement will be prepared in accordance with existing land and resource management plans.

A range of alternatives for timber harvest in the assessment area will be considered. One of the alternatives will be no action. Other alternatives will consider various levels of timber harvest and road construction.

Federal, State and local agencies, potential purchasers, and other individuals or organizations who may be interested in or affected by the decision will be invited to participate in the scoping process, which will begin in October, 1985. This process will include:

1. Identification of the issues to be addressed.
2. Identification of issues to be analyzed in depth.
3. Elimination of insignificant issues, issues covered by previous environmental analysis, and issues not within the scope of this decision.
4. Determination of potential cooperating agencies and assignment of responsibilities.

The Fish and Wildlife Service, Department of Interior, will be invited to participate as a coordinating agency to evaluate potential impacts on threatened and endangered species habitat occurring as a result of this action.

The analysis is expected to take about five months. The draft environmental impact statement should be available for public review by August, 1986. The final environmental impact statement should be issued by December, 1986.

William D. Shenk, Forest Supervisor of the Colville National Forest, is the responsible official.

Written comments and suggestions concerning the analysis should be sent to William D. Shenk, Forest Supervisor, Federal Building, 695 South Main, Colville, Washington 99114, or to Warren N. Current, District Ranger, Sullivan Lake Ranger District, Metaline Falls, Washington 99153.

Questions about the proposed action and environmental impact statement should be directed to Constance J. Mehmel, Sale Planner, Sullivan Lake Ranger District, Metaline Falls, Washington 99153 (telephone: (509) 446-2681).

Dated: September 18, 1985.

William D. Shenk,

Forest Supervisor.

[FR Doc. 85-23097 Filed 9-26-85; 8:45 am]

BILLING CODE 3410-11-M

Threemile Timber Sale, Colville National Forest, Pend Oreille County, WA; Intent To Prepare an Environmental Impact Statement

The U.S. Department of Agriculture, Forest Service, will prepare an environmental impact statement for the development of the Threemile timber sale on the Sullivan Lake Ranger District. The environmental impact statement will be prepared in accordance with existing land and resource management plans.

A range of alternatives for timber harvest in the assessment area will be considered. One of the alternatives will be no action. Other alternatives will consider various levels of timber harvest and road construction.

Federal, State and local agencies, potential purchasers, and other individuals or organizations who may be interested in or affected by the decision will be invited to participate in the scoping process. This process will include:

1. Identification of the issues to be addressed.
2. Identification of issues to be analyzed in depth.
3. Elimination of insignificant issues, issues covered by previous environmental analysis, and issues not within the scope of this decision.
4. Determination of potential cooperating agencies and assignment of responsibilities.

The Fish and Wildlife Service, Department of Interior, will be invited to participate as a coordinating agency to evaluate potential impacts on threatened and endangered species habitat occurring as a result of this action.

Scoping for this project will begin in October, 1985. A public meeting will be scheduled in February, 1986, to finalize issues, concerns and opportunities and to discuss development of alternatives.

The analysis is expected to take about five months. The draft environmental impact statement should be available for public review by September, 1986. The final environmental impact statement should be available by January, 1987.

William D. Shenk, Forest Supervisor of the Colville National Forest, is the responsible official.

Written comments and suggestions concerning the analysis should be sent to William D. Shenk, Forest Supervisor, Federal Building, 695 South Main, Colville, Washington 99114, or to Warren N. Current, District Ranger, Sullivan Lake Ranger District, Metaline Falls, Washington 99153.

Questions about the proposed action and environmental impact statement should be directed to Constance J. Mehmel, Sale Planner, Sullivan Lake Ranger District, Metaline Falls, Washington 99153 (telephone: (509) 446-2681).

Dated: September 18, 1985.

William D. Shenk,

Forest Supervisor.

[FR Doc. 85-23096 Filed 9-26-85; 8:45 am]

BILLING CODE 3410-11-M

Rural Electrification Administration

Tri-State Generation and Transmission Association, Inc.; Information Supplement

AGENCY: Rural Electrification Administration, USDA.

ACTION: Availability of information supplement.

SUMMARY: Notice is hereby given that the Rural Electrification Administration

(REA), pursuant to the National Environmental Policy Act of 1969, as amended, the Council on Environmental Quality Regulations (40 CFR Parts 1500–1508), and REA Environmental Policies and Procedures (7 CFR Part 1794), has prepared an Information Supplement (IS) in connection with the proposed participation and project changes for the Hayden-Blue River 345 kV transmission line project. The proposed facility will be located in Grand, Routt and Summit Counties, Colorado.

FOR FURTHER INFORMATION CONTACT: Mr. Martin G. Seipel, Director, Western Area-Electric, Rural Electrification Administration, Room Number 0207, 14th and Independence Avenue SW., Washington, DC 20250, telephone number (202) 382–8848.

SUPPLEMENTARY INFORMATION: Tri-State Generation and Transmission Association, Inc. (Tri-State), submitted a proposed amended agreement and an environmental document relating to the proposed participation and project changes in the Hayden-Blue River 345 kV transmission line project. The proposed amended agreement will provide different ownership terms, cost, and capacity sharing, and divide construction management responsibilities for the project participants. The proposed agreement provides for Tri-State to be project manager for the northern portion of the line from Hayden to the existing Gore Pass Substation, and provides for Western Area Power Administration (Western) to be project manager for the southern portion of the line from Gore Pass Substation to the proposed Blue River Substation. Under the previous agreement, Tri-State was to be the sole project manager and would have constructed the entire line from Hayden Substation to the proposed Blue River Substation. Under the proposed participation changes, the project participants are sharing the cost as follows: Tri-State, 34 percent; Colorado-Ute Electric Association, Inc. (Colorado-Ute), 22 percent; Platte River Power Authority (Platte River), 22 percent; and Western, 22 percent. The previous capacity participation percentages were: Tri-State, 50 percent; Colorado-Ute, 20 percent; Platte River, 20 percent; and Western, 10 percent. Public Service Company of Colorado (PSC), while not participating in the transmission line construction, will construct the Blue River Substation in Summit County, Colorado.

REA issued a Record of Decision (ROD) for the Hayden-Blue River project on September 30, 1982, following the prepartion of a Draft Environmental Impact Statement (DEIS) and a final Environmental Impact Statement (FEIS) on December 24, 1981, and on July 16, 1982, respectively. REA approved financing assistance for Tri-State's portion of the project on September 30, 1982 and Colorado-Ute's portion on September 23, 1983.

The new participation agreement would allow Tri-State to utilize the existing Gore Pass Substation in the Middle Park area rather than construct a new substation. Tri-State will also construct the first 2.4 km (1.5 miles) of transmission line out of the Hayden Substation using wood-pole H-frame structures insulated at 230 kV instead of steel towers insulated at 345 kV. The use of 230 kV wood-pole H-frame structures is more economical than 345 kV structures because the line would not be uprated to 345 kV operation for at least 15 years; the Hayden Substation cannot presently be enlarged to include 345 kV facilities; and when the line is uprated to 345 kV operation, at least two feasible alternatives call for the removal of the inital section of line from Hayden. The proposed route between Hayden and Gore Pass is the same as the corridor identified in the FEIS and approved in REA's ROD for the Hayden-Blue River project with one exception near the Edna Mine area in Routt County. This exception represents a 0.6 km (0.4 mile) excurion beyond the corridor boundary for a total length of approximately 2.9 km (1.8 miles). Tri-State will utilize the resource information developed in the REA FEIS for routing the transmission line between the Hayden and Gore Pass Substations and will comply with local transmission siting requirements. Western proposes to utilize the resource information developed for REA's FEIS to analyze alternative routings in the Supplemental EIS that it is preparing for the southern portion of the line between the Gore Pass and Blue River Substations.

REA has reviewed the environmental information submitted by Tri-State and has independently evaluated the proposed changes. REA, at its discretion, is issuing this IS to carry out the purpose of the National Environmental Policy Act pursuant to 40 CFR 1502.9(c)(2) and 7 CFR 1794.90(c). The proposed changes will not affect threatened or endangered species, prime farmland, floodplains, wetlands or cultural resources. REA has determined that the environmental consequences of the proposed participation and project changes would not alter the use of the preferred corridors defined in REA's FEIS and approved in REA's ROD; and thus, no significant changes relevant to environmental concerns are expected.

REA considered the following alternatives: (a) Hayden-Blue River project as revised, (b) Hayden-Blue River project as originally proposed, (c) no joint participation in constructing the project, and (d) alternative transformer locations. After reviewing these alternatives, REA determined that the proposed project is revised is an acceptable alternative because it meets Tri-State's, Colorado-Ute's and other participants' needs with minimal adverse impact.

Copies of the IS may be examined at or obtained from the offices of REA in the South Argiculture Building, Room 0207, 14th and Independence Avenue SW., Washington, DC 20250 or at the offices of Tri-State, 12076 Grant Street, Thornton, Colorado 80241, during regular business hours. The IS may also be examined at public libraries in approriate towns in the affected counties. Copies of the IS are being sent to various Federal, State and local agencies and other individuals that have expressed an interest in this project. REA will take no final action with respect to the amended proposal until thirty (30) days after publication of notice in the **Federal Register** and a local newspaper.

This program is listed in the Catalog of Federal Domestic Assistance as 10.850-Rural Electrification Loans and Loan Guarantees.

Dated: September 20, 1985.

Harold V. Hunter,

Administrator.

[FR Doc. 85–23154 Filed 9–26–85; 8:45 am]

BILLING CODE 3410-15-M

COMMISSION ON CIVIL RIGHTS

Connecticut Advisory Committee; Agenda and Notice of Public Meeting

Notice is hereby given, pursuant to the provisions of the Rules and Regulations of the U.S. Commission on Civil Rights, that a meeting of the Connecticut Advisory Committee to the Commission will convene at 3:00 p.m. and adjourn at 5:00 p.m. on October 16, 1985, at the Connecticut Historical Society, 1 Elizabeth Street, Hartford, Connecticut. The purpose of the meeting is to continue program planning for FY '86.

Persons desiring additional information, or planning a presentation to the Committee, should contact Committee Chairperson James Stewart, or Jacob Schlitt, Director of the New England Regional Office, at (617) 223–4671, (TDD 617/223–0344).

The meeting will be conducted pursuant to the provisions of the Rules and Regulations of the Commission.

Dated at Washington, DC, September 24, 1985.

Bert Silver,

Assistant Staff Director for Regional Programs.

[FR Doc. 85–23120 Filed 9–26–85; 8:45 am]

BILLING CODE 6335-01-M

Kansas Advisory Committee; Agenda and Notice of Public Meeting

Notice is hereby given, pursuant to the provisions of the Rules and Regulations of the U.S. Commission on Civil Rights, that a meeting of the Kansas Advisory Committee to the Commission will convene at 10:00 a.m. and adjourn at 1:00 p.m. on October 22, 1985, at the Wichita Royale, 125 N. Market, Wichita, Kansas. The purpose of the meeting is to develop programs and plans for FY '86 and discuss holding community forums on civil rights issues in Western Kansas.

Persons desiring additional information, or planning a presentation to the Committee, should contact Committee Chairperson, Burdett Loomis, or Melvin Jenkins, Director of the Central States Regional Office at (816) 374–5253, (TDD 816/374–5009).

The meeting will be conducted pursuant to the provisions of the Rules and Regulations of the Commission.

Dated at Washington, DC, September 24, 1985.

Bert Silver,

Assistant Staff Director for Regional Programs.

[FR Doc. 85–23121 Filed 9–26–85; 8:45 am]

BILLING CODE 6335-01-M

Maryland Advisory Committee; Agenda and Notice of Public Meeting

Notice is hereby given, pursuant to the provisions of the Rules and Regulations of the U.S. Commission on Civil Rights, that a meeting of the Maryland Advisory Committee to the Commission will convene at 1:30 p.m. and adjourn at 3:30 p.m. on October 21, 1985, at the Omni International Hotel, 101 West Fayette Street, Baltimore, Maryland. The purpose of the meeting will be to review a report of the Committee's community forum on handicap discrimination and develop plans for a possible study of equity issues in special education.

Persons desiring additional information, or planning a presentation to the Committee, should contact Committee Chairperson, Lorretta Johnson, or John Binkley, Director of the Mid-Atlantic Regional Office at (202) 254–6717, (TDD 202/254–5461).

The meeting will be conducted pursuant to the provisions of the rules and regulations of the Commission.

Dated at Washington, DC, September 24, 1985.

Bert Silver,

Assistant Staff Director for Regional Programs.

[FR Doc. 85–23122 Filed 9–26–85; 8:45 am]

BILLING CODE 6335-01-M

Minnesota Advisory Committee; Agenda and Notice of Public Meeting

Notice is hereby given, pursuant to the provisions of the Rules and Regulations of the U.S. Commission on Civil Rights, that a meeting of the Minnesota Advisory Committee to the Commission will convene at 6:00 p.m. and adjourn at 9:00 p.m. on October 21, 1985, at the Hamline University School of Law, 1536 Huwett Avenue, Room 101, St. Paul, Minnesota. The purpose of the meeting will be to discuss committee projects on mental health and affirmative action, as well as to hear from local groups and agencies about other civil rights matters.

Persons desiring additional information, or planning a presentation to the Committee, should contact Committee Chairperson, Talmadge Bartelle, or Clark Roberts, Director of the Midwestern Regional Office at (312) 353–7371, (TDD 312/886–2188).

The meeting will be conducted pursuant to the provisions of the rules and regulations of the Commission.

Dated at Washington, DC, September 24, 1985.

Bert Silver,

Assistant Staff Director for Regional Programs.

[FR Doc. 85–23123 Filed 9–26–85; 8:45 am]

BILLING CODE 6335-01-M

Rhode Island Advisory Committee; Agenda for Notice of Public Meeting

Notice is hereby given, pursuant to the provisions of the Rules and Regulations of the U.S. Commission on Civil Rights, that a meeting of the Rhode Island Advisory Committee to the Commission will convene at 12:00 noon and adjourn at 1:30 p.m. on November 13, 1985, at the Girl Scouts of Rhode Island, 125 Charles Street, Council Room, Providence, Rhode Island. The purpose of the meeting is to discuss the issue of violence against Asians in Providence and continue program planning for FY '86.

Persons desiring additional information, or planning a presentation to the Committee, should contact Committee Chairperson, David Sholes, or Jacob Schlitt, Director of the New England Regional Office at (617) 223–4671, (TDD 617/223–0344).

The meeting will be conducted pursuant to the provisions of the Rules and Regulations of the Commission.

Dated at Washington, DC, September 24, 1985.

Bert Silver,

Assistant Staff Director for Regional Programs.

[FR Doc. 85–23124 Filed 9–26–85; 8:45 am]

BILLING CODE 6335-01-M

DEPARTMENT OF COMMERCE

International Trade Administration

Consolidated Decision on Applications for Duty-Free Entry of Electron Microscopes

This a decision consolidated pursuant to section 6(c) of the Educational, Scientific, and Cultural Materials Importation Act of 1966 (Pub. L. 89–651, 80 Stat. 897; 15 CFR Part 301). Related records can be viewed between 8:30 A.M. and 5:00 P.M. in Room 1523, U.S. Department of Commerce, 14th and Constitution Avenue, N.W., Washington, DC.

Docket No.: 85–179. Applicant: George Washington University Medical Center, Washington, DC 20037. Instrument: Electron Microscope, Model JEM–1200EX with Accessories. Manufacturer: JEOL, Ltd., Japan. Intended use: See notice at 50 FR 24552. Instrument ordered: February 25, 1985.

Docket No.: 85–180. Applicant: University of Southern California, Los Angeles, CA 90089–1141. Instrument: Electron Microscope, Model JEM–1200EX with Accessories. Manufacturer: JEOL, Ltd., Japan. Intended use: See notice at 50 FR 24552. Instrument ordered: February 5, 1985.

Docket No.: 85–184. Applicant: Brandeis University, Waltham, MA 02254. Instrument: Electron Microscope, Model EM 420 with Accessories. Manufacturer: N.V. Philips, The Netherlands. Intended use: See notice at 50 FR 26395. Instrument ordered: March 28, 1985.

Docket No.: 85–185. Applicant: University of Michigan, Ann Arbor, MI 48109–2029. Instrument: Electron Microscope, Model EM 10CA with Accessories. Manufacturer: Carl Zeiss, Inc., West Germany. Intended use: See notice at 50 FR 24553. Instrument ordered: March 27, 1985.

Docket No.: 85-198. Applicant: University of Pittsburgh, Pittsburgh, PA 15261. Instrument: Electron Microscope, Model JEM-2000FX with Accessories. Manufacturer: JEOL, Japan. Intended use: See notice at 50 FR 26394. Instrument ordered: September 21, 1984.

Docket No.: 85-201. Applicant: The Pennsylvania State University (MSHMC), Hershey, PA 17033. Instrument: Electron Microscope, Model EM 10-CAS with Accessories. Manufacturer: Carl Zeiss, Inc., West Germany. Intended use: See notice at 50 FR 26394. Instrument ordered: March 22, 1985.

Docket No.: 85-202. Applicant: Columbia University (College of Physicians & Surgeons), New York, NY 10032, Instrument: Electron Microscope, Model EM 10CA and Accessories. Manufacturer: Carl Zeiss, West Germany. Intended use: See notice at 50 FR 26394. Instrument ordered: April 18, 1985.

Docket No.: 85-203. Applicant: The Jackson Laboratory, Bar Harbor, ME 04609. Instrument: Electron Microscope, Model JEM-100CXII and Accessories. Manufacturer: JEOL, Co., Ltd., Japan. Intended use: See notice at 50 FR 26394. Instrument ordered: February 8, 1985.

Docket No.: 85-214. Applicant: University of Pittsburgh, Pittsburgh, PA 15261. Instrument: Electron Microscope, Model JEM-200CX with Accessories. Manufacturer: JEOL, Japan. Intended use: See notice at 50 FR 28000. Application received by Commissioner of Customs: June 3, 1985.

Docket No.: 85-221. Applicant: Boston University School of Medicine, Boston, MS 02118. Instrument: Electron Microscope, Model JEM-100CXII. Manufacturer: JEOL, Ltd., Japan. Intended use: See notice at 50 FR 28001. Instrument ordered: October 24, 1984.

Docket No.: 85-224. Applicant: Oakland University, Rochester, MI 48063. Instrument: Electron Microscope, Model EM 410LS with Accessories. Manufacturer: N.V. Philips, The Netherlands. Intended use: See notice at 50 FR 29243. Instrument ordered: March 25, 1985.

Comments: None received.

Decision: Approved. No instrument of equivalent scientific value to the foreign instrument, for such purposes as these instruments are intended to be used, was being manufactured in the United States at the time the instruments were ordered.

Reasons: Each foreign instrument is a conventional transmission electron microscope (CTEM) and is intended for research or scientific educational uses requiring a CTEM. We know of no CTEM, or of any other instrument suited to these purposes, which was being manufactured in the United States either at the time of order of each instrument or at the time of receipt of application by the U.S. Customs Service.

(Catalog of Federal Domestic Assistance Program No. 11.105, Importation of Duty-Free Educational and Scientific Materials)

Frank W. Creel,

Director, Statutory Import Programs Staff.

[FR Doc. 85-23176 Filed 9-26-85; 8:45 am]

BILLING CODE 3510-DS-M

[Case No. 656]

Josef Kubicek et al.; Order Amending Temporary Denial of Export Privileges

In the Matter of Josef Kubicek, individually and doing business as, Exclusitrade, Inc. and J.O.K., Inc., with locations at 18 La Vista Verde Rancho Palos Verdes, California 90274, and 2001 Artesia Boulevard, Redondo Beach, California 90278, and Lakeside Office Park, No. 4, Wakefield, Massachusetts 01890, William Carlton Dart, individually and doing business as, Display Systems, Inc. and Perpetuum, Inc., with locations at 2000 Martin Avenue, Santa Clara, California 95050, Robert William Haire, Sr., individually and doing business as, Display Systems, Inc., 2000 Martin Avenue, Santa Clara, California 95050, and Exclusitrade, Inc., with locations at 18 La Vista Verde, Rancho Palos Verdes, California 90274, and 2001 Artesia Boulevard, Redondo Beach, California 90278, and Lakeside Office Park, No. 4, Wakefield, Massachusetts 01880, Raymond Shields Spitz, Star Route 45E, Spirit Lake, Idaho 83869.

By order issued on November 6, 1984, (19 FR 45468, November 16, 1984) ("Order"), respondents William Carlton Dart and Robert William Haire, Sr., both individually and doing business as Display Systems Inc. and Perpetuum, Inc. (all such individual and corporate respondents hereinafter referred to collectively as "Respondents"), are temporarily denied, pursuant to § 388.19 of the Export Administration Regulations (currently codified at 15 CFR Parts 368-399 (1985)) ("Regulations"), all privileges of participating in any manner or capacity in the export or reexport of U.S.-origin commodities or technical data under the Regulations. Under a March 5, 1985 Amendment to the Order (50 FR 9473, March 8, 1985), Respondents were permitted to export certain commodities to the People's Republic of China. The U.S. Department of Commerce ("Department") and Respondents have now moved jointly to modify the Order further, as set forth below. Josef Kubicek, individually and doing business as Exclusitrade, Inc. and J.O.K., Inc., and Raymond Shields Spitz, who are also denied export privileges under the Order, as amended, are not parties to this further requested modification.

The modification jointly requested by the Department and Respondents would, subject to the safeguards agreement described below:

1. Authorize Respondents to export under general license GTDR, without applying for individual validated licenses as otherwise required under paragraph 2 below, from the United States to the Republic of Korea and to Taiwan, technical data, consisting of drawings, artwork and written specifications for, or relating to, the manufacture of liquid crystal displays and liquid crystal display systems; and

2. Authorize Respondents to apply for individual validated licenses covering the export from the United States of U.S.-origin commodities and technical data subject to the jurisdiction of the Export Administration Act of 1979, currently codified at 50 U.S.C. app. Sections 2401-2420 (1982), as amended by the Export Administration Amendments Act of 1985, Pub. L. 99-64, 99 Stat. 120 (July 12, 1985) ("Act"). This authorization to apply for individual validated licenses would extend also to commodities and technical data (other than GTDR exports permitted under paragraph 1 above) which, but for the Order, could be exported under general licensing provisions. All such applications shall be accompanied by a copy of the written contract, as prepared in accordance with Respondents' customary business practices, underlying the transaction.

The joint motion by the Department and Respondents further provides that the export of any commodities and technical data authorized for export under this modification must be in accordance with the terms of an agreement on safeguards ("Agreement"), entered into by the Department and Respondents of September 18, 1985. A copy of the Agreement has been submitted for the record.

Based on the representations made by the Department and by Respondents, I find that the requested motion is justified, and that granting it will not jeopardize the purpose of the Order.

Accordingly, it is hereby Ordered that the Order of November 6, 1984 is further amended, as an additional modification of the Order's denial of all of Respondents' export privileges, and subject to the condition that for each export permitted below Respondents shall comply with the safeguards set forth in the Agreement, to permit Respondents:

(a) To export under general license GTDR, without applying for individual validated licenses as otherwise required under paragraph (b) below, from the United States to the Republic of Korea and to Taiwan, technical data, consisting of drawings, artwork and written specifications for, or relating to, the manufacture of liquid crystal displays and liquid crystal display systems; and

(b) To apply for individual validated licenses covering the export from the United States of U.S.-origin commodities and technical data subject to the jurisdiction of the Act and the Regulations, including commodities and technical data (other than GTDR exports permitted under paragraph (a) above) which, but for the Order, could be exported under general licensing provisions, provided, that every such application shall be accompanied by a copy of the written contract, as prepared in accordance with Respondents' customary business practices, underlying the transaction.

This Amendment of the Order for the exports in paragraphs (a) and (b) above, subject to the condition regarding safeguards, shall apply only to Respondents, as the parties with the Department to the joint motion, and shall not apply to the other individuals and corporations that are denied export privileges under the Order.

This Amendment of the Order is effective immediately and shall be published in the **Federal Register.**

Dated: September 20, 1985, 4:05 pm, E.d.t.

Thomas W. Hoya,

Hearing Commissioner.

[FR Doc. 85-23175 Filed 9-26-85; 8:45 am]

BILLING CODE 3510-DT-M

COMMITTEE FOR PURCHASE FROM THE BLIND AND OTHER SEVERELY HANDICAPPED

Procurement List 1985; Proposed Additions

AGENCY: Committee for Purchase from the Blind and Other Severely Handicapped.

ACTION: Proposed additions to procurement list.

SUMMARY: The Committee has received proposals to add to Procurement List 1985 a commodity to be produced by and a service to be provided by workshops for the blind and other severely handicapped.

DATE: Comments must be received on or before: October 30, 1985.

ADDRESS: Committee for Purchase from the Blind and Other Severely Handicapped, Crystal Square 5, Suite 1107, 1755 Jefferson Davis Highway, Arlington, Virginia 22202-3509.

FOR FURTHER INFORMATION CONTACT: C.W. Fletcher, (703) 557-1145.

SUPPLEMENTARY INFORMATION: This notice is published pursuant to 41 U.S.C. 47(a)(2), 85 Stat. 77 and 41 CFR 51-2.6. Its purpose is to provide interested persons an opportunity to submit comments on the possible impact of the proposed actions.

Additions

If the Committee approves the proposed additions, all entities of the Federal Government will be required to procure the commodity and service listed below from workshops for the blind or other severely handicapped.

It is proposed to add the following commodity and service to Procurement List 1985, October 19, 1984 (49 FR 41195):

Commodity

Kit Bag, Flyer's (Coated Nylon) 8460-00-883-8673

Service

Janitorial Service, Federal Building, 240 West Third Street, Williamsport, Pennsylvania.

C.W. Fletcher,

Executive Director.

[FR Doc. 85-23164 Filed 9-26-85; 8:45 am]

BILLING CODE 6820-33-M

Procurement List 1985 Additions

AGENCY: Committee for Purchase from the Blind and Other Severely Handicapped.

ACTION: Additions to procurement list.

SUMMARY: This action adds to Procurement List 1985 a service to be provided by workshops for the blind and other severely handicapped.

EFFECTIVE DATE: September 27, 1985.

ADDRESS: Committee for Purchase from the Blind and Other Severely Handicapped, Crystal Square 5, Suite 1107, 1755 Jefferson Davis Highway, Arlington, Virginia 22202-3509.

FOR FURTHER INFORMATION CONTACT: C.W. Fletcher, (703) 557-1145.

SUPPLEMENTARY INFORMATION: On July 5, 1985, the Committee for Purchase from the Blind and Other Severely Handicapped published a notice (50 FR 27650) of proposed additions to Procurement List 1985, October 19, 1984 (49 FR 41195).

Addition

After consideration of the relevant matter presented, the Committee has determined that the service listed below is suitable for procurement by the Federal Government under 41 U.S.C. 46-48c, 85 Stat. 77 and 41 CFR 51-2.6.

I certify that the following action will not have a significant impact on a substantial number of small entities. The major factors considered were:

a. The action will not result in any additional reporting, recordkeeping or other compliance requirements.

b. The action will not have a serious economic impact on any contractors for the service listed.

c. The action will result in authorizing small entities to provide the service procured by the Government.

Accordingly, the following service is hereby added to Procurement List 1985:

Service

Commissary Shelf Stocking and Custodial, Fort Monmouth (Ocean Port), New Jersey

C.W. Fletcher,

Executive Director.

[FR Doc. 85-23165 Filed 9-26-85; 8:45 am]

BILLING CODE 6820-33-M

DEPARTMENT OF DEFENSE

Department of the Air Force

Air Force Academy Board of Visitors; Meeting

Pursuant to section 9355, Title 10, United States Code, the Air Force Academy Board of Visitors will meet at the Air Force Academy, Colorado Springs, Colorado, 31 Oct-2 Nov 1985. The purpose of the meeting is to consider morale and discipline, the curriculum, instruction, physical equipment, fiscal affairs, academic methods, and other matters relating to the Academy.

This meeting will be closed to the public to discuss matters analogous to those listed in subsections (2), (4), and (6) of section 552b(c), Title 5, United States Code. These closed sessions will include: attendance at cadet classes and panel discussions with groups of cadets and military staff and faculty officers involving personal information and opinions, the disclosure of which would result in a clearly unwarranted invasion of personal privacy. Closed sessions will also include executive sessions involving discussions of personal information, including financial information, and information relating solely to internal personnel rules and practices of the Board of Visitors and the Academy. Meeting sessions will be held in the Superintendent's Conference Room, Harmon Hall, USAF Academy.

For further information, contact Major Randall R. Cantrell, Headquarters, U.S. Air Force (MPPA), Washington, D.C. 20330, at (202) 697-7116.

Patsy J. Conner,

Air Force Federal Register Liaison Officer.

[FR Doc. 85-23177 Filed 9-26-85; 8:45 am]

BILLING CODE 3910-01-M

USAF Scientific Advisory Board; Meeting

September 16, 1985.

The USAF Scientific Advisory Board Fall General Membership Meeting will be held at Fort McNair, Washington, DC on October 23 and 24, 1985. The meeting will convene from 9:30 a.m. to 4:30 p.m. on October 23 and from 8:30 a.m. to 3:15 p.m. on October 24.

The purpose of the meeting will be to receive classified briefings on results of recent SAB studies, a briefing on the recently-completed Air Force Innovation Study, and a briefing on USAF Special Operations Forces In-Theater Operations.

The meeting concerns matters listed in section 552b(c) of Title 5, United States Code, specifically subparagraph (1) thereof, and accordingly, will be closed to the public.

For further information, contact the Scientific Advisory Board Secretariat at 202-697-8845.

Patsy J. Conner,

Air Force Federal Register Liaison Officer.

[FR Doc. 85-23178 Filed 9-26-85; 8:45 am]

BILLING CODE 3910-01-M

DEPARTMENT OF ENERGY

Civilian Radioactive Waste Management Transportation Institutional Workshop

AGENCY: Office of Civilian Radioactive Waste Management, DOE.

ACTION: Notice of Transportation Institutional Workshop.

The Office of Civilian Radioactive Waste Management (OCRWM), U.S. Department of Energy, is holding a Transportation Institutional Workshop in Atlanta, Georgia on November 19-21, 1985. The OCRWM Draft Transportation Institutional Plan that will be released for public comment by the end of September 1985, will be discussed, as will specific issues related to transportation procedures and policies under the provisions of the Nuclear Waste Policy Act of 1982. A working group format will be used at the meeting to promote maximum exchange of views and expertise. The objective is to foster broad-based participation in planning for NWPA transportation.

Further details of the meeting can be obtained by writing or calling Mr. Robert Philpott, Office of Civilian Radioactive Waste Management, U.S. Department of Energy, RW-33, Forrestal Building, 1000 Independence Avenue, SW., Washington, DC 20585 (202) 252-9620.

Issued in Washington, DC, September 24, 1985.

Robert H. Bauer,

Associate Director for Resource Management, Office of Civilian Radioactive Waste Management.

[FR Doc. 85-23214 Filed 9-26-85; 8:45 am]

BILLING CODE 6450-01-M

Civilian Radioactive Waste Management Draft Transportation Institutional Plan

AGENCY: Office of Civilian Radioactive Waste Management, DOE.

ACTION: Notice of Availability for Comment on the Draft Transportation Institutional Plan.

The Office of Civilian Radioactive Waste Management (OCRWM), U.S. Department of Energy, is developing a Transportation Institutional Plan related to responsibilities assigned by the Nuclear Waste Policy Act of 1982. The document will contain information on the plans and strategies for interaction with institutions affected by the transport of high-level waste under the provisions of the Act.

For planning purposes, the institutions have been divided into three main categories: intergovernmental including other Federal agencies as well as State, Tribal, and local governments; the utilities and industry; and the public. Interaction with these groups will identify both opportunities for cooperative effort and transportation issues of concern. A primary purpose of the Draft Transportation Institutional Plan will be to establish a process for addressing and resolving these concerns.

Copies of the Draft Transportation Institutional Plan may be obtained after September 27, 1985, by telephoning (202) 252-5575, by direct pickup from, or by request in writing to:

Office of Public Affairs, U.S. Department of Energy, Room 1E-218, Forrestal Building, 1000 Independence Avenue, SW., Washington, D.C. 20585

Copies of the document will also be available by writing:

Technical Information Division, Office of Scientific and Technical Information, Technical Information Center, P.O. Box 62, Oak Ridge, TN 37831

All comments on the Draft Transportation Institutional Plan should be submitted in writing no later than December 31, 1985, to Mr. Robert Philpott, Office of Civilian Radioactive Waste Management, U.S. Department of Energy, RW-33, Forrestal Building, 1000 Independence Avenue, SW., Washington, DC 20585. The Department expects to prepare a Final Transportation Institutional Plan by the Spring of 1986.

Issued in Washington, DC, September 24, 1985.

Robert H. Bauer,

Associate Director for Resource Management, Office of Civilian Radioactive Waste Management.

[FR Doc. 85-23215 Filed 9-26-85; 8:45 am]

BILLING CODE 6450-01-M

Financial Assistance Award (Grant); Tufts University

AGENCY: Department of Energy.

ACTION: Notice of Restriction of Eligibility for Grant Award.

SUMMARY: The proposed work will be conducted at Tufts University, Electro-Optics Technical Center, Hilligan Hall, Medford, MA 02155. The proposed period of performance is thirty six months (36) beginning September 30, 1985 through September 30, 1988. The proposed cost of the work is $529,520.

Grant No. DE-FG03-85SF15927

Scope of Project

The major thrust of the research is to address four principal areas: (a) Development of polycrystalline WO_3 films with decreased free electron scattering; (b) development of high integrity, high resistivity, thin ion conducting films; (c) development of optically passive, counterelectrode layers; and (d) developoment of prototype small-area window structures which meet desired performance criteria.

The Council will survey related research to determine the extent of completed and ongoing research in this area. The final agenda will be developed with the use of an industry based review panel, include estimates of time and staff, and delineate the possible role of the Department of Energy.

FOR FURTHER INFORMATION CONTACT: Jane Hadly, Contract Specialist, Contract Management Division, U.S. Department of Energy, San Francisco

Operations Office, 1333 Broadway, Oakland, CA 94612.

Issued in San Francisco, California, September 30, 1985.

Vito A. Magliano,

Acting Manager.

[FR Doc. 85-23153 Filed 9-26-85; 8:45 am]

BILLING CODE 6450-01-M

Office of Assistant Secretary for International Affairs and Energy Emergencies

Atomic Energy Agreement; Proposed Subsequent Arrangements; Canada

Pursuant to section 131 of the Atomic Energy Act of 1954, as amended (42 U.S.C. 2160) notice is hereby given of proposed "subsequent arrangements" under the Agreement for Cooperation Between the Government of the United States of America and the Government of Canada concerning Civil Uses of Atomic Energy, as amended, the Additional Agreement for Cooperation Between the Government of the United States of America and the European Atomic Energy Community (EURATOM) concerning Peaceful Uses of Atomic Energy, as amended, and the Agreement for Cooperation Between the Government of the United States of America and the Government of Japan concerning Civil Uses of Atomic Energy, as amended.

The subsequent arrangements to be carried out under the above-mentioned agreements involve approval of the following sales: Contract S-CA-379, to Eldorado Resources, Ltd., Port Hope, Ontario, Canada, 21.927 grams of uranium enriched to 2.38% in U-235, 43.678 grams of uranium, enriched to 1.35% in U-235, and 489.03 grams of natural uranium, for use as standard reference materials. Contract S-EU-858, to the Bureau of Analysed Samples, Ltd., England, 148.4 grams of natural uranium, for use as standard reference material. Contract S-JA-361, to Japan Nuclear Fuel Co., Ltd., Japan, 593.6 grams of natural uranium, for use as standard reference material.

In accordance with section 131 of the Atomic Energy Act of 1954, as amended, it has been determined that these subsequent arrangements will not be inimical to the common defense and security.

These subsequent arrangements will take effect no sooner than fifteen days after the date of publication of this notice.

Dated: September 23, 1985.

For the Department of Energy.

George J. Bradley, Jr.,

Acting Assistant Secretary for International Affairs and Energy Emergencies.

[FR Doc. 85-23152 Filed 9-26-85; 8:45 am]

BILLING CODE 6450-01-M

Atomic Energy Agreements; Proposed Subsequent Arrangement; Government of Canada

Pursuant to section 131 of the Atomic Energy Act of 1954, as amended (42 U.S.C. 2160) notice is hereby given of a proposed "subsequent arrangement" under the Agreement for Cooperation between the Government of the United States of America and the Government of Canada concerning Civil Uses Atomic Energy, as amended.

The subsequent arrangement to be carried out under the above-mentioned agreement involves approval of the following sale: Contract Number S-CA-376, to the National Research Council of Canada, Ottawa, Canada, one microcurie of plutonium-236, for use as a tracer in analyzing picocurie levels of other plutonium isotopes.

In accordance with section 131 of the Atomic Energy Act of 1954, as amended, it has been determined that this subsequent arrangement will not be inimical to the common defense and security.

This subsequent arrangement will take effect no sooner than fifteen days after the date of publication of this notice.

For the Department of Energy.

Dated: September 23, 1985.

George J. Bradley, Jr.,

Acting Assistant Secretary for International Affairs and Energy Emergencies.

[FR Doc. 85-23091 Filed 9-26-85; 8:45 am]

BILLING CODE 6450-01-M

Economic Regulatory Administration

[Docket PP-39EA]

Application for Authorization To Transmit Electric Energy to Canada

AGENCY: Economic Regulatory Administration, DOE.

ACTION: Notice of application by Boise Cascade, Corp. for authorization to export electric energy to a foreign country.

SUMMARY: Boise Cascade has filed an application with the Economic Regulatory Administration (ERA) for authority to export electric energy to its Canadian subsidiary, Boise Cascade Canada, Ltd., located in Fort Francis, Ontario. Sale of this electric energy will commence on or before January 1, 1986, and terminate on December 31, 1990.

FOR FURTHER INFORMATION CONTACT:

Anthony J. Como, Economic Regulatory Administration (RG-22), Department of Energy, 1000 Independence Avenue, SW., Washington, D.C. 20585, (202) 252-5935

Lise Courtney M. Howe, Office of General Counsel (GC), Department of Energy, 1000 Independence Avenue, SW., Washington, D.C. 20585, (202) 252-2900.

SUPPLEMENTARY INFORMATION: On September 11, 1985, Boise Cascade filed an application with ERA seeking authorization to export electric power and energy to its Canadian subsidiary, Boise Cascade Canada, LTD.

The applicant is located in the City of International Falls, Minnesota and plans to transmit 21.5 megawatts of firm power and associated energy to its Canadian subsidiary located in Fort Frances, Ontario. This export is scheduled to start on or before January 1, 1986, and will terminate on December 31, 1990. Details of the agreement between the applicant and its Canadian subsidiary were made part of the application.

The proposed export would be transmitted over an existing 6.6 kilovolt transmission line extending approximately 0.20 miles between Boise Cascade's U.S. and Canadian plants. The construction and operation of this international transmission line was authorized previously by the Presidential permit in ERA Docket PP-39 (formerly Federal Power Commission Docket E-7286).

Any person desiring to be heard or to protest said application should file a petition to intervene or protest with the Office of Coal & Electricity, Department of Energy, 1000 Independence Avenue, SW., Washington, DC 20585, in accordance with §§ 385.211 or 385.214 of the Rules of Practice and Procedure (18 CFR 385.215, 385.211, 385.214).

Any such petitions and protests should be filed within 30 days of the publication of this notice. Protests will be considered by DOE in determining the appropriate action to be taken, but will not serve to make protestants parties to the proceeding. Any person wishing to become a party must file a petition to intervene. Copies of this application are on file with DOE and will, upon request, be made available for public inspection and copying at the DOE Freedom of Information Reading Room, Room 1E-190, 1000 Independence Avenue, SW, Washington, DC from 8:00

a.m. to 4:00 p.m., Monday through Friday.

Issued in Washington, DC on Septemer 16, 1985.

Marshall Staunton,

Acting Administrator, Economic Regulatory Administration.

[FR Doc. 85-23092 Filed 9-26-85; 8:45 am]

BILLING CODE 6450-01-M

Federal Energy Regulatory Commission

[Docket Nos. ER85-773-000, et al.]

Arkansas Power & Light Co. et al.; Electric Rate and Corporate Regulation Filings

Take notice that the following filings have been made with the Commission:

1. Arkansas Power & Light Company

[Docket No. ER85-773-000]

September 20, 1985.

Take notice that Arkansas Power & Light Company (AP&L) filed on September 17, 1985, a proposed Peaking Power Agreement which is a supplement to the Power Coordination Interchange and Transmission Agreement between City of west Memphis, Arkansas and Arkansas Power & Light Company dated June 25, 1982. The agreement provides for the sale to the City of 22 MW of Peaking capacity and 5,280 MWH of Peaking Energy during the months of May through September.

The proposed Peaking Power Agreement will effect a savings of approximately $1,168,000 per year for the City of West Memphis based on the actual billings for the twelve month period ended July 31, 1985.

Comment date: October 2, 1985, in accordance with Standard Paragraph E at the end of this notice.

2. Duke Power Company

[Docket Nos. ER82-732-000 ER82-732-001, ER83-123-000 and ER83-123-001]

September 23, 1985.

Take notice that on September 3, 1985, Duke Power Company (the Company) tendered for filing a compliance report of amounts due under the fuel clause provisions of a settlement agreement between the Company and its wholesale customers. The Commission approved the agreement on June 20, 1983. The report reflects refunds being made to South Carolina municipal customers now being served under the Catawba Nuclear Station Interconnection Agreement. The Company has previously advised the Commission that service under the Catawba Interconnection Agreement would begin in July 1985 for the South Carolina municipalities who are members of the Piedmont Municipal Power Agency (Docket No. 85-602-000, Letter Order dated August 2, 1985). In a letter dated March 11, 1983, the Company advised the Commission of refunds made to the North Carolina municipalities and North and South Carolina cooperative previously served under Rate Schedule No. 10 and Rate Schedule No. 11 in August 1983 and December 1983, respectively.

Comment date: October 4, 1985, in accordance with Standard Paragraph H at the end of this notice.

3. Northern States Power Company

[Docket No. FR85-759-000]

September 23, 1985.

The notice that Northern States Power Company (NSP) on September 12, 1985, tendered for filing Supplement No. 8 to the Integrated Transmission Agreement between Cooperative Power Association (CPA) and Northern States Power Company (Supplement).

The Supplement, dated July 10, 1985, revises four exhibits to the Intergrated Transmission Agreement (ITA) dated August 25, 1967. The ITA is on file with the Commission and is designated as Rate Schedule FERC No. 342. The four exhibits revised by this Supplement contain diagrams and descriptions of the interconnection facilities covered by the ITA. The changes are necessary in order to reflect the transmission and substation facility additions and the sale to CPA in 1984 of various properties which have occurred since the exhibits were last updated.

Comment date: October 3, 1985, in accordance with Standard Paragraph E at the end of this notice.

4. Northern States Power Company

[Docket No. ER85-760-000]

September 23, 1985.

Take Notice that Northern States Power Company (NSP) on September 12, 1985 tendered for filing the Diversity Exchange Agreement Between Northern States Power Company and United Power Association (Diversity Exchange Agreement).

The Diversity Exchange Agreement is an initial rate schedule filing. The Diversity Exchange Agreement profides that NSP will supply United Power Association power and energy during the winter season beginning in November 1993 for a minimum period of fifteen years. The Agreement also provides that in consideration of the power and energy provided by NSP, United Power Association will make available to NSP power and energy during the summer season for the same period of time.

NSP requests the Diversity Exchange Agreement become effective on May 1, 1993; and therefore, requests waiver of the Commission's notice requirements.

Comment date: October 3, 1985, in accordance with Standard Paragraph E at the end of this notice.

5. Northern States Power Company

[Docket No. ER85-761-000]

September 23, 1985.

Take Notice that Northern States Power Company (NSP) on Sept. 12, 1985, tendered for filing the Diversity Exchange Agreement Between Northern States Power Company and Minnkota Power Cooperative (Diversity Exchange Agreement).

The Diversity Exchange Agreement is an initial rate schedule filing. The Diversity Exchange Agreement provides that NSP will supply Minnkota Power Cooperative power and energy during the winter season beginning in November 1991 for a minimum period of ten years. The Agreement also provides that in consideration of the power and energy provided by NSP, Minnkota Power Cooperative will make available to NSP power and energy during the summer season for the same period of time.

NSP requests the Diversity Exchange Agreement become effective on May 1, 1991, and therefore, requests wavier of the Commission's notice requirements.

Comments date: October 3, 1985, in accordance with Standard paragraph E at the end of this notice.

6. Pacific Power & Light Company, an Assumed Business Name of PacifiCorp.

[Docket No. ER85-757-000]

September 23, 1985.

Take Notice that Pacific Power & Light Company (Pacific), an assumed business name of PacificCorp, on September 12, 1985, tendered for filing, in accordance with § 35.13a(d)(5) of the Commission's Regulations, Pacific's Revised Appendix 1 for state of Washington. The Revised Appendix 1 calculates an average system cost for the state of Washington applicable to the exchange of power between Bonneville Power Administration (Bonneville) and Pacific.

Pacific requests waiver of the Commission's notice requirements to permit this rate schedule to become effective October 1, 1984, which it claims is the date of commencement of service.

Copies of the filing were supplied to Bonneville, the Washington Utilities and Transportation Commission, and

Bonneville's Direct Service Industries Customers.

Comment date: Octobert 3, 1985, in accordance with Standard Paragraph E at the end of this notice.

7. Pacific Power & Light Company, an Assumed Business Name of PacifiCorp.

[Docket No. ER85-758-000]

September 23, 1985.

Take Notice that Pacific & Light Company (Pacific), an assumed business name of PacificCorp, on September 12, 1985, tendered for filing, in accordance with § 35.13a(d)(5) of the Commission's Regulations, Pacific's Revised Appendix 1 for the state of Idaho. The Revised Appendix 1 calculates an average system cost for the state of Idaho applicable to the exchange of power between Bonneville Power Administration (Bonneville) and Pacific.

Pacific requests waiver of the Commission's notice requirements to permit this rate schedule to become effective October 1, 1984, which it claims is the date of commencement of service.

Copies of the filing were supplied to Bonneville, the Idaho Public Utilities Commission, and Bonneville's Direct Service Industrial Customers.

Comment date: October 3, 1985, in accordance with Standard Paragraph E at the end of this notice.

8. Pacific Power & Light Company, an Assumed Business Name of PacificCorp.

[Docket No. ER85-762-000]

September 23, 1985.

Take Notice that Pacific Power & Light Company (Pacific), an assumed business name of PacificCorp, on September 12, 1985, tendered for filing, in accordance with § 35.13a(d)(5) of the Commission's Regulations, Pacific's Revised Appendix 1 for the state of Oregon. The Revised Appendix 1 calculates an average system cost for the state of Oregon applicable to the exchange of power between Bonneville Power Administration (Bonneville) and Pacific.

Pacific requests waiver of the Commission's notice requirements to permit this rate schedule to become effective October 1, 1984, which it claims is the date of commencement of service.

Copies of the filing were supplied to Bonneville, the Public Utility Commissioner of Oregon, and Bonneville's Direct Service Industrial Customers.

Comment date: October 3, 1985, in accordance with Standard Paragraph E at the end of this notice.

9. Puget Sound Power & Light Company

[Docket No. ER85-771-000]

September 20, 1985.

Take notice that on September 16, 1985, Puget Sound Power and Light Company (Puget) tendered for filing Appendix 1 to the Residential Purchase and Sale Agreement, Contract No. DE-MS79-81BP90604, between Puget and the Bonneville Power Administration. In addition, Puget tendered for filing BPA report dated August 26, 1985 pertaining to the above filing.

Comment date: October 2, 1985, in accordance with Standard Paragraph E at the end of this notice.

10. Puget Sound Power & Light Company

[Docket No. ER85-774-000]

September 23, 1985.

Take notice that on September 18, 1985, Puget Sound Power and Light Company (Puget) tendered for filing Appendix 1 to the Residential Purchase and Sale Agreement, Contract No. DE-MS79-81BP90604, between Puget and the Bonneville Power Administration, including the ECAC ASC adjustment demonstrated on Schedule 4. In addition, Puget tendered for filing BPA report dated September 3, 1985 pertaining to the above filing.

Comment date: October 3, 1985, in accordance with Standard Paragraph E at the end of this notice.

11. South Carolina Generating Company

[Docket No. ER85-603-002]

September 23, 1985.

Take notice that on September 13, 1985, South Carolina Generating Company (GENCO) tendered for filing in accordance with the Commission's order of August 15, 1985 seven copies of further revised schedules 3 and 8. (Supplement Nos. 5 and 3 respectively, to GENCO's Rate Schedule FERC No. 1). The remaining schedules that accompanied GENCO's June 27, 1985 filing did not include Account No. 407 and are not being refiled at this time in accordance with the Commission's request that Account No. 407 be deleted.

Comment date: October 1, 1985, in accordance with Standard Paragraph H at the end of this notice.

12. Northwestern Public Service Company

[Docket No. EC85-22-000]

September 23, 1985.

Take notice that Northwestern Public Service Company (NWPS) filed an application on September 16, 1985 pursuant to 18 CFR 33.1, et seq. requesting that authority be granted under Title 16, U.S.C. 824b.(a) allowing NWPS to purchase, acquire, hold and sell securities of other public utilities as part of a planned expansion of corporate investments. NWPS proposes to limit its holding or ownership of any given class of securities to an amount not to exceed one percent (1%) of the capital stock or funded debt outstanding. Additionally, NWPS is requesting a modification of the reporting requirement under Title 18, CFR 33.8 to allow an annual update and status report only. The application sets forth the limitations and requirements protecting the public interest. The application is on file with the Commission and open to public inspection.

Comment date: October 7, 1985, in accordance with Standard Paragraph E at the end of this notice.

Standard Paragraphs

E. Any person desiring to be heard or to protest said filing should file a motion to intervene or protest with the Federal Energy Regulatory Commission, 825 North Capitol Street, NE., Washington, DC 20426, in accordance with Rules 211 and 214 of the Commission's Rules of Practice and Procedure (18 CFR 385.211 and 385.214). All such motions or protests should be filed on or before the comment date. Protests will be considered by the Commission in determining the appropriate action to be taken, but will not serve to make protestants parties to the proceeding. Any person wishing to become a party must file a motion to intervene. Copies of this filing are on file with the Commission and are available for public inspection.

H. Any person desiring to be heard or to protest this filing should file comments with the Federal Energy Regulatory Commission, 825 North Capitol Street, NW., Washington, D.C. 20426, on or before the comment date. Comments will be considered by the Commission in determining the appropriate action to be taken. Copies of this filing are on file with the Commission and are available for public inspection.

Kenneth F. Plumb,

Secretary.

[FR Doc. 85-23131 Filed 9-26-85; 8:45 am]

BILLING CODE 6717-01-M

[Docket Nos. ID-1972-002, et al.]

Interlocking directorate applications; John F. Opeka et al.

September 23, 1985.

Take notice that the following filings have been made with the Commission:

1. John F. Opeka

[Docket No. ID-1972-002]

Take notice that on September 16, 1985, John F. Opeka, pursuant to section 305(b) of the Federal Power Act, submitted for filing a supplemental application for authority to hold the following position:

Assistant Treasurer, Connecticut Yankee Atomic Power Company, Public Utility

Comment date: October 3, 1985, in accordance with Standard Paragraph E at the end of this notice.

2. C. Thayer Browne

[Docket No. ID-1971-001]

Take notice that on September 16, 1985, C. Thayer Browne, pursuant to section 305(b) of the Federal Power Act, submitted for filing a supplemental application for authority to hold the following position:

Assistant Treasurer, Connecticut Yankee Atomic Power Company, Public Utility

Comment date: October 3, 1985, in accordance with Standard Paragraph E at the end of this notice.

Standard Paragraphs

E. Any person desiring to be heard or to protest said filing should file a motion to intervene or protest with the Federal Energy Regulatory Commission, 825 North Capitol Stret, NE., Washington, D.C. 20426, in accordance with Rules 211 and 214 of the Commission's Rules of Practice and Procedure (18 CFR 385.211 and 385.214). All such motions or protest should be filed on or before the comment date. Protests will be considered by the Commission in determining the appropriate action to be taken, but will not serve to make protestants parties to the proceeding. Any person wishing to become a party must file a motion to intervene. Copies of this filing are on file with the Commission and are available for public inspection.

Kenneth F. Plumb,

Secretary.

[FR Doc. 85-23125 Filed 9-26-85; 8:45 am]

BILLING CODE 6717-01-M

[Docket No. RP84-53-003]

Ozark Gas Transmission System; Filing

September 23, 1985.

Take notice that Ozark Gas Transmission System (Ozark) on September 18, 1985, tendered for filing a Request To Charge Rates Lower Than Those Currently In Effect Under Its FERC Gas Tariff. Ozark also filed Second Revised Sheet No. 5 to its FERC Gas Tariff. Ozark proposes to place its reduced rate into effect retroactively as of March 1, 1984, and to collect said reduced rate subject to refund pending issuance of a final Commission decision in this docket.

Copies of the filing were served upon Ozark's jurisdictional customers, parties to this proceeding and the appropriate state regulatory commissions.

Any person desiring to be heard or to protest said filing should file a motion to intervene or a protest with the Federal Energy Regualtory Commission, 825 North Capitol Street, NE., Washington, D.C. 20426, in accordance with Rules 211 and 214 of the Commission's Rules of Practice and Procedure (18 CFR 385.211, 385.214). All such motions or protests should be filed on or before September 27, 1985. Protests will be considered by the Commission in determining the appropriate action to be taken, but will not serve to make protestants parties to the proceeding. Any person wishing to become a party must file a motion to intervene. Copies of this filing are on file with the Commission and are available for public inspection.

Kenneth F. Plumb,

Secretary.

[FR Doc. 85-23128 Filed 9-26-85; 8:45 am]

BILLING CODE 6717-01-M

[Docket No. SA85-51-000]

Pacific Gas and Electric Petition for Exemption From Incremental Pricing

September 24, 1985.

Take notice that on August 26, 1985, Pacific Gas and Electric Company (Pacific) filed a petition with the Commission pursuant to section 206(d) of the Natural Gas Policy Act of 1978 (NPGA). Pacific seeks an order from the Director of the Commission's Office of Pipeline and Producer Regulation, exempting from incremental pricing for a 15 year period, natural gas sales to be made by Pacific under Pacific's Rate Schedules G-59, G-90 and other schedules to enhanced oil recovery customers.

Pacific states that it is artificially constrained by incremental pricing regulations in attempting to sell gas to the enhanced oil recovery market, and that in the absence of the relief requested, Pacific and its ratepayers will be disadvantaged relative to competing gas suppliers and alternative fuels not subject to incremental pricing regulations. Pacific asserts that an incremental pricing exemption is warranted to insure fair competition in the enhanced oil recovery market, and that failure to grant the requests may result in hardship, inequity, and unfair distribution of burdens.

Pacific requests expedited consideration of its petition. Pacific maintains that expedition is necessary to give potential enhanced oil recovery customers sufficient lead time to obtain commitments for construction of necessary facilities. Pacific also maintains that expedited consideration is necessary to prevent a loss of enhanced oil recovery customers resulting from their procurement of alternative long term sources of supply.

The procedures applicable to the conduct of this proceeding are set forth in Subpart K of the Commission's Rules of Practice and Procedure. Any person desiring to participate in this proceeding must file with the Federal Energy Regulatory Commission, 825 North Capitol Street, NE., Washington, D.C. 20426, a motion to intervene must be filed within 15 days after publication of this notice in the **Federal Register.**

Kenneth F. Plumb,

Secretary.

[FR Doc. 85-23129 Filed 9-26-85; 8:45 am]

BILLING CODE 6717-01-M

[Docket No. GP84-46-000]

Petitions to Reopen and Vacate Final Well Category Determinations and Request to Withdraw

September 24, 1985

In the matter of Commonwealth of Pennsylvania, Department of environmental Resources, Section 102 Determinations, Victory Development Company, Koop, #1 Well, FERC JD No. 83-30338, #1 Defense & Emergency Well, FERC JD No. 83-30335, #3 Defense & Emergency Well, FERC JD No. 83-30337, #1 Lions recreation Center Well, FERC JD No. 83-3039.

Take notice tha on June 20, 1985, Victory Development Company filed with the Commission pursuant to § 275.205 of the Commission's regulations a petition to reopen and vacate final well category determinations under section 102 of the Natural Gas Policy Act of 1978 (NGPA) for the wells listed in the caption of this notice and to withdraw its applications for the determinations

Victory states that the wells do not qualify under section 102 of the NGPA because they are located within 2.5 miles of a marker well drilled by T.W. Phillips Gas & Oil Company in the 1940's. victory further states that no gas has been sold from any of the wells and therefore no refunds are required.

Any person desiring to be heard or to protest Victory'a peition should filed a motion to intervene (18 CFR 385.214) or protest (18 CFR 385.211) with the Federal Energy Regulatory Commission, 825 North Capitol Street NW., Washington, DC. 20426 within 30 days after this notice is published in the **Federal Register**. All protests filed will be considered by the Commission but will not make the protestant a party to the proceeding. Any person wishing to become a party must file a motion to intervene.

Kenneth F. Plumb,
Secretary.

[FR Doc. 85-23127 Filed 9-26-85; 8:45 am]

BILLING CODE 6717-01-M

[Docket No. CI85-4-000]

Shell Offshore, Inc. and Shell Western E & P, Inc.; Quarterly Status Conference

September 24, 1985.

A quarterly status conference is to be held in the above-captioned docket pursuant to Commission order of September 26, 1984, to evaluate whether the implementation of the special marketing program is achieving the Commission's purposes. The conference will be held at the offices of the Commission at 825 North Capitol Street, NE., Washington, D.C. at 2:00 p.m. on October 1, 1985. All interested persons and Staff are invited to attend.

Kenneth F. Plumb,
Secretary.

[FR Doc. 85-23130 Filed 9-26-85; 8:45 am]

BILLING CODE 6717-01-M

Office of Energy Research

Energy Research Advisory Board, Solar Panel; Open Meeting

Notice is hereby given of the following meeting:

Name: Solar Panel of the Energy Research Advisory Board (ERAB).

Date and time: October 10, 1985—8:30 a.m.-4:00 p.m.

Place: Department of Energy, 1000 Independence Avenue, SW, Room 4A-110, Washington, DC 20585.

Contact: William L. Woodard, Department of Energy, Office of Energy Research, 1000 Independence Avenue, SW, Washington, DC 20585, (202) 252-5767.

Purpose of the Parent Board

To advise the Department of Energy (DOE) on the overall research and development conducted in DOE and to provide long-range guidance in these areas to the Department.

Purpose of the Panel

The Solar Panel is a subgroup of ERAB and reports to the parent Board. The purpose of the Panel is to assess in more detail the appropriateness of consolidating the Department's solar research activities into fewer field offices, national laboratories, and research centers, as was recommended in the Board's 1982 report, "Solar Energy Research and Development: Federal and Private Sector Roles."

Tentative Agenda

- Introduction and Discussion of Charge.
- Program Briefings by Selected DOE Laboratories Involved in Solar Research.
- Informal Discussion.
- Consideration of Draft Letter Report.
- Public Comment (10 minute rule).

Public Participation

The meeting is open to the public. Written statements may be filed with the Panel either before or after the meeting. Members of the public who wish to make oral statements pertaining to agenda items should contact William Woodard at the address or telephone number listed above. Requests must be received 5 days prior to the meeting and reasonable provisions will be made to include the presentation on the agenda. The Chairperson of the Panel is empowered to conduct the meeting in a fashion that will facilitate the orderly conduct of business.

Transcripts

Available for public review and copying at the Freedom of Information Public Reading Room, 1E-190, Forrestal Building, 1000 Independence Avenue, SW, Washington, DC between 9:00 a.m. and 4:00 p.m., Monday through Friday, except Federal holidays.

Issued at Washington, DC on September 17, 1985.

Charles E. Cathey,
Deputy Director, Science and Technology Affairs Staff, Office of Energy Research.

[FR Doc. 85-23213 Filed 9-26-85; 8:45 am]

BILLING CODE 6450-01-M

ENVIRONMENTAL PROTECTION AGENCY

[OPTS-59733; TSH-FRL 2904-5]

Certain Chemicals Premanufacture Notices

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice.

SUMMARY: Section 5(a)(1) of the Toxic Substances Control Act (TSCA) requires any person who intends to manufacture or import a new chemical substance to submit a premanufacture notice (PMN) to EPA at least 90 days before manufacture or import commences. Statutory requirements for section 5(a)(1) premanufacture notices are discussed in EPA statements of the final rule published in the **Federal Register** of May 13, 1983 (48 FR 21722). In the **Federal Register** of November 11, 1984, (49 FR 46066) (40 CFR 723.250), EPA published a rule which granted a limited exemption from certain PMN requirements for certain types of polymers. PMNs for such polymers are reviewed by EPA within 21 days of receipt. This notice announces receipt of ten such PMNs and provides a summary of each.

DATES: Close of Review Period:.

Y 85-157 and 85-158, October 3, 1985
Y 85-159, October 7, 1985
Y 85-160, 85-161, 85-162, and 85-163, October 8, 1985
Y 85-164, 85-165, and 85-166, October 9, 1985.

FOR FURTHER INFORMATION CONTACT: Wendy Cleland-Hamnett, Chemical Control Division (TS-794), Office of Toxic Substances, Environmental Protection Agency, Rm. E-611, 401 M St., SW., Washington, DC 20460, (202-382-3725).

SUPPLEMENTARY INFORMATION: The following notice contains information extracted from the non-confidential version of the submission by the manufacturer on the exemptions received by EPA. The complete non-confidential document is available in the Public Reading Room E-107 at the above address between 8:00 a.m. and 4:00 p.m., Monday through Friday, excluding legal holidays.

Y 85-157

Manufacturer. Confidential.

Chemical. (G) Polyester of carbomonocyclic acid and alkylene glycols.

Use/Production. (S) Industrial extrusion into textile fiber for apparel and home furnishing fabric. Prod. range. Confidential.

Toxicity Data. No data on the PMN substance submitted.

Exposure. No data submitted.

Environmental Release/Disposal. No data submitted.

Y 85–158

Manufacturer. Confidential.

Chemical. (G) Polyester of carbomonocyclic ester and alkylene glycols.

Use/Production. (S) Industrial extrusion into textile fiber for apparel and home furnishing fabric. Prod. range. Confidential.

Toxicity Data. No data on the PMN substance submitted.

Exposure. No data submitted.

Environmental Release/Disposal. No data submitted.

Y 85–159

Manufacturer. Confidential.

Chemical. (G) Dimer acids, dicarboxylic acid, ethylenediamine, diamine polyamide resin.

Use/Production. (S) Industrial hot melt adhesive and is intended for use in bonding plastics in automobile applications. Prod. range. Confidential.

Toxicity Data. No data on the PMN substance submitted.

Exposure. Manufacture: dermal and inhalation, a total of 4 workers.

Environmental Release/Disposal. Less than 0.1 kg/batch released to water and <2 kg/batch to land. Disposal by sanitary landfill.

Y 85–160

Manufacturer. S.C. Johnson & Son, Inc.

Chemical. (G) An ammonia/water soluble random copolymer of butyl acrylate and acrylic acid.

Use/Production. (G) Used as component in the manufacture of a water borne emulsion for water pressure sensitive adhesives to be used on tapes, labels and decals. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Confidential.

Environmental Release/Disposal. Confidential. Disposal by POTW.

Y 85–161

Manufacturer. S.C. Johnson & Son, Inc.

Chemical. (G) Styrene-acrylate random emulsion copolymer.

Use/Production. (G) Open, non-dispersive use. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Confidential.

Environmental Release/Disposal. Confidential. Disposal by POTW.

Y 85–162

Manufacturer. S.C. Johnson & Son, Inc.

Chemical. (G) Ammonium salt solution of a terpolymer of styrene, alpha methyl styrene and acrylic acid.

Use/Production. (G) A resin solution in the form of ammonium salt for formulating aqueous gravure inks and overprint varnishes. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Confidential.

Environmental Release/Disposal. Confidential. Disposal by POTW.

Y 85–163

Manufacturer. S.C. Johnson & Son, Inc.

Chemical. (G) A terpolymer of 2-ethylhexyl acrylate, butyl acrylate, and hydroxyethyl methacrylate.

Use/Production. (G) The material is a high solids, hydroxyl functional polymer to be cross linked with isocyanate functional crosslinkers to be used in high solids, packaging and laminating adhesives. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Confidential.

Environmental Release/Disposal. Confidential. Disposal by POTW.

Y 85–164

Importer. Urethane Concepts Inc.

Chemical. Further clarification needed before information can be released to the public files.

Use/Production. (S) Industrial manufacturing of flexible and rigid polyurethane foam. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Manufacture: dermal, a total of 10–50 workers, up to 8 hrs/da, up to 240 da/yr.

Environmental Release/Disposal. No data submitted.

Y 85–165

Importer. Urethane Concepts Inc.

Chemical. Further clarification is needed before information can be released to the public files.

Use/Production. (S) Industrial manufacturing of flexible and rigid polyurethane foam. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Manufacture: dermal, a total of 10–50 workers, up to 8 hrs/da, up to 240 da/yr.

Environmental Release/Disposal. No data submitted.

Y 85–166

Importer. Urethane Concepts Inc.

Chemical. Further clarification is needed before information can be released to the public files.

Use/Production. (S) Industrial polyol component in flexible polyurethane form. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Manufacture: dermal, a total of 10–50 workers, up to 8 hrs/da, up to 240 da/yr.

Environmental Release/Disposal. No data submitted.

Dated: September 23, 1985.

Linda A. Travers,

Acting Director, Information Management Division.

[FR Doc. 85–23113 Filed 9–26–85; 8:45 am]

BILLING CODE 6560-50-M

[OPTS-51590; TSH-FRL 2904-7]

Certain Chemicals Premanufacture Notices

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice.

SUMMARY: Section 5(a)(1) of the Toxic Substances Control Act (TSCA) requires any person who intends to manufacture or import a new chemical substance to submit a permanufacture notice (PMN) to EPA at least 90 days before manufacture or import commences. Statutory requirements for section 5(a)(1) premanufacture notices are discussed in EPA statements of the final rule published in the **Federal Register** of May 13, 1983 (48 FR 21722). This notice announces receipt of fifty-one PMNs and provides a summary of each.

DATES: Close of Review Period:

P 85–1479, December 10, 1985

P 85–1439, 85–1440, 85–1441, 85–1442, 85–1443, 85–1444, 85–1445 and 85–1446, December 11, 1985

P 85–1447, 85–1448, 85–1449, 85–1450, 85–1451, 85–1452, and 85–1453, December 14, 1985

P 85–1454, 85–1455, 85–1456, 85–1457, 85–1458, 85–1459, 85–1460, 85–1461, 85–1462, 85–1463, 85–1464, 85–1465, 85–1466, and 85–1467, December 15, 1985

P 85–1468, 85–1469, 85–1470, 85–1471, 85–1472, 85–1473, 85–1474, 85–1475, 85–1476, 85–1477, 85–1478, and 85–1480, December 16, 1985

P 85–1481, 85–1482, 85–1483, 85–1484, 85–1485, 85–1486, 85–1487, 85–1488, and 85–1489, December 17, 1985

Written comments by:

P 85–1479, November 10, 1985

P 85–1439, 85–1440, 85–1441, 85–1442, 85–1443, 85–1444, 85–1445, and 85–1446, November 11, 1985

P 85-1447, 85-1448, 85-1449, 85-1450, 85-1451, 85-1452, and 85-1453, November 14, 1985
P 85-1454, 85-1455, 85-1456, 85-1457, 85-1458, 85-1459, 85-1460, 85-1461, 85-1462, 85-1463, 85-1464, 85-1465, 85-1466, and 85-1467, November 15, 1985
P 85-1468, 85-1469, 85-1470, 85-1471, 85-1472, 85-1473, 85-1474, 85-1475, 85-1476, 85-1477, 85-1478, and 85-1480, November 16, 1985
P 85-1481, 85-1482, 85-1483, 85-1484, 85-1485, 85-1486, 85-1487, 85-1488, and 85-1489, November 17, 1985

ADDRESS: Written comments, identified by the document control number "[OPTS-51590]" and the specific PMN number should be sent to: Document Control Officer (TS-793), Chemical Information Branch, Information Management Division, Office of Toxic Substance, Environmental Protection Agency, Rm. E-201, 401 M St., SW, Washington, DC 20460, (202) 382-3532.

FOR FURTHER INFORMATION CONTACT: Wendy Cleland-Hamnett, Premanufacture Notice Management Branch, Chemical Control Division (TS-794), Office of Toxic Substances, Environmental Protection Agency, Rm. E-611, 401 M St., SW., Washington, DC 20460, (202) 382-3725.

SUPPLEMENTARY INFORMATION: The following notice contains information extracted from the non-confidential version of the submission provided by the manufacturer on the PMNs received by EPA. The complete non-confidential document is available in the Public Reading Room E-107 at the above address.

P 85-1439

Manufacturer. The Dow Chemical Company.

Chemical. Further clarification needed before information can be released to the public files.

Use/Production. (G) Solvent. Prod. range. Confidential.

Toxicity Data. Acute oral: >4,000 mg/kg: Acute oral: >2,000 mg/kg: Irritation: Skin—Moderate to severe; Eye—Moderate to severe.

Exposure. Manufacture: dermal.

Environmental Release/Disposal. Release to air and water Disposal by incineration, on-site industrial waste treatment plant and navigable waterway after treatment.

P 85-1440

Manufacturer. Lilly Industrial Coatings, Inc.

Chemical. Further clarification needed before information can be released to the public files.

Use/Production. (G) Industrial liquid paints. Prod. range. 36,000-63,000 kg/yr.

Toxicity Data. No data submitted.

Exposure. Manufacture and processing: a total of 28 workers, up to 12 hrs/da, up to 24 da/yr.

Environmental Release/Disposal. Less than 1 to 15 kg/batch released to air. Disposal by publicly owned treatment works (POTW) and incineration.

P 85-1441

Importer. Marubeni America Corporation.

Chemical. (S) Pyridinium,1,1'-[sulfonylbis[3,1-phenyleneimino[6-[[3-[[3-carboxy-4,5-dihydro-5-oxo-1-(4-sulfophenyl)-1H-pyrazol-4-yl]azo]-4-sulfphenyl]amino]-1,3,5-triazine-4,2-diyl]]]bis [3-carboxy-, dihydroxide, tetrasodium salt.

Use/Import. (S) Dye for cellulosic fibers. Import range. 10,000 kg/yr.

Toxicity Data. Acute oral: >5,000 mg/kg: Inhalation: Ames test: Negative; TLm 48 hours (Orange medaka): >1,000 parts per million (ppm).

Exposure. Processing: dermal.

Environmental Release/Disposal. No data submitted.

P 85-1442

Importer. Marubeni America Corporation.

Chemical. (S) Bonzoic acid, 2,5-bis[[4-[[4-[(4,8-disulfo-2-naphthalenyl) azo]-3 methylphenyl]amino]-6-(4-morpholinyl)-1,3,5-triazin-2-yl]amino]-, pentasodium salt.

Use/Import. (S) Dye for cellulosic fibers. Import range. 10,000 kg/yr.

Toxicity Data: Acute oral: >5,000 mg/kg; Ames test: Negative; TLm 48 hours (Orange medaka): >1,000 ppm.

Exposure: Processing: dermal.

Environmental Release/Disposal. No data submitted.

P 85-1443

Importer. Marubeni America Corporation.

Chemical. (S) Chromate (7-) bis[1-[4-[[3-(acetylamino]-4-[(4,8-disulfo-2-naphthalenyl)azo]phenyl]amino]-6-[[6-[(2-carboxyphenyl)azo]-5-hydroxy-7-sulfo-2-naphthalenyl]amino]-1,3,5-triazin-2-yl]-3-carboxypyridiniumato(6-)]-, heptasodium, dihydrate.

Use/Import. (S) Dye for cellulosic fibers. Import range. 10,000 kg/yr.

Toxicity Data: Acute oral: >5,000 mg/kg; Ames test: Negative; TLm 48 hours (Orange medaka): >1,000 ppm.

Exposure: Processing: dermal.

Environmental Release/Disposal. No data submitted.

P 85-1444

Importer. Marubeni America Corporation.

Chemical. (S) 2,7-Naphthalenedisulfonic acid, 5-[[4-chloro-6-[(2-sulfoethyl)amino]-1,3,5-triazin-2-yl]amino]-4-hydroxy-3-[(2-sulfophenyl)azo]-, tetrasodium salt.

Use/Import. (S) Dye for cellulosic fibers. Import range. 10,000 kg/yr.

Toxicity Data: Acute oral: >5,000 mg/kg; Ames test: Negative; TLm 48 hours (Orange medaka): >1,000 ppm.

Exposure: Processing: dermal.

Environmental Release/Disposal. No data submitted.

P 85-1445

Manufacturer. Confidential.

Chemical. (G) Urethane-modified polyester-acrylic copolymer.

Use/Production. (S) Site-limited and industrial resin used in automotive paint. Prod. range. 70,000-210,000 kg/yr.

Toxicity Data: No data submitted.

Exposure: Manufacture and processing: dermal and inhalation, a total of 6 workers, up to 0.5 hrs/da, up to 250 da/yr.

Environmental Release/Disposal. 0.2 to 180 kg/batch of resin and 0.2 to 1 kg/batch of paint released. Disposal by landfill.

P 85-1446

Manufacturer: Miranol Chemical Company, Inc.

Chemical. Further clarification needed before information can be released to public files.

Use/Production. (S) Heavy-duty concentrated alkaline cleaners, vibratory barrel cleaning, polishing and solvent emulsion cleaners for industrial, commercial and consumer use. Prod. range. 30,000-100,000 kg/yr.

Toxicity Data: No data submitted.

Exposure: Manufacture: a total of 5 workers, up to 2 hrs/da, up to 7 da/yr.

Environmental Release/Disposal. 4 kg/batch released to water. Disposal by POTW.

P 85-1447

Manufacturer: Confidential.

Chemical. (S) Polymer of methyl methacrylate, ethyl acrylate and 2-hydroxy ethyl acrylate.

Use/Production. (G) Used in an open system. Prod. range. 20,000-32,000 kg/yr.

Toxicity Data: No data submitted.

Exposure: Manufacture dermal, a total of 6 workers, up to 1 hr/da, up to 20 da/yr.

Environmental Release/Disposal. Minimal release to air. Disposal by licensed landfill.

P 85-1448

Manufacturer: Ashland Chemical Company.

Chemical. Further clarification needed before information can be released to public files.

Use/Production. (G) Adhesive component/open, non-dispersive use. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Manufacture and processing: dermal, a total of 6 workers, up to 2 hrs/da, up to 50 da/yr.

Environmental Release/Disposal. Release to land. Disposal by incineration or secure landfill.

P 85–1449

Manufacturer. Confidential.

Chemical. (G) Polymer of bisanhydride of Bisphenol-A, aliphatic diamine and an aromatic diamine.

Use/Production. (S) Electronics/appliances and transportation for industrial, commercial and consumer use. Prod. range. 200,000–900,000 kg/yr.

Toxicity Data. No data submitted.

Exposure. Manufacture: dermal, a total of 24 workers, up to 8 hrs/da, up to 100 da/yr.

Environmental Release/Disposal. Trace to 5 kg/batch released to land. Disposal by POTW and navigable waterway.

P85–1450

Manufacturer. Confidential.

Chemical. (S) Ethylene glycol benzoate trialkylacetate with carbon numbers C_{18}–C_{19}.

Use/Production. (G) Plasticizer for polyvinyl chloride. Prod. range. Confidential.

Toxicity Data. No data on the PMN substance submitted.

Exposure. Manufacture: dermal.

Environmental Release/Disposal. Release to air, water and land. Disposal by secondary biological treatment.

P 85–1451

Manufacturer. Quality Chemicals, Inc.

Chemical. (S) 2,2′, 4-tris-(2-chlorophenyl)-5-(3,4-dimethoxyphenyl)-4′, 5′-diphenyl-1,1′ bi-1*H*-imidazole.

Use/Production. (S) Site-limited photographic chemical. Prod. range. 5,000–8,000 kg/yr.

Toxicity Data. No data submitted.

Exposure. Manufacture: dermal, a total of 6 workers, up to 8 hrs/da, up to 8 da/yr.

Environmental Release/Disposal. 1 to 5 kg/batch incinerated and 3 to 5 kg/batch released to sewer. Disposal by POTW.

P 85–1452

Manufacturer. Confidential.

Chemical. Further clarification needed before information can be released to the public files.

Use/Production. (G) Cross linker in resinous systems. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Confidential.

Environmental Release/Disposal. Confidential.

P 85–1453

Manufacturer. Confidential.

Chemical. Further clarification needed before information can be released to the public files.

Use/Production. (G) Cross linker in resinous systems. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Confidential.

Environmental Release/Disposal. Confidential.

P 85–1454

Manufacturer. Confidential.

Chemical. (G) Polyurea polyurethane polymer.

Use/Production. (G) Paint additive. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Confidential.

Environmental Release/Disposal. Confidential.

P 85–1455

Manufacturer. Confidential.

Chemical. (G) Fatty polyol.

Use/Import. (G) Chemical intermediate for coatings, elastomers, adhesives and foams. Import range. Confidential.

Toxicity Data. Acute oral: 5.0 g/kg; Acute dermal: 2.0 g/kg; Irritation: Skin—Non-irritant; Eye—Non-irritant.

Exposure. Use: dermal.

Environmental Release/Disposal. Disposal by landfill.

P 85–1456

Importer. Confidential.

Chemical. Further clarification needed before information can be released to the public files.

Use/Import. (S) Dye. Import range. Confidential.

Toxicity Data. No data on the PMN substance submitted.

Exposure. Use: dermal, up to 2–3 hrs/da, up to 2 da/wk.

Environmental Release/Disposal. No data submitted.

P 85–1457

Manufacturer. E. I. du Pont de Nemours & Company, Inc.

Chemical. (G) Aliphatic ammonium salt of substituted aromatic acid.

Use/Production. (G) Contained use. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Manufacture and processing: dermal, a total of 12 workers.

Environmental Release/Disposal. Confidential. Disposal by incineration.

P 85–1458

Manufacturer. E. I. du Pont de Nemours & Company, Inc.

Chemical. (G) Acrylic polymer containing aromatic carboxyesters.

Use/Production. (G) Open, non-dispersive use. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Manufacture and processing: dermal, a total of 8 workers.

Environmental Release/Disposal. Release to land. Disposal by incineration.

P 85–1459

Manufacturer. E. I. du Pont de Nemours & Company, Inc.

Chemical. (G) Acrylic polymer containing aromatic carboxyesters.

Use/Production. (G) Open, non-dispersive use. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Manufacture and processing: dermal, a total of 9 workers.

Environmental Release/Disposal. Release to land. Disposal by incineration and landfill.

P 85–1460

Manufacturer. Confidential.

Chemical. (S) Polymer of bis-(4-phenoxyphenyl) methanone and 1,4-benzenedicarbonyl dichloride.

Use/Production. (S) Thermoplastic engineering resin for extrusion of fiber, film, wire and cable insulation, tubing and other profiles, molding of connectors, valves seats, seals, crimp devices, and other molded shapes for industrial, commercial and consumer use. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Confidential.

Environmental Release/Disposal. Confidential. Disposal by POTW.

P 85–1461

Manufacturer. Confidential.

Chemical. (S) Bis(4-phenoxyphenyl) methanone.

Use/Production. (S) Site-limited and industrial monomer for the production of aromatic polyketones. Prod. range. Confidential.

Toxicity Data. No data on the PMN substance submitted.

Exposure. Confidential.

Environmental Release/Disposal. No release.

P 85-1462

Importer. Confidential.

Chemical. Further clarification needed before information can be released to the public files.

Use/Import. (S) Industrial production of urethane-elastomer articles. Import range. Confidential.

Toxicity Data. No data submitted.

Exposure. No data submitted.

Environmental Release/Disposal. No data submitted.

P 85-1463

Importer. Confidential.

Chemical. Further clarification needed before information can be released to the public files.

Use/Import. (S) Industrial production or urethane-elastomer articles. Import range. 2,000–5,000 kg/yr.

Toxicity Data. No data submitted.

Exposure. No data submitted.

Environmental Release/Disposal. No data submitted.

P 85-1464

Importer. Ciba-Geigy Corporation.

Chemical. (G) Substituted pyrazol azo benzene sulfonic acid.

Use/Import. (G) Textile dye. Import range. 4,700 kg/yr.

Toxicity Data. Acute oral: 2,404 mg/kg; Irritation: Skin—Moderate; Eye—Slight; BOD_5: 130 mg/gO_2; Waste treatment plant bacteria: >3,000 mg/l; Inhalation: TL_{50} (Trout): 175 mg/L; Ames test: Negative; COD: 1,064 mg/gO_2.

Exposure. Processing: inhalation, a total of 2 workers, up to 0.5 hr/da, up to 2 da/yr.

Environmental Release/Disposal. 0.3 Kg/batch released to water. Disposal by navigable waterway.

P 85-1465

Manufacturer. Confidential.

Chemical. (G) (Dialkylamino, alkyl)aryl and N-(alkysulfonyl amino and halo)phenyl substituted oxopentanamide.

Use/Production. (G) Contained use in an article. Prod. range. 3,000 kg/yr.

Toxicity Data. No data submitted.

Exposure. Manufacture and processing: a total of 92 workers, up to 1.2 hrs/da, up to 7 da/yr.

Environmental Release/Disposal. Less than 0.07 ppm/batch released to water. Less than 8 to >20 kg/batch incinerated with disposal by navigable waterway.

P 85-1466

Manufacturer. Confidential.

Chemical. (G) (Dialkyl)phenoxy, (N-dialkylamino and alkyl)aryl and (cyanophenylureido)aryl substituted hexanamide.

Use/Production. (G) Contained use in article. Prod. range. 600 kg/yr.

Toxicity Data. No data on the PMN substance submitted.

Exposure. Manufacture and processing: dermal and inhalation, a total of 88 workers, up to 1.5 hrs/da, up to 5 da/yr.

Environmental Release/Disposal. Less than 0.02 ppm/batch released to water. Less than 2 to >6 kg/batch incinerated with disposal by navigable waterway.

P 85-1467

Manufacturer. Product Research & Chemical Corporation.

Chemical. (S) Polymer of Spenkel P49-A6-60 and diethyl toluene diamine.

Use/Production. (S) Industrial polyurethane elastomer curing agent. Prod. range. 14,000–25,000 kg/yr.

Toxicity Data. No data on the PMN substance submitted.

Exposure. Manufacture: dermal, a total of 20 workers, up to 3.0 hrs/da, up to 70 da/yr.

Environmental Release/Disposal. 2 kg/batch released to land. Disposal by approved landfill.

P 85-1468

Manufacturer. The Dow Chemical Company.

Chemical. Further clarification needed before information can be released to the public files.

Use/Production. (S) Commercial coatings. Prod. range. Confidential.

Toxicity Data. Acute oral: >2,000 g/kg; Acute dermal: >2,000 mg/kg; Irritation: Skin—Non-irritant; Eye—Non-irritant;

Exposure. Manufacture: dermal.

Environmental Release/Disposal. Less than 1 to >5 kg/batch released to air and land. Disposal by incineration and landfill.

P 85-1469

Manufacturer. The Dow Chemical Company.

Chemical. Further clarification needed before information can be released to the public files.

Use/Production. (S) Commercial coatings. Prod. range. Confidential.

Toxicity Data. Acute oral: >2,000 g/kg; Acute dermal: >2,000 mg/kg; Irritation: Skin—Non-irritant; Eye—Non-irritant.

Exposure. Manufacture: dermal.

Environmental Release/Disposal. Less than 1 to >5 kg/batch released to air and land. Disposal by incineration and landfill.

P 85-1470

Manufacturer. The Dow Chemical Company.

Chemical. (G) Methylene diphenylene diisocyanate polyol prepolymer.

Use/Production. (S) Polyurethane elastomers for industrial components. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. dermal and inhalation.

Environmental Release/Disposal. Less than 1 kg/batch released to air. Disposal by incineration.

P 85-1471

Manufacturer. The Dow Chemical Company.

Chemical. (G) Methylene diphenylene diisocyanate polyol prepolymer.

Use/Production. (S) Polyurethane elastomers for industrial components. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Manufacturer: dermal and inhalation.

Environmental Release/Disposal. Less than 1 kb/batch released to air. Disposal by incineration.

P 85-1472

Manufacturer. The Dow Chemical Company.

Chemical. (G) Methylene diphenylene diisocyanate polyol prepolymer.

Use/Production. (S) Polyurethane elastomers for industrial components. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Manufacturer: dermal and inhalation.

Environmental Release/Disposal. Less than 1 kb/batch released to air. Disposal by incineration.

P 85-1473

Manufacturer. The Dow Chemical Company.

Chemical. (G) Methylene diphenylene diisocyanate polyol prepolymer.

Use/Production. (S) Polyurethane elastomers for industrial components. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Manufacturer: dermal and inhalation.

Environmental Release/Disposal. Less than 1 kb/batch released to air. Disposal by incineration.

P 85-1474

Manufacturer. The Dow Chemical Company.

Chemical. (G) Polyumeric diisocyanate polyol prepolymer.

Use/Production. (S) Polyurethane elastomers for industrial components. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Manufacturer: dermal and inhalation.

Environmental Release/Disposal. Less than 1 kb/batch released to air. Disposal by incineration.

P 85–1475

Manufacturer. The Dow Chemical Company.

Chemical. (G) Methylene diphenylene diisocyanate polyol prepolymer.

Use/Production. (S) Polyurethane elastomers for industrial components. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Manufacturer: dermal and inhalation.

Environmental Release/Disposal. Less than 1 kb/batch released to air. Disposal by incineration.

P 85–1476

Manufacturer. The Dow Chemical Company.

Chemical. (G) Methylene diphenylene diisocyanate polyol prepolymer.

Use/Production. (S) Polyurethane elastomers for industrial components. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Manufacturer: dermal and inhalation.

Environmental Release/Disposal. Less than 1 kb/batch released to air. Disposal by incineration.

P 85–1477

Manufacturer. The Dow Chemical Company.

Chemical. (G) Methylene diphenylene diisocyanate polyol prepolymer.

Use/Production. (S) Polyurethane elastomers for industrial components. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Manufacture: dermal and inhalation.

Environmental Release/Disposal. Less than 1 kg/batch released to air. Disposal by incineration.

P 85–1478

Manufacturer. The Dow Chemical Company.

Chemical. (G) Acyclic polymaine.

Use/Production. (S) Industrial chemical intermediate. Prod. range. Confidential.

Toxicity Data. Acute oral: Between 500–1,000 mg/kg; Irritation: Skin—Irritant.

Exposure. Manufacture: dermal.

Environmental Release/Disposal. Release to air, water and land. Disposal by incineration, landfill and navigable waterway after treatment.

P 85–1479

Manufacturer. Confidential.

Chemical. (G) Mixed alkyl phosphate ester.

Use/Production. (G) An additive used in the energy production industry. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Manufacturer: dermal, a total of 1 worker, up to 3–4 hrs/da.

Environmental Release/Disposal. 25 kg/da released.

P 85–1480

Manufacturer. Condidential.

Chemical. (G) Substituted bis(phenyl)isobenzofuranone.

Use/Production. (G) Captive intermediate used in manufacturing a minor component for paper coatings. Prod. range. Confidential.

Toxicity Data. No data submitted.

Exposure. Confidential.

Environmental Release/Disposal. Confidential. Disposal by POTW

P 85–1481

Importer. Urethane Concepts, Inc.

Chemical. (G) Polyether triol.

Use/Import. (S) Industrial polyol component in rigid polyurethane foam. Import range. Confidential.

Toxicity Data. No data submitted.

Exposure. Processing: dermal, a total of 10–50 workers, up to 8 hrs/da, up to 240 da/yr.

Environmental Release/Disposal. No data submitted

P 85–1482

Importer. Urethane Concepts, Inc.

Chemical. Further clarification needed before information can be released to the public files.

Use/Import. (S) Industrial viscosity aid in polyurethane foam and polyol in polyurethane elastomer production. Import range. Confidential.

Toxicity Data. No data submitted.

Exposure. Processing: dermal, a total of 10–50 workers, up to 8 hrs/da, up to 240 da/yr.

Environmental Release/Disposal. No data submitted.

P 85–1483

Importer. Urethane Concepts, Inc.

Chemical. Further clarification needed before information can be released to the public files.

Use/Import. (S) Manufacturing of flexible and rigid polyurethane foam. Import range. Confidential.

Toxicity Data. No data submitted.

Exposure. Processing: dermal, a total of 10–50 workers, up to 8 hrs/da, up to 240 da/yr.

Environmental Release/Disposal. No data submitted.

P 85–1484

Importer. Urethane Concepts, Inc.

Chemical. Further clarification needed before information can be released to the public files.

Use/Import. (S) Industrial viscosity cutter in spray foam formultion. Import range. Confidential.

Toxicity Data. No data submitted.

Exposure. Processing: dermal, a total of 10–50 workers, up to 8 hrs/da, up to 240 da/yr.

Environmental Release/Disposal. No data submitted.

P–1485

Importer. Urethane Concepts, Inc.

Chemical. Further clarification needed before information can be released to public files.

Use/Import. (S) Industrial additive for flame laminable flexible polyether foam. Import range. Confidential.

Toxicity Data. No data submitted.

Exposure. Processing: dermal, a total of 10–50 workers, up to 8 hrs/da, up to 240 da/yr.

Environmental Release/Disposal. No data submitted.

P 85–1486

Importer. Urethane Concepts, Inc.

Chemical. Further clarification needed before information can be released to the public files.

Use/Import. (S) Industrial flame retardant additive in rigid foam manufacture. Import range. Confidential.

Toxicity Data. No data submitted.

Exposure. Processing: dermal, a total of 10–50 workers, up to 8 hrs/da, up to 240 da/yr.

Environmental Release/Disposal. No data submitted.

P 85–1487

Importer. Urethane Concepts, Inc.

Chemical. Further clarification needed before information can be released to the public files.

Use/Import. (S) Industrial polyol component in rigid polyurethane foam. Import range. Confidential.

Toxicity Data. No data submitted.

Exposure. Processing: dermal, a total of 10–50 workers, up to 8 hrs/da, up to 240 da/yr.

Environmental Release/Disposal. No data submitted.

85–1488

Importer. Urethane Concepts, Inc.

Chemical. Further clarification needed before information can be released to the public files.

Use/Import. (S) Industrial polyol component in rigid polyurethane foam. Import range. Confidential.

Toxicity Data. No data submitted.

Exposure. Processing: dermal, a total of 10–50 workers, up to 8 hrs/da, up to 240 da/yr.

Environmental Release/Disposal. No data submitted.

P 85–1489

Importer. Urethane Concepts, Inc.

Chemical. (G) Polyether triol.

Use/Import. (S) Industrial polyol component in rigid polyurethane foam. Import range. Confidential.

Toxicity Data. No data submitted.

Exposure. Processing: dermal, a total of 10–50 workers, up to 8 hrs/da, up to 240 da/yr.

Environmental Release/Disposal. No data submitted.

Dated: September 23, 1985.

Linda A. Travers,

Acting Director, Information Management Division.

[FR Doc. 85–23115 Filed 9–26–85; 8:45]

BILLING 6560-50-M

[OPP-30081B; PH-FRL 2904-4]

Cypermethrin; Extension of Conditional Registration

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice.

SUMMARY: This notice amends the conditional registrations, pursuant to section 3(c)(7)(C) of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), for products containing the synthetic pyrethroid cypermethrin for use on cotton to control various cotton insects. The Agency has determined that the benefits of use will outweight the risks during the period of the extension of the conditional registration and that the extension is in the public interest.

EFFECTIVE DATE: September 27, 1985.

ADDRESS: Written comments may be submitted to the: Hearing Clerk (A–110), Environmental Protection Agency, Room 3708, 401 M Street SW., Washington, D.C. 20460.

FOR FURTHER INFORMATION CONTACT: George T. LaRocca, Product Manager (PM) 15, Registration Division, Environmental Protection Agency, 401 M Street SW., Washington, D.C. 20460

Office location and telephone number: Rm. 207, CM #2, 1921 Jefferson Davis Highway, Arlington, VA 22202 (703–557–2690).

SUPPLEMENTARY INFORMATION: In the Federal Register on January 9, 1985 (50 FR 1112), EPA announced its decision to extend ICI Americas, Inc. (ICI), and FMC Corp. (FMC) conditional registrations of products containing the synthetic pyrethroid active ingredient (±) *alpha*-cyano(3-phenoxyphenyl)methyl(±)-*cis, trans*-3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropanecarboxylate (cypermethrin) for use on cotton to control various cotton insects, for a period which extended to December 31, 1986, to allow time for the submission and evaluation of a full field study (due in April 1986). That document set forth EPA's evaluation of the data that had been submitted, the product's regulatory history, and other facts about cypermethrin.

In order to obtain the necessary data, ICI and FMC had conducted a 1-year baseline study on a proposed test site in 1984 in the State of Maryland. On May 10, 1985, ICI was issued an experimental use permit (EUP) to allow for further testing in the State of Maryland. The State of Maryland Department of Agriculture denied ICI's request for further testing of cypermethrin in Maryland until the safe use of cypermethrin can be demonstrated to the satisfaction of the State Secretary of Agriculture. The action by the State of Maryland precipitates the need for initiating a new study (including baseline data), making it impossible to meet the previous deadline of April 1986. On June 12, 1985 and June 14, 1985, ICI and FMC, respectively, submitted a letter to the Agency requesting an extension of time to December 31, 1988, for submittal of the field-monitoring study.

To further support the request for an extension of time, on July 1, 1985, ICI submitted a letter to EPA with justification for the time periods requested and a letter from the State of Alabama giving ICI permission to conduct the full field study in the State of Alabama.

Based on the information submitted by ICI and FMC and all available data, the Agency has determined that the requested extensions are proper for consideration under FIFRA section 3(c)(7)(C). Therefore, EPA concluded that the expiration date for the registrations should be extended to December 1, 1988, to allow time for the submission and evaluation of the full field study and an EPA decision whether the products may be registered under FIFRA section 3(c)(5).

EPA concluded that use of cypermethrin will not cause unreasonable adverse effects to the environment during the period of conditional registration, and the Agency finds that to extend the conditional registration of cypermethrin is in the public interest.

In accordance with section 3(c)(2) of FIFRA, a copy of the approval label and the list of data references used to support the continued conditional registration are available for public inspection in the office of the Product Manager listed above. The data and other scientific information used to support registration, except for the material specifically protected by section 10 of FIFRA, are available for public inspection in the Program Management and Support Division (TS-769C), Office of Pesticide Programs, Environmental Protection Agency, Rm. 236, CM#2, Arlington, VA 22202. Request for data must be made in accordance with the provisions of the Freedom of Information Act and must be addressed to the Freedom of Information Office (A–101), 401 M Street SW., Washington, D.C. 20460. Such requests should identify the product names and registration number(s) and specify the data or information desired.

Dated: September 17, 1985.

Steven Schatzow,

Director, Office of Pesticide Programs.

[FR Doc. 85–23114 Filed 9–26–85; 8:45 am]

BILLING CODE 6560-SD-M

[ER-FRL-2903-5]

Environmental Impact Statements; Availability

Responsible agency: Office of Federal Activities, General Information (202) 382–5073 or (202) 382–5075. Availability of Environmental Impact Statements filed September 16, 1985 through September 20, 1985 pursuant to 40 CFR 1506.9.

EIS No. 850395, Final, BLM, ID, NV, Jarbidge Resource Area, Resource Management Plan and Wilderness Designation, Due: October 28, 1985, Contact: Ted Milesnick (208) 334–1582.

EIS No. 850396, Final, FHW, MI, M-44/East Beltline Avenue Reconstruction, I-96 to Plainfield Avenue, Kent County, Due: October 28, 1985, Contact: Ken Barkema (517) 377–1852.

EIS No. 850397, Final, COE, TX, Wright Patman Lake and Dam (Formerly Texarkana Lake), Operation and Maintenance, Sulphur River, Bowie, Cass, Morris, Titus and Red River Counties, Due: October 28, 1985, Contact: Joe Paxton (817) 334–2095.

EIS No. 850398, Final, IBR, SD, Lake Andes-Wagner Unit, Water Resource Project, Pick-Sloan Missouri Basin Program, Approval, Lake Francis Case, Missouri River, Charles Mix Co. Due: October 28, 1985, Contact: John Lawson (406) 657–6164.

EIS No. 850399, Final, FHW, TX, US 183 Upgrading, TX–71 to Ranch to

Market Road 620, Travis and Williamson Counties, Due: October 28, 1985, Contact: Gamaliel E. Olvera (512) 482–5966.

EIS No. 850400, Final, FHW, NC, Benjamin Parkway Extension, Benjamin Parkway to Airport Parkway, Guilford County, Due: October 28, 1985, Contact: Kenneth Bellamy (919) 755–4346.

EIS No. 850401, Final, SCS, MO, Big Creek-Hurricane Creek Watershed Protection and Flood Prevention Plan, Carroll and Livingston Cos., Due: October 28, 1985, Contact: Paul Larson (314) 875–5214.

EIS No. 850402, Draft, BLM, WY, MT, ND, SD, Bairoil/Dakota Carbon Dioxide Projects, Approval, R–O–W Grants and Issuance of Permits, November 12, 1985, Contact: Janis VanWyhe (303) 236–1080.

EIS No. 850403, Draft, COE, OR, West Hayden Island Marine Industrial Park Development, Section 10 and 404 Permits, Multnomah County, Due: November 12, 1985, Contact: David Kurkoski (503) 221–6094.

EIS No. 850404, Final, AFS, MS, Mississippi National Forests, Land and Resource Management Plan, Due: October 28, 1985, Contact: John Alcock (404) 881–4177.

EIS No. 850405, Final, UMT, IL, Chicago Southwest Corridor Transit Improvements, Cook County, Due: October 28, 1985, Contact: James Ryan (202) 426–9271.

EIS No. 850406, Final, EPA, MD, Parkway Wastewater Treatment Facilities Upgrading, Grant, Prince George's County, Due: October 28, 1985, Contact: Tom Slenkamp (215) 597–9169.

EIS No. 850407, DSuppl, AFS, IN, Hoosier Natl. Forest, Land and Resource Mgmt. Plan, Off-Road Vehicle Policy, Due: December 27, 1985, Contact: Harold Godlevske (812) 275–5987.

EIS No. 850408, Final, AFS, IN, Hoosier Natl. Forest, Land and Resource Management Plan, Due: October 28, 1985, Contact: Harold Godlevske (812) 275–5987.

EIS No. 850409, Final, BLM, OR, Two Rivers Planning Area, Resource Mgmt. Plan, John Day and Deschutes Rivers, Due: November 15, 1985, Contact: Brian Cunninghame (503) 447–4115.

EIS No. 850410, FSuppl, FERC, AK, Bradley Lake Hydroelectric Project, Construction and Operation, License, Kenai Peninsula, Due: October 28, 1985, Contact: Peter Foo'te (202) 376–9053.

EIS No. 850411, Final, OSM, NM, La Plata Mine Mining and Transportation Corridor Plan, Approval and Permit, San Juan County, Due: October 28, 1985, Contact: Allen Klein (303) 844–5656.

EIS No. 850412, Draft, BLM, AZ, Eastern Arizona Grazing Management Program, Due: December 6, 1985, Contact: Jerrold Coolidge (602) 428–4040.

Dated: September 24, 1985.

Allan Hirsch,

Director, Office of Federal Activities.

[FR Doc. 85–23197 Filed 9–26–85; 8:45 am]

BILLING CODE 6560–50–M

[ER–FRL–2903–6]

Environmental Impact Statements and Regulations; Availability of EPA Comments

Availability of EPA comments prepared September 9, 1985 through September 13, 1985 pursuant to the Environmental Review Process (ERP) under section 309 of the Clean Air Act and section 102(2)(c) of the National Environmental Policy Act as amended. Requests for copies of EPA comments can be directed to the Office of Federal Activities at (202) 382–5075/76. An explanation of the ratings assigned to draft environmental impact statements (EISs) was published in FR dated October 19, 1984 (49 FR 41108).

Draft EISs

ERP No. D–CDB–C89023–NY, Rating LO, Rochester Science Park Development, Expansion and Replacement, CDBG, NY. Summary: EPA stated that it has no objections to the project as proposed. EPA did recommend that Alter. B be selected as the preferred alternative, although EPA believes that either alternative would not create significant adverse environmental impacts. Mitigation measures, as proposed, would provide adequate safeguards for the protection of the environment.

ERP No. D–COE–G36030–LA, Rating EC1, Aloha—Rigolette Area Agriculture Flood Control Plan, expressed environmental concerns with the proposed action as described. EPA is not opposed to the implementation of the flood damage reduction features of the preferred action; however, EPA believes the inclusion of the no development easements in the plan would significantly reduce the potential for increased flooding loss to life and human resources, substantially increase the overall environmental benefits, and clearly establish a reliable mechanism to protect and preserve the remaining bottomland hardwoods in the project area. EPA, therefore, supports Plan 19C and recommends that it be selected as the preferred action.

ERP No. D–FHW–D40209–PA, Rating EC2, I–95 Completion, Between Benjamin Franklin and Walt Whitman Bridges, Right-of-Way Improvements, PA. Summary: EPA's review of the DEIS identified a number of environmental concerns and found an incomplete discussion of the alternatives and their benefit/cost ratios. EPA suggested a supplement to the DEIS with a more detailed discussion of the alternatives.

ERP No. D–FHW–L40148–OR, Rating EC2, 185th Ave. Improvements, Rock Creek Blvd. to Tualatin Valley Hwy., 404 Permit, OR. Summary: EPA's review indicated that the project could have significant traffic noise impacts and requested that additional noise levels estimates and mitigation measures be included in the FEIS. Growth induced nonpoint source water pollution impacts were also requested to be evaluation. Park, Old Wickford, Naval Gardens, and Gould Island, Disposal of Surplus Gov't Property, RI. Summary: EPA requested that the Supplemental Final EIS identify the toxic and hazardous waste sites on Gould Island, address a schedule for clean up, and a commitment to conduct the approved remedial action. EPA also recommended that the negotiated sale for the Hoskins Park Housing Area include conditions such as conservation easements to protect surrounding wetlands; cite the federal, state and local laws, and ordinances in place to protect the wetlands; and, require that the Hoskins Park pumping facility be operational before occupany of the development.

ERP No. RD–NHT–A52159–00, Rating LO, 1986 Model Year Passenger Cars, Corporate Average Fuel Economy Stds. Summary: While agreeing with the conclusion that the proposed action should have little impact on air quality, EPA suggested that additional data that supports the conclusion be included in the Final EIS.

Final EISs

ERP No. F–AFS–E65028–SC, Sumter Nat'l Forest, Land and Resource Mgmt. Plan, SC. Summary: EPA's concerns expressed in its comments on the DEIS have been adequately addressed in the Final EIS.

ERP No. F–FHW–D40195–DC, Whitehurst Freeway/US 29 Corridor Modifications, Improvement or Replacement, Washington, DC. Summary: EPA's review found the FEIS to eliminate all previous concerns and there are now no objections to the project implementation. Construction, Cairo Bridge to Reelfoot Lake, 404 Permit, KY. Summary: EPA requested that additional information be presented concerning possible water quality/ wetland impacts before the Federal Highway Adminsitration completes the Record of Decision.

ERP No. F–FHW–F40216–IL, Lake Front Hwy./FAP 437 Construction, IL Tri-State Tollway/I–94 to Grand Ave./ IL–132, 404 Permit, IL. Summary: EPA's review of the FEIS did not identify any significant environmental impacts requiring changes to the proposed project.

ERP No. F–FHW–K40104–CA, CA–55/ Newport Blvd. Improvement, CA–1/ Pacific Coast Hwy. to US 73/Corona Del Mar Freeway, 404 Permit, CA. Summary: The FEIS adequately responded to EPA's comments made on the DEIS.

ERP No. F–FHW–K40124–CA, I–5/ Santa Ana Freeway and CA–55/ Newport-Costa Mesa Freeway Improvements, I–5 and I–55 Interchange Reconstruction, CA–22 to I–405, CA. Summary: The DEIS adequately responded to EPA comments on the DEIS.

ERP No. F–FHW–L40133–OR, Murray Blvd. Widening, Sunset Hwy./US 26 to Jenkins Rd., Improvements, Right-of-Way Acquisition, OR. Summary: EPA made no formal comments. EPA reviewed the FEIS and found the project to be satisfactory.

ERP No. F–SFW–L64028–AK, Alaska Peninsular Nat'l Wildlife Refuge Mgmt., Comprehensive Conservation Plan and Wilderness Designation, AK. Summary: EPA made no formal comments. EPA reviewed the FEIS and fond the project to be satisfactory.

Dated: Septemer 24, 1985.

Allan Hirsch,

Director, Office of Federal Activities.

[FR Doc. 85–23198 Filed 9–26–85; 8:45 am]

BILLING CODE 6560–50–M

FEDERAL HOME LOAN BANK BOARD

Glen Ellyn Savings & Loan Association, Glen Ellyn, IL; Appointment of Receiver

Notice is hereby given that pursuant to the authority contained in section 406(c)(1)(B) of the National Housing Act, as amended, 12 U.S.C. 1729(c)(1)(B) (1982), the Federal Home Loan Bank Board appointed the Federal Savings and Loan Insurance Corporation as sole receiver for Glen Ellyn Savings and Loan Association, Glen Ellyn, Illinois, on September 20, 1985.

Dated: September 23, 1985.

Nadine Y. Penn,

Acting Secretary.

[FR Doc. 85–23100 Filed 9–26–85; 8:45 am]

BILLING CODE 6720–01–M

FEDERAL RESERVE SYSTEM

Bank of Boston Corporation et al.; Applications To Engage de Novo in Permissible Nonbanking Activities

The companies listed in this notice have filed an application under § 225.23(a)(1) of the Board's Regulation Y (12 CFR 225.23(a)(1)) for the Board's approval under section 4(c)(8) of the Bank Holding Company Act (12 U.S.C. 1843(c)(8)) and § 225.21(a) of Regulation Y (12 CFR 225.21(a)) to commence or to engage *de novo*, either directly or through a subsidiary, in a nonbanking activity that is listed in § 225.25 of Regulation Y as closely related to banking and permissible for bank holding companies. Unless otherwise noted, such activities will be conducted throughout the United States.

Each application is available for immediate inspection at the Federal Reserve Bank indicated. Once the application has been accepted for processing, it will also be available for inspection at the offices of the Board of Governors. Interested persons may express their views in writing on the question whether consummation of the proposal can "reasonably be expected to produce benefits to the public, such as greater convenience, increased competition, or gains in efficiency, that outweigh possible adverse effects, such as undue concentration of resources, decreased or unfair competition, conflicts of interests, or unsound banking practices." Any request for a hearing on this question must be accompanied by a statement of the reasons a written presentation would not suffice in lieu of a hearing, identifying specifically any questions of fact that are in dispute, summarizing the evidence that would be presented at a hearing, and indicating how the party commenting would be aggrieved by approval of the proposal.

Unless otherwise noted, comments regarding the applications must be received at the Reserve Bank indicated or the offices of the Board of Governors not later than October 17, 1985.

A. Federal Reserve Bank of Boston (Richard E. Randall, Vice President) 600 Atlantic Avenue, Boston, Massachusetts 02106:

1. *Bank of Boston Corporation*, Boston, Massachusetts; to engage *de novo* through its subsidiary, BancBoston FBC Inc., Boston, Massachusetts, in data processing services and management consulting services to depository institutions pursuant to § 225.25(b) (7) and (11), respectively, of Regulation Y.

B. Federal Reserve Bank of New York (William L. Rutledge, Vice President) 33 Liberty Street, New York, New York 10045:

1. *Irving Bank Corporation*, New York, New York; to engage *de novo* through its subsidiary, Irving Trust Company Florida, Miami, Florida, in performing or carrying on any one or more of the functions or activities that may be performed or carried on by a trust company, including activities of a fiduciary, investment advisory, agency or custodial nature, in the manner authorized by Florida law.

C. Federal Reserve Bank of Kansas City (Thomas M. Hoenig, Vice President) 925 Grand Avenue, Kansas City, Missouri 64198:

1. *J.R. Montgomery Bancorporation* Lawton, Oklahoma; to engage *de novo* through its subsidiary, JRMB Insurance Company, Lawton, Oklahoma, in acting as agent with respect to insurance limited to assuring repayment of the outstanding balance due on a specific extension of credit by a bank holding company or its subsidiary in the event of the death of the debtor, pursuant to section 4(c)(8)(A) of the Act.

Board of Governors of the Federal Reserve System, September 23, 1985.

James McAfee,

Associate Secretary of the Board.

[FR Doc. 85–23179 Filed 9–26–85; 8:45 am]

BILLING CODE 6210–01–M

Pennbancorp et al.; Formations of; Acquisitions by; and Mergers of Bank Holding Companies

The companies listed in this notice have applied for the Board's approval under section 3 of the Bank Holding Company Act (12 U.S.C. 1842) and § 225.14 of the Board's Regulation Y (12 CFR 225.14) to become a bank holding company or to acquire a bank or bank holding company. The factors that are considered in acting on the applications are set forth in section 3(c) of the Act (12 U.S.C. 1842(c)).

Each application is available for immediate inspection at the Federal Reserve Bank indicated. Once the application has been accepted for processing, it will also be available for inspection at the offices of the Board of Governors. Interested persons may express their views in writing to the Reserve Bank or to the offices of the Board of Governors. Any comment on an application that requests a hearing must include a statement of why a written presentation would not suffice in lieu of a hearing, identifying specifically any questions of fact that are in dispute and summarizing the evidence that would be presented at a hearing.

Unless otherwise noted, comments regarding each of these applications must be received not later than October 18, 1985.

A. **Federal Reserve Bank of Cleveland** (Lee S. Adams, Vice President) 1455 East Sixth Street, Cleveland, Ohio 44101:

1. *Pennbancorp*, Titusville, Pennsylvania; to merge with GNB Corporation, Uniontown, Pennsylvania. Comments on this application must be received not later than October 21, 1985. Comments on this application must be received not later than October 21, 1985.

B. **Federal Reserve Bank of Chicago** (Franklin D. Dreyer, Vice President) 230 South LaSalle Street, Chicago, Illinois 60690:

1. *Rosendale Bancshares, Inc.*, Rosendale, Wisconsin; to become a bank holding company by acquiring at least 89.72 percent of the voting shares of Rosendale State Bank, Rosendale, Wisconsin.

C. **Federal Reserve Bank of Dallas** (Anthony J. Montelaro, Vice President) 400 South Akard Street, Dallas, Texas 75222:

1. *Lamar Financial Corporation*, Paris, Texas; to become a bank holding company by acquiring 80 percent of the voting shares of Lamar National Bank, Paris, Texas.

2. *Rockdale Bancshares, Inc.*, Rockdale, Texas; to become a bank holding company by acquiring 100 percent of the voting shares of Community Bank and Trust, Rockdale, Texas.

D. **Federal Reserve Bank of San Francisco** (Harry W. Green, Vice President) 101 Market Street, San Francisco, California 94105:

1. *United American Bancorp*, Shelton, Washington; to become a bank holding company by acquiring 100 percent of the voting shares of Hood Canal State Bank, Shelton, Washington. Comments on this application must be received not later than October 21, 1985.

Board of Governors of the Federal Reserve System, September 23, 1985.

James McAfee,

Associate Secretary of the Board.

[FR Doc. 85-23180 Filed 9-26-85; 8:45 am]

BILLING CODE 6210-01-M

Post-Och Kreditbanken, Pkabanken; Application To Engage de Novo in Permissible Nonbanking Activities

The company listed in this notice has filed an application under § 225.23(a)(1) of the Board's Regulation Y (12 CFR 225.23(a)(1)) for the Board's approval under section 4(c)(8) of the Bank Holding Company Act (12 U.S.C. 1843(c)(8)) and § 225.21(a) of Regulation Y (12 CFR 225.21(a)) to commence or to engage *de novo*, either directly or through a subsidiary, in a nonbanking activity that is listed in § 225.25 of Regulation Y as closely related to banking and permissable for bank holding companies. Unless otherwise noted, such activities will be conducted throughout the United States.

The application is available for immediate inspection at the Federal Reserve Bank indicated. Once the application has been accepted for processing, it will also be available for inspection at the offices of the Board of Governors. Interested persons may express their views in writing on the question whether consummation of the proposal can "reasonably be expected to produce benefits to the public, such as greater convenience, increased competition, or gains in efficiency, that outweigh possible adverse effects, such as undue concentration of resources, decreased or unfair competition, conflicts of interests, or unsound banking practices." Any request for a hearing on this question must be accompanied by a statement of the reasons a written presentation would not suffice in lieu of a hearing, identifying specifically any questions of fact that are in dispute, summarizing the evidence that would be presented at a hearing, and indicating how the party commenting would be aggrieved by approval of the proposal.

Unless otherwise noted, comments regarding the application must be received at the Reserve Bank indicated or the offices of the Board of Governors not later than October 16, 1985.

A. **Federal Reserve Bank of New York** (William L. Rutledge, Vice President) 33 Liberty Street, New York, New York 10045:

1. *Post-Och Kreditbanken, Pkbanken*, Stockholm, Sweden; to engage *de novo* through its subsidiary, Pkfinans International Corporation, Greenwich, Connecticut, in making, or acquiring, for its own account or for the account of others, commercial loans and other extensions of credit as are made by a commercial finance, equipment, finance or factoring company, including *inter alia* equipment, inventory and accounts receivable financing; making leases of real and personal property that are the functional equivalent of extensions of credit; acting as agent, broker or adviser with respect to such financing and leasing activities; and servicing loans and other extensions of credit.

Board of Governors of the Federal Reserve System, September 23, 1985.

James McAfee,

Associate Secretary of the Board.

[FR Doc. 85-23181 Filed 9-26-85; 8:45 am]

BILLING CODE 6210-01-M

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Office of the Secretary

Agency Forms Submitted to the Office of Management and Budget for Clearance

Each Friday the Department of Health and Human Services (HHS) publishes a list of information collection packages it has submitted to the Office of Management and Budget (OMB) for clearance in compliance with the Paperwork Reduction Act (44 U.S.C. Chapter 35). The following are those packages submitted to OMB since the last list was published on September 20, 1985.

Health Care Financing Administration

Subject: Information Collection Requirements in 42 CFR Parts 405.1202, 1221, 1223, 1228 and 1229—Conditions of Participation for Home Health Agencies—HCFA-R-39—Extension (0938-0365).

Respondents: Businesses or other for-profit institutions, small businesses or organizations.

Subject: Medicare Contractor Administrative Budget and Cost Reporting System—HCFA-1523/1524—Revision (0938-0350/0351).

Respondents: Non-profit institutions.

Subject: Home Health Agency Cost Report—HCFA-1728—Revision (0938-0022).

Respondents: Businesses or other for-profit institutions.

OMB Desk Officer: Fay S. Iudicello.

Public Health Service

Food and Drug Administration

Subject: Infant Feeding Practice Study—New.

Respondents: Individuals or households.

OMB Desk Officer: Bruce Artim.

Health Resources and Services Administration

Subject: Health Resources and Services Administration Competing Training Grant Application and Supplements—Revision (0915-0060).

Respondents: Non-profit institutions.

Subject: Health Resources and Services Administration Noncompeting

Training Grant Application and Supplements—Revision (0915-0061).
Respondents: Non-profit institutions.
OMB Desk Officer: Fay S. Iudicello.

Office of the Secretary

Subject: Requirements Contained in Community Services Block Grant Legislation—Reinstatement.
Respondents: States and Indian tribes.
OMB Desk Officer: Judy A. McIntosh.

Copies of the above information collection clearance packages can be obtained by calling the HHS Reports Clearance Officer on 202-245-6511.

Written comments and recommendations for the proposed information collections should be sent directly to the appropriate OMB Desk Officer designated above at the following address: OMB Reports Management Branch, New Executive Office Building, Room 3208, Washington, D.C. 20503, Attn: (name of OMB Desk Officer).

Agency Forms Withdrawn from the Office of Management and Budget Clearance Process.

The Department of Health and Human Services has withdrawn the following information collection package previously submitted to OMB for approval under the Paperwork Reduction Act.

Public Health Service

Subject: Malaria Survey Among U.S. Travelers—Reinstatement (0920-0154).
Reference: Federal Register/Volume 50, No. 178/Page 37437/Friday, September 13, 1985.

Dated: September 23, 1985
K. Jacqueline Holz,
Deputy Assistant Secretary for Management Analysis and Systems.

[FR Doc. 85-23106 Filed 9-26-85; 8:45 am]
BILLING CODE 4150-04-M

Food and Drug Administration

Advisory Committees; Meetings

AGENCY: Food and Drug Administration.

ACTION: Notice.

SUMMARY: This notice announces forthcoming meetings of public advisory committees of the Food and Drug Administration (FDA). This notice also summarizes the procedures for the meetings and methods by which interested persons may participate in open public hearings before FDA's advisory committees.

MEETINGS: The following advisory committee meetings are announced:

Ophthalmic Devices Panel

Date, time, and place. October 17 and 18, 9 a.m., Auditorium, 200 Independence Ave. SW., Washington, DC.

Type of meeting and contact person. Open public hearing, October 17, 9 a.m. to 10 a.m.; open committee discussion, 10 a.m. to 1 p.m.; closed committee deliberations, 2 p.m. to 5 p.m.; open public hearing, October 18, 9 a.m. to 10 a.m.; open committee discussion, 10 a.m. to 1 p.m.; closed committee deliberations, 2 p.m. to 3 p.m.; open committee discussion, 3 p.m. to 5 p.m.; Mary Elizabeth Jacobs, Center for Devices and Radiological Health (HFZ-460), Food and Drug Administration, 8757 Georgia Ave., Silver Spring, MD 20910, 301-427-7940.

General function of committee. The committee reviews and evaluates available data on the safety and effectiveness of devices currently in use and makes recommendations for their regulation. The committee also reviews data on new devices and makes recommendations regarding their safety and effectiveness and their suitability for marketing.

Agenda—Open public hearing. Interested persons may present data, information, or views, orally or in writing, on issues pending before the committee. Those desiring to make formal presentations should notify the contact person before October 1, and submit a brief statement of the general nature of the evidence or arguments they wish to present, the names and addresses of proposed participants, and an indication of the approximate time required to make their comments.

Open committee discussion. On October 17, the committee will discuss general issues relating to approvals of premarket approval applications (PMA's) for intraocular lenses (IOL's) and neodymium:yttrium-aluminum-garnet (Nd:YAG) lasers, and may discuss specific PMA's for these devices. If discussion of all pertinent IOL or Nd:YAG laser issues is not completed, discussion will be continued the following day. On October 18, the committee will discuss PMA's for contact lenses and other ophthalmic devices and requirements for PMA approval.

Closed committee deliberations. On October 17, the committee will discuss trade secret or confidential commercial information relevant to PMA's for IOL's and Nd:YAG lasers. On October 18, the committee may discuss trade secret or confidential commercial information relevant to PMA's for contact lenses or other ophthalmic devices. These portions of the meeting will be closed to permit discussion of this information (5 U.S.C. 552b(c)(4)).

Immunology Devices Panel

Date, time, and place. October 24 and 25, 9 a.m., Rm. 703-727A, 200 Independence Ave. SW., Washington, DC.

Type of meeting and contact person. Open public hearing, October 24, 9 a.m. to 10 a.m.; open committee discussion, 10 a.m. to 2 p.m.; closed presentation of data, 2 p.m. to 5 p.m.; open committee discussion, October 25, 9 a.m. to 5 p.m.; Strikrishna Vadlamudi, Center for Devices and Radiological Health (HFZ-440), Food And Drug Administration, 8757 Georgia Ave., Silver Spring, MD 20910, 301-427-7550.

General function of the committee. The committee reviews and evaluates available data on the safety and effectiveness of devices and makes recommendation for their regulation.

Agenda—Open public hearing. Interested persons may present data, information, or views, orally or in writing, on issues pending before the committee. Those desiring to make formal presentations should notify the contact person before October 10, and submit a brief statement of the general nature of the evidence or arguments they wish to present, the names and addresses of proposed participants, and an indication of the approximate time required to make their comments.

Open committee discussion. The committee will discuss premarket approval applications for tumor marker in vitro diagnostic assays.

Closed committee deliberations. The committee will review and discuss trade secret or confidential commercial information regarding premarket approval applications for tumor marker in vitro diagnostic assays. This portion of the meeting will be closed to permit discussion of their information (5 U.S.C. 552b(c)(4)).

Each public advisory committee listed above may have as many as four separable portions: (1) An open public hearing, (2) an open committee discussion, (3) a closed presentation of data, and (4) a closed committee deliberation. Every advisory committee meeting shall have an open public hearing portion. Whether or not it also includes any of the other three portions will depend upon the specific meeting involved. The dates and times reserved for the separate portions of each committee meeting are listed above.

The open public hearing portion of each meeting shall be at least 1 hour long unless public participation does not last that long. It is emphasized, however, that the 1 hour time limit for an open public hearing represents a minimum rather than a maximum time for public participation, and an open public hearing may last for whatever longer period the committee chairman determines will facilitate the committee's work.

Public hearings are subject to FDA's guideline (Subpart C of 21 CFR Part 10) concerning the policy and procedures to expedite electronic media coverage of FDA's public administrative proceedings, including hearings before public advisory committees under 21 CFR Part 14. Under 21 CFR 10.205, representatives of the electronic media may be permitted, subject to certain limitations, to videotape, film, or otherwise record FDA's public administrative proceedings, including presentations by participants.

Meetings of advisory committees shall be conducted, insofar as is practical, in accordance with the agenda published in this **Federal Register** notice. Changes in the agenda will be announced at the beginning of the open portion of a meeting.

Any interested person who wishes to be assured of the right to make an oral presentation at the open public hearing portion of a meeting shall inform the contact person listed above, either orally or in writing, prior to the meeting. Any person attending the hearing who does not in advance of the meeting request an opportunity to speak will be allowed to make an oral presentation at the hearing's conclusion, if time permits, at the chairman's discretion.

Persons interested in specific agenda items to be discussed in open session may ascertain from the contact person the approximate time of discussion.

A list of committee members and summary minutes of meetings may be requested from the Dockets Management Branch (HFA-305), Rm. 4-62, Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, between 9 a.m. and 4 p.m., Monday through Friday.

The Commissioner, with the concurrence of the Chief Counsel, has determined for the reasons stated that those portions of the advisory committee meetings so designated in this notice shall be closed. The Federal Advisory Committee Act (FACA), as amended by the Government in the Sunshine Act (Pub. L. 94-409), permits such closed advisory committee meetings in certain circumstances. Those portions of a meeting designated as closed, however, shall be closed for the shortest possible time, consistent with the intent of the cited statutes.

The FACA, as amended, provides that a portion of a meeting may be closed where the matter for discussion involves a trade secret; commercial or financial information that is privileged or confidential; information of a personal nature, disclosure of which would be a clearly unwarranted invasion of personal privacy; investigatory files compiled for law enforcement purposes; information the premature disclosure of which would be likely to significantly frustrate implementation of a proposed agency action; and information in certain other instances not generally relevant to FDA matters.

Examples of portions of FDA advisory committee meetings that ordinarily may be closed, where necessary and in accordance with FACA criteria, include the review, discussion, and evaluation of drafts of regulations or guidelines or similar preexisting internal agency documents, but only if their premature disclosure is likely to significantly frustrate implementation of proposed agency action; review of trade secrets and confidential commercial or financial information submitted to the agency; consideration of matters involving investigatory files compiled for law enforcement purposes; and review of matters, such as personnel records or individual patient records, where disclosure would constitute a clearly unwarranted invasion of personal privacy.

Examples of portions of FDA advisory committee meetings that ordinarily shall not be closed include the review, discussion, and evaluation of general preclinical and clinical test protocols and procedures for a class of drugs or devices; consideration of labeling requirements for a class of marketed drugs or devices; review of data and information on specific investigational or marketed drugs and devices that have previously been made public; presentation of any other data or information that is not exempt from public disclosure pursuant to the FACA, as amended; and, notably deliberative sessions to formulate advice and recommendations to the agency on matters that do not independently justify closing.

This notice is issued under section 10(a)(1) and (2) of the Federal Advisory Committee Act (Pub. L. 92-463, 86 Stat. 770-776 (5 U.S.C. App. I)), and FDA's regulations (21 CFR Part 14) on advisory committees.

Dated: September 23, 1985.

Frank E. Young,

Commissioner of Food and Drugs.

[FR Doc. 85-23072 Filed 9-26-85; 8:45 am]

BILLING CODE 4160-01-M

[Docket No. 85P-0235]

Petition Requesting Exclusivity for Ibuprofen

AGENCY: Food and Drug Administration.

ACTION: Notice.

SUMMARY: The Food and Drug Administration (FDA) is announcing the filing of a petition requesting a period of marketing exclusivity for ibuprofen. FDA is giving notice of the filing of this petition to all interested persons because, if FDA decides to grant the petition, this decision may affect the date when abbreviated new drug applications (ANDA's) for ibuprofen may be made effective.

DATE: Comments by October 28, 1985.

ADDRESS: Requests for a copy of the petition and written comments regarding the petition to the Dockets Management Branch (HFA-305), Food and Drug Administration, Rm. 4-62, 5600 Fishers Lane, Rockville, MD 20857.

FOR FURTHER INFORMATION CONTACT: Ed Farha, Center for Drugs and Biologics (HFN-364), Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20857, 301-443-6490.

SUPPLEMENTARY INFORMATION: On September 24, 1984, the President signed into law the Drug Price Competition and Patent Term Restoration Act of 1984. This act amends the Federal Food, Drug, and Cosmetic Act (the act) authorizing, among other things, the agency to accept ANDA's for most previously approved new drug products. This legislation also provides for extending the term of a patent which claims a product, use, or method of manufacture that was subject to a regulatory review period in accordance with the act. Further, this new legislation also provides for periods of exclusive marketing of certain new drug products submitted in an application (or a supplement to an application) under section 505(b) of the act (21 U.S.C. 355(b)). An ANDA or paper new drug application (NDA) for such a drug may not be submitted (under some provisions) or made effective (under other provisions) until the period of "exclusive" marketing ends.

The new drug products that have been granted "exclusivity" under one of the several exclusivity provisions of this new legislation are set forth in the volume entitled "Approved Prescription Drug Products with Therapeutic Equivalence Evaluations" (the list) and its monthly supplements. In addition, the period of "exclusivity" is shown. Further, the list also shows those products that are covered by a patent and when the patent expires.

The agency believes that all patent and exclusivity information appearing in the list is correct, and expects that such information appearing in any future supplements to the list will also be correct. However, interested persons may disagree with the agency's findings and believe that FDA has excluded patent or exclusivity information that should have been included, or included patent or exclusivity information that should have been excluded. Accordingly, FDA has established a policy that, when an interested person submits a citizen petition requesting such inclusion or exclusion, the agency will publish a notice in the **Federal Register** of the availability of the petition. This publication is constructive notice to all interested persons that they may be affected by the petition and gives them an opportunity to submit their comments on the petition to the agency. Persons potentially affected include holders of approved ANDA's or approved paper NDA's the effective dates of which might be changed by a decision to grant the petition, persons who have pending ANDA's or paper NDA's or who contemplate submitting such applications that, when approved, would have effective dates that will be determined by the decision on the petition or, in some cases, persons whose right to submit such applications may be affected. Where a petition seeks a change in a decision to grant exclusivity, the applicant granted exclusivity has an obvious interest in the issue.

In accordance with this policy, FDA is announcing the filing of a petition submitted by Boots Pharmaceuticals, Inc. (Boots), requesting exclusivity for ibuprofen. Specifically, Boots requests that the agency:

1. Recognize and effectuate 2-year nonpatent exclusivity under section 505(j)(4)(D)(v) and (c)(3)(D)(v) of the act for 600 milligram, 400 milligram, and 300 milligram forms of ibuprofen by including notice in the list and by refusing to make effective ANDA's and paper NDA's for these dosage forms of ibuprofen until September 24, 1986; and

2. Refrain from making effective any ANDA or paper NDA for any 300 milligram, 400 milligram, or 600 milligram ibuprofen until the issues raised by this petition are resolved.

FDA is reviewing the merits of this petition and, by this notice, is giving anyone who may be affected by this petition an opportunity to submit comments within 30 days.

Interested persons may, on or before October 28, 1985, submit to the Dockets Management Branch (address above) written comments on the petition. These comments will be considered in preparing an agency response to the petition. Two copies of any comments are to be submitted, except that individuals may submit one copy. Comments are to be identified with the docket number found in brackets in the heading of this document. The petition and received comments may be seen in the Dockets Management Branch between 9 a.m. and 4 p.m., Monday through Friday. Requests for a single copy of the petition should be sent to the Dockets Management Branch.

Dated: September 20, 1985.

Mervin H. Shumate,

Acting Associate Commissioner for Regulatory Affairs.

[FR Doc. 85-23073 Filed 9-26-85; 8:45 am]

BILLING CODE 4160-01-M

National Institutes of Health

Consensus Development Conference on Health Implications of Smokeless Tobacco Use; Meeting

Notice is hereby given of the NIH Consensus Development Conference on "Health Implications of Smokeless Tobacco Use," sponsored by the National Cancer Institute, the National Institute of Dental Research and the Office of Medical Applications of Research. The conference will be held January 13-15, 1986, in the Masur Auditorium of the Warren Grant Magnuson Clinical Center (Building 10) at the National Institutes of Health, 900 Rockville Pike, Bethesda, Maryland 20892.

Serious questions have been raised regarding health and behavioral effects from the use of smokeless tobacco products. This meeting has been scheduled to examine these issues.

Following two days of presentations by scientific and medical experts and discussion by the audience, a Consensus Panel will consider the scientific evidence presented.

The panel members, including medical experts and the lay public, will formulate a draft statement responding to the following key questions:

What are the current trends in use of smokeless tobacco in the United States?

Does the use of smokeless tobacco increase the risk of oral or other cancers?

Does the use of smokeless tobacco increase the risk of periodontal disease or other oral and health problems?

What are the behavioral consequences of smokeless tobacco use?

What issues regarding the health consequences of smokeless tobacco use require further research?

On the third and final day of the conference, Consensus Panel Chairman Dr. Brian MacMahon, Professor and Chairman of the Department of Epidemiology at Harvard School of Public Health, will read the draft consensus statement before the conference audience and invite comments and questions. Information on the program may be obtained from Ms. Barbara McChesney, Prospect Associates, 2115 East Jefferson Street, Suite 401, Rockville, Maryland 20852, (301) 468-6555.

Dated: September 19, 1985.

Betty J. Beveridge,

Committee Management Officer, NIH.

[FR Doc. 85-23090 Filed 9-26-85; 8:45 am]

BILLING CODE 4140-01-M

Dental Research Programs Advisory Committee; Meeting

Pursuant to Pub. L. 92-463 notice is hereby given of the meeting of the Dental Research Programs Advisory Committee from 9:00 a.m. to recess on October 24 and from 9:00 a.m. to adjourhment on October 25, 1985, Wilson Hall, Shannon Building, National Institutes of Health, Bethesda, Maryland.

The entire meeting will be open to the public to discuss research progress and ongoing plans and programs. Attendance by the public will be limited to space available.

Dr. Anthony Rizzo, Deputy Associate Director for Extramural Programs, NIDR, NIH, Westwood Building, Room 504, Bethesda, MD 20892 (telephone: 301/496-7748) will provide a summary of the meeting, roster of committee members and substantive program information upon request.

(Catalog of Federal Domestic Assistance Program Nos. 13.121—Diseases of the Teeth and Supporting Tissues: Caries and Restorative Materials; Periodontal and Soft Tissue Diseases; 13.122—Disorders of Structure, Function, and Behavior, Craniofacial Anomalies, Pain Control, and Behavioral Studies; 13-845-Dental Research Institutes, National Institutes of Health)

Dated: September 19, 1985.

Betty J. Beveridge,

NIH Committee Management Officer.

[FR Doc. 85-23088, Filed 9-26-85; 8:45 am]

BILLING CODE 4140-01-M

National Heart, Lung, and Blood Institute; Pulmonary Diseases Advisory Committee; Meeting

Pursuant to Pub. L. 92-463, notice is hereby given of the meeting of the Pulmonary Diseases Advisory Committee, National Heart, Lung, and Blood Institute on November 7-8, 1985 at the National Institutes of Health, 9000 Rockville Pike, Building 31, Conference Room 4, Bethesda, Maryland 20892.

The entire meeting, from 8:30 a.m. on November 7 to adjournment on November 8, will be open to the public. The Committee will discuss the current status of the Division of Lung Diseases' programs and Committee plans for fiscal year 1987. Attendance by the public will be limited to the space available.

Ms. Terry Bellicha, Chief, Public Inquiry Reports Branch, National Heart, Lung, and Blood Institute, Building 31, Room 4A-21, National Institutes of Health, Bethesda, Maryland 20892, phone (301) 496-4236, will provide a summary of the meeting and a roster of the Committee members.

Dr. Suzanne S. Hurd, Executive Secretary of the Committee, Westwood Building, Room 6A16, National Institutes of Health, Bethesda, Maryland 20892, phone (301) 496-7208, will furnish substantive program information.

(Catalog of Federal Domestic Assistance Program No. 13.838, Lung Diseases Research, National Institutes of Health)

Dated: September 19, 1985.

Betty J. Beveridge,

Committee Management Officer.

[FR Doc. 85-23089 Filed 9-26-85; 8:45 am]

BILLING CODE 4140-01-M

DEPARTMENT OF THE INTERIOR

Fish and Wildlife Service

Issuance of Permit for Marine Mammals

On August 9, 1985, a notice was published in the **Federal Register** (Vol. 50, No. 154) that an application had been filed with the Fish and Wildlife Service by Miyajima Public Aquarium (PPT-696262) for a permit to take 4 Alaskan sea otters (*Enhydra lutris lutris)* for public display.

Notice is hereby given that on September 13, 1985, as authorized by the provisions of the Marine Mammal Protection Act of 1972 (16 USC 1361-1407), the Fish and Wildlife Service issued the requested permit subject to certain conditions set forth therein.

The permit is available for public inspection during normal business hours at the Fish and Wildlife Service's Permit Office in Room 605, 1000 North Glebe Road, Arlington, Virginia 22201.

Dated: September 20, 1985.

R.K. Robinson,

Chief, Branch of Permits, Federal Wildlife Permit Office.

[FR Doc. 85-23155 Filed 9-26-85; 8:45 am]

BILLING CODE 4310-55-M

Issuance of Permit for Marine Mammals

On August 9, 1985, a notice was published in the **Federal Register** (Vol. 50, No. 154) that an application had been filed with the Fish and Wildlife Service by Otaru Public Aquarium (PRT-685320) for a permit to take 4 Alaskan sea otters (*Enhydra lutris lutris)* for the purpose of public display.

Notice is hereby given that on September 11, 1985, as authorized by the provisions of the Marine Mammal Protection Act of 1972 (16 USC 1361-1407), the Fish and Wildlife Service issued the requested permit subject to certain conditions set forth therein.

The permit is available for public inspection during normal business hours at the Fish and Wildlife Service's Permit Office in Room 605, 1000 North Glebe Road, Arlington, Virginia 22201.

Dated: September 20, 1985.

R.K. Robinson,

Chief Branch of Permits, Federal Wildlife Permit Office

[FR Doc. 85-23156 Filed 9-26-85; 8:45 am]

BILLING CODE 4310-55-M

Bureau of Land Management

Garnet Resource Management Plan/Environmental Impact Statement; Butte District, MT

AGENCY: Bureau of Land Management, Interior.

ACTION: Notice of Availability of Proposed Final Resource Management Plan/Environmental Impact Statement.

SUMMARY: Pursuant to section 202(f) of the Federal Land Policy and Management Act of 1976 and section 102(2)(c) of the National Environmental Policy Act of 1969, a proposed final Resource Management Plan/Environmental Impact Statement (RMP/EIS) has been prepared for the Garnet Resource Area. The RMP/EIS addresses future management options for approximately 145,660 surface acres and 213,385 acres of federal mineral estate administered by the Bureau of Land Management in west-central Montana. The affected counties are Missoula, Granite, and Powell.

Public Participation. The draft RMP/EIS was available for public review from December 14, 1984, to March 13, 1985. Letters and/or oral statements received from 48 agencies, organizations and individuals were considered during preparation of the proposed final FMP/EIS.

Copies of the proposed final RMP/EIS are available at the Butte District Office, 106 North Parkmont, P.O. Box 3388, Butte, MT 59702, phone (406) 494-5059, and at the Garnet Resource Area Office, 3255 Fort Missoula Road, Missoula, MT 59801, phone (406) 329-3914. Public reading copies are available for review at the following locations:

Office of Public Affairs, Interior Building, 18th and C Streets, NW., Washington, DC 20240

BLM, Montana State Office, Public Affairs Office, 222 North 32nd Street, Billings, Montana 59107

BLM, Butte District Office, 106 North Parkmont, Butte, Montana 59702

BLM, Garnet Resource Area Office, 3255 Fort Missoula Road, Missoula, Montana 59801

With the exception of wilderness recommendations for the Wales Creek, Hoodoo Mountain, and Quigg West Wilderness Study Areas (WSAs), all parts of this proposed plan may be protested. A final legislative EIS dealing with wilderness recommendations for these WSAs will be available in 1986. Protests should be sent to the Director (202), Bureau of Land Management, 1800 C Street, NW., Washington, DC 20240, prior to October 27, 1985—the end of the 30-day protest period—and should include the following information:

1. The name, mailing address, telephone number, and interest of the person filing the protest.
2. A statement of the issue or issues being protested.
3. A statement of the part or parts of the plan being protested.
4. A copy of all documents addressing the issue or issues that were submitted during the planning process by the protesting party, or an indication of the date the issue or issues were discussed for the record.
5. A concise statement explaining why the State Director's decision is believed to be wrong.

At the end of the 30-day protest period, the proposed plan, excluding any

portion under protest, will become final. Approval will be withheld on any portion of the plan under protest until final action has been completed.

Any significant change to the proposed plan made as a result of a protest will be made available for public review and comment prior to final approval and implementation.

SUPPLEMENTARY INFORMATION: The proposed RMP/EIS was developed following analysis of five alternatives, with one representing a continuation of present management direction (no action). The others emphasize environmental protection, partial wilderness, resource production, and an intermediate or balanced approach (the proposed RMP).

One Area of Critical Environmental Concern (ACEC) is being proposed in the final RMP/EIS. This area is referred to as Limestone Cliffs and consists of 20 acres located in T. 11 N., R. 13 W., M.P.M., Section 4, SE¼SE¼SE¼ and Section 9, NE¼NE¼NE¼. The site encompasses a unique geologic (limestone) feature which has been intensively studied and mapped by high schools and universities throughout the Northwest. This area would be closed to motorized vehicle use except along Rattler Gulch Road. Also, there would be no timber harvested and a withdrawal from mineral entry would be pursued.

The plan focuses on resolving five resource management issue groups: renewable resources; nonrenewable resources; special attention resources; land ownership and administration; and recreation, cultural and esthetic resources.

FOR FURTHER INFORMATION CONTACT: Project Manager, Garnet RMP, Garnet Resource Area, 3255 Fort Missoula Road, Missoula, MT 59801, Telephone (406) 329-3914.

Marvin LeNoue,

Acting State Director.

September 3, 1985.

[FR Doc. 85-21963 Filed 9-26-85; 8:45 am]

BILLING CODE 4310-DN-M

Sale of Public Lands in Hot Springs and Washakie Counties, WY; Termination of Realty Action

AGENCY: Bureau of Land Management, Interior.

ACTION: Termination of realty action—sale of public lands in Hot Springs and Washakie Counties, Wyoming.

SUMMARY: The Notice of Realty Action—Rescheduling of sale of public lands in Hot Springs and Washakie Counties, published in the **Federal Register**, Volume 50, No. 59 on March 27, 1985, at page 12088 is hereby terminated for the reason that no bids were received on two of five sale parcels.

The public sale parcels were offered on April 18, 1985 and the two unsold parcels were offered in May and June, with the last offering on July 24, 1985. The lack of interest in the sale parcels does not warrant continuing the public sale.

Dated: September 17, 1985.

Chester E. Conard,

District Manager, Worland, Wyoming.

[FR Doc. 85-23186 Filed 9-26-85; 8:45 am]

BILLING CODE 4310-22-M

New Mexico; Availability of Proposed Rio Puerco Resource Management Plan and Final Environmental Impact Statement

AGENCY: Bureau of Land Management, Interior.

ACTION: Notice of Availability.

SUMMARY: The Bureau of Land Management announces the availability of the Rio Puerco Proposed Resource Management Plan (RMP) and Final Environmental Impact Statement (EIS). This document identifies and analyzes the future options for managing 896,490 acres of public land and 1,962,753 acres of Federal mineral estate in central and north-central New Mexico. The Plan also contains recommendations that the Areas of Critical Environmental Concern (ACEC) be designated.

The Draft Rio Puerco RMP/EIS was made available for public review and comment in April of 1985. Comments received on the Draft were considered in preparing the Proposed RMP/Final EIS. Any person who participated in the planning process and has an interest that is or may be affected by approval of the Proposed RMP may file a protest.

DATE: Protests must be filed by October 31, 1985.

ADDRESS: Comments should be sent to: Director, Bureau of Land Management, Department of the Interior, 18th and C Streets NW., Washington, D.C. 20240.

FOR FURTHER INFORMATION CONTACT: Herrick E. Hanks, Area Manager, Rio Puerco Resource Area, Bureau of Land Management, P.O. Box 6770, Albuquerque, New Mexico 87197-6770. Telelphone (505) 766-3114.

SUPPLEMENTARY INFORMATION: The Proposed RMP provides a comprehensive framework for managing and allocating public land and resources within the Rio Puerco Resource Area during the next ten to twenty years. The document is primarily focused on resolving seven key resource management issues that were identified with public involvement early in the planning process. These issues are: (1) Special Management Areas; (2) Off-Road Vehicle Designations; (3) Vegetative Uses; (4) Land Ownership Adjustments; (5) Fuelwood Supply; (6) Rights-of-way Corridors; and (7) Coal Leasing Suitability Assessment. The "Continuing Management Guidance" section of the Proposed RMP describes those aspects of current management which are not at issue and will continue after the RMP is approved. The continuing managmeent guidance was developed primarily from laws, regulations, and manuals, as well as from previous land use plans and grazing EIS's.

The Proposed Plan is a slightly modified version of the Preferred Alternative (Alternative D) presented in the Draft RMP/EIS. Slight changes were made to the ORV Designations and Special Management Areas issue resolutions of the Preferred Alternative as a result of comments received on the Draft RMP/EIS. The Proposed Plan will protect important environmental values and sensitive resources while at the same time allowing development of resources which provide commercial goods and service.

Areas of Critical Environmental Concern: Nine ACEC's were recommended for designation in the Draft RMP/EIS and were described in the **Federal Register** on April 2, 1985. As a result of the comments received on the Draft RMP/EIS, the Proposed Plan recommends the designation of one additional ACEC which is discussed briefly below. Comments on this proposed ACEC must be submitted by November 30, 1985 to the Rio Puerco Area Manager at the previously identified Address. Approval of the RMP will constitite designation of the ten proposed ACEC's.

The Pronoun Cave Complex (1,194 acres) contains nine known vertical caves which contain valued late glacial paleontological fauna. These caves also provide important habitat for several species of bats. A management agreement would be developed with the New Mexico Museum of Natural History and motorized vehicle use would be limited to exisitng roads and trails.

Dated: September 16, 1985.

Monte G. Jordan,

Associate State Director.

[FR Doc. 85-23188 Filed 9-26-85; 8:45 am]

BILLING CODE 4310-FB-M

Availability of the Proposed Two Rivers Resource Management Plan and Final Environmental Impact Statement; Proposed Area of Critical Environmental Concern (ACEC) Designations; Prineville District, OR

AGENCY: Bureau of Land Management, Department of the Interior.

ACTION: Notice.

SUMMARY: Pursuant to section 102(2)(C) of the National Environmental Policy Act of 1969, section 202(a) of the Federal Land Policy and Management Act of 1976, and 43 CFR Part 1600, the Bureau of Land Management has prepared a proposed Resource Management Plan, known as the Two Rivers RMP, and a final Environmental Impact Statement covering 324,705 acres in 7 counties north of Central Oregon. Included in the proposed Two Rivers RMP are 5 areas recommended for Area of Critical Environmental Concern (ACEC) designation.

The draft Two Rivers RMP/EIS was made available to members of the public in April 1985. Comments on the draft were considered in preparing the final EIS. Any person who participated in the planning process and has an interest which is or may be adversely affected by approval of the Two Rivers RMP may protest. A protest may raise only those issues which were submitted for the record during the planning process.

DATES AND ADDRESSES: Comments for the District Manager's consideration in the development of the decisions should be submitted to the District Manager by November 15, 1985. Copies of the Proposed Plan and Final EIS are available at the following locations: Prineville District Office BLM, 185 East Fourth Street, Prineville, Oregon 97754 (503) 447-4115. Public Affairs BLM, Oregon State Office, 825 N.E. Multnomah, Portland, Oregon 97208, (503) 231-6277; Public Affairs BLM, Interior Building, 18th and C Streets, Washington, DC 20240, (202) 343-9435. Protests must be filed on or before November 15, 1985. To be timely, protests should be filed with the Director (202), Bureau of Land Management, U.S. Department of the Interior, Washington, DC 20240. The procedures for filing a protest are listed in the Proposed Plan and in 43 CFR 1610.5-2.

SUPPLEMENTARY INFORMATION: The final EIS discusses five alternative plans for managing natural resources in the Two Rivers Planning Area over the next 10 to 15 years. One alternative has been identified as the proposed Resource Management Plan for the Two Rivers Planning Area.

The alternative plans included in the EIS are designed to resolve the planning issues identified earlier through public involvement. The general topics covered are livestock grazing management, riparian management, wildlife habitat management, land tenure and access, forestry, minerals management, recreation and the management of special areas.

The objectives of the proposed plan for each of these resource programs are as follows:

1. Maintain forage production and livestock use at 17,778 AUMs. Maintain current livestock grazing levels and meet riparian and upland vegetation management objectives.

2. Manage riparian areas along the Deschutes and John Day rivers and their major tributaries to full potential, with a minimum of 60 percent of the vegetative potential to be achieved within 20 years.

3. Provide forage to meet management objective numbers of the Oregon Department of Fish and Wildlife for deer and elk. Manage upland vegetation to achieve maximum wildlife habitat diversity. Manage all streams with fisheries or fisheries potential to achieve a good to excellent aquatic habitat condition.

4. Place emphasis on retaining and expanding, by exchange of public land, holdings in: (1) Areas of national significance, (2) areas where management is cost effective, and (3) where land is most appropriately managed in public ownership due to significant multiple resource values. Public lands having no reasonable opportunity for exchange would be offered for sale if they are: (1) Difficult and uneconomical to manage and are not needed by another agency; (2) no longer needed for the specific purpose for which they were acquired or for any other Federal purpose: (3) provide greater benefits to the public in private ownership. The transfer of public lands to other public land management agencies would occur if more efficient management of the land would result.

Authorize agricultural use of public lands if proposals are consistent with the management and protection of other values. Pursue attempts to acquire limited public access through exchange or negotiated easement, consistent with management objectives.

5. Intensively manage commercial forestlands suitable for timber production but recognize harvest restrictions or exclusions to protect riparian vegetation, wildlife, visual and other resource values.

6. Keep public lands open for exploration and development of mineral resources and related rights of way. Retain restrictive stipulations for oil and gas exploration and development on 132,000 acres of public land.

7. Designate public lands as open to off road vehicles except in areas where that use would not be appropriate or where significant damage to soils, vegetation, wildlife or other natural values is resulting from that use.

Areas which have high or moderate quality collectible mineral resources, including plant and invertebrate fossils, would be available for rockhound purposes and would be recognized in land use decisions. Public use areas would be reviewed on a case by case basis to insure that no significant conflict exists with the protection of other natural values.

8. Designate areas with identified outstanding natural or cultural values as areas of critical environmental concern. Maintain or improve other unique wildlife or ecological values.

The following areas would be designated as areas of critical environmental concern under the proposed RMP:

Designate and manage The Island in The Cove Palisades State Park, (250 acres of public land) as an Area of Critical Environmental Concern: Research Natural Area. This includes 80 acres of USFS land and will necessitate a cooperative management agreement.

The designation and management of this area will be designed to protect and preserve what is considered to be the best remaining example of the western juniper/big sagebrush/bluebunch wheatgrass ecotype plant association in the region. It is also a raptor, deer, and waterfowl use area and contains outstanding scenic vistas of Lake Billy Chinook and the Cascades.

The Horn Butte Curlew Area which totals 6,000 acres will be designated as an Area of Critical Environmental Concern. The designation and management of this area will be designed to protect and preserve the important nesting habitat for the long billed curlew which exists as a result of a bluebunch wheatgrass, Sandburg bluegrass, needlegrass, snakewood and gray rabbitbrush habitat type.

Twelve and one-half acres of public land within the Governor Tom McCall Preserve at Rowena would be designated as an Area of Critical Environmental Concern: Outstanding Natural Area. The important botanicall and scenic qualities of 76 additional acres (in two parcels) outside this preserve, but within the Columbia Gorge, will also be preserved with a designation as an Area of Critical Environmental Concern: Outstanding

Natural Area. The designation and management of these areas will be designed to protect and preserve the Idaho fescue/hawkweed and Columbia Gorge forest complex ecotypes or plant associations which exist in the areas. Four rare plants are also within this preserve. High visual qualities are also present and can be seen from both Oregon and Washington highways within the gorge.

The 335 acre Spanish Gulch Mining District will be designated as an Area of Critical Environmental Concern to protect and maintain significant historical values.

This mining district is an important historic gold mining area dating back to the mid 1800's. Remnants of early mining activities include an old stamp mill, mineshafts and several old cabins.

As the respective management plans for these areas are developed, additional protective measures may be proposed.

For further information contact: Brian Cunninghame, Project Manager, Bureau of Land Management, 185 East 4th Street, Prineville, Oregon 97754; telephone (503) 447-4115.

Dated: September 20, 1985.

Gerald E. Magnuson,

District Manager.

[FR Doc. 85-23189 Filed 9-26-85; 8:45 am]

BILLING CODE 4310-33-M

[DES 85-44]

National Park Service

Availability of Draft Environmental Impact Statement and Notice of Public Hearings; George Washington Memorial Parkway, VA; Spout Run Parkway to Theodore Roosevelt Memorial Bridge Section

Summary: The National Park Service has prepared a draft environmental impact statement (EIS) for alternatives to make structural roadway changes to improve traffic flow and safety in a section of the George Washington Memorial Parkway between Theodore Roosevelt Bridge (I-66) and Spout Run Parkway, including Spout Run Parkway to its western terminus before Lee Highway (29-211).

Public hearings will be held to solicit public opinion concerning the alternatives and impacts presented in the draft EIS. Meeting times and places are as follows: October 8, 1985, at 7:00 p.m. in the cafeteria of Cooper Intermediate School, 977 Balls Hill Road, McLean, Virginia; October 9, 1985, at 7:00 p.m. in the Professional Center Conference Room, 3rd floor of George Mason University Metro Campus, 3401 North Fairfax Drive, Arlington, Virginia; and October 10, 1985 at 7:00 p.m. in the 10th floor meeting room, National Capital Planning Commission, 1325 G Street, NW, Washington, DC.

Public reading copies of the unabridged draft EIS will be available for review at the following locations: Arlington Central Library, Virginiana Section, 1015 N. Quincy Street; Cherrydale Branch Library, 2190 N. Military Road, Arlington; Fairfax City Regional Library, 4000 Chain Bridge Road, Fairfax; Reston Regional Library, 2355-A Hunters Woods Plaza, Reston; Dolley Madison Library, 1244 Oak Ridge Avenue, Vienna; Potomac Library, 1000 Falls Road, Potomac, Maryland; and Martin Luther King Library, Washingtoniana Division, 901 G Street, NW. Washington, DC.

Written comments on the draft EIS are invited and will be accepted until November 26, 1985. Send comments to Superintendent, George Washington Memorial Parkway, Turkey Run Headquarters, McLean, Virginia 22101.

Copies of the summary and full EIS are available by writing to the above address.

Supplementary Information: Alternative A of the draft EIS is essentially a continuation of existing management where current road base reconstruction and resurfacing activities would be completed. Safety improvements, such as reflective markings, guardrails, signing, and skid-resistant surfacing, would be installed, and Rosslyn Circle would be improved. These actions would apply also to alternatives B-D.

Major actions associated with the preferred alternative, Alternative B, for inbound lanes include reconstructing the Lorcom Lane/Spout Run Parkway intersection; lengthening the merge lane from Spout Run to the George Washington Memorial Parkway; lengthening the deceleration lanes for the off-ramps to Key and Roosevelt Bridges; and reconstructing the off-ramp to Key Bridge thereby adding direct access to Lee Highway. Rosslyn Circle would be improved by removal of old bus turnarounds, rehabilitation of the roadway, and landscaping. In addition, the left turn lane from North Lynn Street to the on-ramp for outbound George Washington Memorial Parkway would be widened and realigned. Actions affecting outbound lanes include lengthening the acceleration lane from Roosevelt Bridge on-ramp to George Washington Memorial Parkway; improving access to Theodore Roosevelt Island parking lot; adding a third continuous lane from Key Bridge ramp to Spout Run exit.

Alternative B was selected as the preferred alternative because it improves traffic movement and safety and enhances the parkway's scenic and recreational values with less adverse environmental impact than alternatives C or D.

Actions associated with Alternative C for inbound lanes include installing a traffic signal at the Lorcom Lane/Spout Run intersection; adding a third lane from Spout Run to the Key Bridge off-ramp; reconstructing and reopening the Key Bridge off-ramp for morning rush hours; replacing the off-ramp to Roosevelt Bridge with a new stacking lane giving direct access to U.S. 50 into Rosslyn. Alternative C actions for outbound George Washington Memorial Parkway include those mentioned in B plus addition of new third and fourth lanes from Key Bridge ramp to Spout Run and a new merge lane north of Spout Run exit. Also, the outbound turn from Spout Run Parkway to Lorcom Lane would be redesigned.

Alternative D inbound actions which differ from C include adding a third lane from Spout Run to the Roosevelt Bridge ramp, and adding a new off-ramp to Rosslyn Circle. Alternative D differs only in that the new third lane would extend from the Roosevelt Bridge to Spout Run, while the fourth would begin at Key Bridge.

Dated: September 24, 1985.

Manus J. Fish, Jr.,

Regional Director, National Capital Region.

[FR Doc. 85-23196 Filed 9-26-85; 8:45 am]

BILLING CODE 4310-70-M

Cape Cod National Seashore Advisory Commission; Meeting

Notice is hereby given in accordance with the Federal Advisory Committee Act (Pub. L. 92-463, 86 Stat. 770 (5 U.S.C. App. 1 section 10), that a meeting of the Cape Cod National Seashore Advisory Commission will be held Friday, October 18, 1985.

The Commission was established pursuant to Pub. L. 91-383 to meet and consult with the Secretary of the Interior on general policies and specific matters relating to the development of Cape Cod National Seashore.

The meeting will convene at Park Headquarters at 1:30 p.m. to discuss:

1. Alteration and Expansion of Commercial Use—Surfside Colony, Wellfleet.
2. Ponds Management Planning.

The meeting is open to the public. It is expected that 15 persons will be able to attend the session in addition to the Commission members.

Interested persons may make oral/written presentations to the Commission or file written statements. Such requests should be made to the official listed below at least seven days prior to the meeting.

Further information concerning this meeting may be obtained from Herbert Olsen, Superintendent, Cape Cod National Seashore, So. Wellfleet, MA 02663. Telephone: (617) 349-3785. Minutes of the meeting will be available for public information and copying two weeks after the meeting at the office of the Superintendent, Cape Cod National Seashore, So. Wellfleet, Massachusetts.

Herbert Olsen,

Superintendent, Cape Cod National Seashore.

September 20, 1985.

[FR Doc. 85-23194 Filed 9-26-85; 8:45 am]

BILLING CODE 4310-70-M

Martin Luther King, Jr., National Historic Site and Preservation District Advisory Commission; Meeting

Notice is hereby given in accordance with the Federal Advisory Commission Act that a meeting of the Martin Luther King, Jr., National Historic Site Advisory Commission has been rescheduled from Monday, October 7, 1985, at 10:30 a.m., to Wednesday, October 16, 1985 at 7:00 p.m., at the Martin Luther King, Jr., Center for Non-Violent Social Change, Inc., Freedom Hall, Room 261, 449 Auburn Avenue, NE, Atlanta, Georgia 30312.

The purpose of the Martin Luther King, Jr., National Historic Site Advisory Commission is to consult with the Secretary of the Interior on matters of planning, development and administration of the Martin Luther King, Jr., National Historic Site. The purpose of this meeting will be to update the Commission on park planning and operations. A presentation of the final recommendations for the Development Program/Advisory Commission Study will be made.

The members of the Commission are as follows:

Mr. William Allison, Chairman
Mr. John H. Calhoun
Dr. Elizabeth A. Lyon
Mr. C. Randy Humphrey
Mrs. Christine King Farris
Mr. Handy Johnson, Jr.
Mr. James Patterson
Mrs. Freddye Scarborough Henderson
Mrs. Millicent Dobbs Jordan
Mr. John W. Cox
Reverend Joseph L. Roberts, Jr.
Mrs. Coretta Scott King, Ex-Officio Member, Director, National Park Service, Ex-Officio Member

The meeting will be open to the public; however, facilities and space for accommodating members of the public are limited. Any member of the public may file with the Commission a written statement concerning the matters to be discussed.

Persons wishing further information concerning the meeting or who wish to submit written statements may contact Randolph Scott, Superintendent, Martin Luther King, Jr., National Historic Site, 522 Auburn Avenue, NE, Atlanta, Georgia 30312; Telephone 404/221-5190. Minutes of the meeting will be available approximately 4 weeks after the meeting.

Dated: September 18, 1985.

Frank Catroppa,

Regional Director, Southeast Region.

[FR Doc. 85-23193 Filed 9-26-85; 8:45 am]

BILLING CODE 4310-70-M

National Capital Region, Public Affairs; Public Meeting

The National Park Service is seeking public comments and suggestions on the planning of the 1985 Christmas Pageant of Peace, which opens December 12 on the Ellipse, south of the White House.

A public meeting will be held at the National Capital Region Building, 1100 Ohio Drive, SW, Room 234, at 10 a.m. on November 1, 1985.

Interested persons who would like to comment at the meeting should notify the National Park Service by October 25, by calling the Office of Public Affairs between 9 a.m. and 4 p.m., weekdays at 426-6700. Persons who cannot attend the meeting can send written comments to Regional Director, National Capital Region, 1100 Ohio Drive, SW, Washington, DC. 20242. Written comments will be accepted until October 17, 1985.

Dated: September 19, 1985.

Manus J. Fish, Jr.,

Regional Director, National Capital Region.

[FR Doc. 85-23195 Filed 9-26-85; 8:45 am]

BILLING CODE 4310-70-M

INTERSTATE COMMERCE COMMISSION

[Ex Parte No. 290 (Sub-2)]

Railroad Cost Recovery Procedures

AGENCY: Interstate Commerce Commission.

ACTION: Notice of approval of rail cost adjustment factor and decision.

SUMMARY: The Commission has decided to approve the cost index filed by the Association of American Railroads (AAR) under the procedures of Ex Parte No. 290 (Sub-No. 2), *Railroad Cost Recovery Procedures*. The application of the index provides for a fourth quarter 1985 Rail Cost Adjustment Factor (RCAF) of 1.012. This RCAF shows a decrease of .028 in railroad input prices from the third quarter level of 1.040. Since the fourth quarter 1985 RCAF, remains below the level of a prior RCAF no rate actions are ordered.

EFFECTIVE DATE: October 1, 1985.

FOR FURTHER INFORMATION CONTACT: Robert C. Hasek, (202) 275-0938; Douglas Galloway, (202) 275-7278.

SUPPLEMENTARY INFORMATION: By decision served January 2, 1985, (50 FR 87, January 2, 1985) we outlined the procedures for the calculation of the all inclusive index of railroad input costs and the methodology for the computation of the RCAF. These procedures replaced an interim methodology which was formerly used. AAR is required to calculate the forecasted index on a quarterly basis and submit it on the fifth day of the last month of each calendar quarter.

We have reviewed AAR's calculations of the index for the fourth quarter of 1985 and find that, with one exception, these calculations comply with the rules contained in our decision served January 2, 1985. These rules call for the lease rental portion of the equipment rents component of the index to be calculated using actual data. AAR states that it has been unable to develop a meaningful lease rentals index using actual data and anticipates seeking modification of our rule concerning this component. We will consider that submission when it is received. At this time we will continue to accept use of the Producer Price Index for Industrial Commodities, less Fuel, Power and Related Products as a surrogate for the lease rental portion of the equipment rents component of the index. We have previously observed that the lease rental portion of the index is only 2.4 percent of the total and is not likely to have a major effect on the RCAF.

We find the RCAF for the fourth quarter of 1985 is 1.012. This is a decrease of .028 from the third quarter of 1985. No rate actions are ordered.

The indices and RCAF derived from AAR's fourth quarter calculations are shown in Table A (see appendix). Table B (see appendix) shows the second quarter 1985 index calculated on both an actual basis and a forecasted basis for comparative purposes.

This decision will not significantly affect the quality of the human environment or the conservation of energy resources. This proceeding will not have a significant adverse impact on a substantial number of small entities because these procedures simplify a formerly complex and burdensome rate increase procedure.

Authority: 49 U.S.C. 10321, 10707a, 5 U.S.C. 553.)

Dated: September 13, 1985.

By the Commission, Chairman Taylor, Vice Chairman Gradison, Commissioners Sterrett, Andre, Simmons, Lamboley and Strenio. Commissioner Sterrett did not participate in the disposition of this proceeding.

James H. Bayne,

Secretary.

TABLE A.—EX PARTE 290 (SUB-2) ALL INCLUSIVE INDEX OF RAILROAD INPUT COSTS

Line No.	Index component	1983 weights (percent)	1984 weights (percent)	Third quarter 1985 forecast	Fourth quarter 1985 forecast
1	Labor	50.4	50.5	144.9	139.0
2	Fuel	10.8	10.8	91.4	87.1
3	Materials and supplies.	7.5	7.8	108.5	107.7
4	Equipment Rents.	9.6	9.4	151.7	151.8
5	Depreciation.	7.7	7.4	117.4	116.0
6	Other items [1].	14.0	14.1	119.8	120.0
7	Weighted Average.	100.0	100.0	131.4	127.8
	a. 1980 = 100				
	b. Linked Index [2].			125.7	122.3
8	Rail Cost Adjustment Factor [3] (10/1/82 = 100). 120.9 = 100			1.040	1.012

[1] Other items are a combination of Purchased Services, Casualties and Insurance, General and Administrative, Other Taxes and Loss and Damage, all of which are measured by the Producer Price Index for Industrial Commodities, less Fuel, Power and Related Products.

[2] Linking is necessitated by a change to 1984 weights beginning with the fourth quarter 1985. The following formula was used for the fourth quarter 1985 index:

$$\frac{\text{4th quarter 1985 index (1984 weights)}}{\text{3rd quarter 1985 index (1984 weights)}} \times \text{3rd quarter 1985 index (linked index)} = \text{Linked index (1980 weights to 1984 weights)}$$

or

$$\frac{127.8}{131.3} \times 125.7 = 122.3$$

[3] The denominator was rebased to an October 1, 1982 level in accordance with the requirements of the Staggers Rail Act of 1980.

TABLE B.—COMPARISON OF SECOND QUARTER 1985 INTERIM INDEX CALCULATED ON BOTH A FORECASTED AND AN ACTUAL BASIS

Line No.	Index component	1983 weights	Second quarter 1985 forecast	Second quarter 1985 actual
1	Labor	50.4	146.5	146.5
2	Fuel	10.8	89.6	39.9
3	Materials and Supplies	7.5	108.3	108.3
4	Equipment Rents	9.6	151.2	151.4
5	Depreciation	7.7	115.1	116.0
6	Other	14.0	120.1	119.7
7	Weighted Average	100.0	131.7	132.3
	a. 1980 = 100			
	b. Linked Index		126.0	126.6
8	Rail Cost Adjustment Factor		1.042	[1] 1.047

[1] For comparative purposes only, an RCAF for the second quarter 1985 has been calculated using actual data. The published RCAF for the second quarter 1985 was computed using forecasted data.

[FR Doc. 85-23132 Filed 9-26-85; 8:45 am]

BILLING CODE 7035-01-M

DEPARTMENT OF LABOR

President's Committee on the International Labor Organization; Meeting

In accordance with section 10(a) of the Federal Advisory Committee Act (Pub. L. 92-463), announcement is hereby given of a meeting of the President's Committee on the ILO:

Name: President's Committee on the International Labor Organization.

Date: October 17, 1985.

Time: 2:00 p.m.

Place: Department of Labor, Third & Constitution Avenue NW., Room S-2508, Washington, DC 20210.

This meeting will be closed to the public under the authority of section 10(d) of the Federal Advisory Committee Act. During its closed session, the Committee will discuss national security matters.

All communications regarding this Committee should be addressed to: Mr. Robert W. Searby, Counselor to the Committee, Department of Labor, Third & Constitution Ave., NW., Room S-2235, Washington, DC 20210, telephone (202) 523-6043.

Signed at Washington, DC this 20th day of September, 1985.

William E. Brock,

Secretary of Labor.

[FR Doc. 85-23158 Filed 9-26-85; 8:45 am]

BILLING CODE 4510-28-M

Office of the Secretary

Agency Recordkeeping/Reporting Requirements Under Review by the Office of Management and Budget (OMB)

Background: The Department of Labor, in carrying out its responsibilities under the Paperwork Reduction Act (44 U.S.C. Chapter 35), considers comments on the reporting and recordkeeping requirements that will affect the public.

List of Recordkeeping/Reporting Requirements Under Review: On each Tuesday and/or Friday, as necessary, the Department of Labor will publish a list of the Agency recordkeeping/reporting requirements under review by the Office of Management and Budget (OMB) since the last list was published. The list will have all entries grouped into new collections, revisions, extensions, or reinstatements. The Department Clearance Officer will, upon request, be able to advise members of the public of the nature of the particular submission they are interested in.

Each entry may contain the following information:

The Agency of the Department issuing this recordkeeping/reporting requirement.

The title of the recordkeeping/reporting requirement.

The OBM and Agency identification numbers, if applicable.

How often the recordkeeping/reporting requirement is needed.

Who will be required to or asked to report or keep records.

Whether small businesses or organizations are affected.

An estimate of the total number of hours needed to comply with the recordkeeping/reporting requirments.

The number of forms in the request for approval, if applicable.

An abstract describing the need for and use of the information collection.

Comments and Questions: Copies of the recordkeeping/reporting requirements may be obtained by calling the Department Clearance Officer, Paul E. Larson, Telephone 202 523-6331. Comments and questions about the items on this list should be directed to Mr. Larson, Office of Information Management, U.S. Department of Labor, 200 Constitution Avenue, NW, Room N-

1301, Washington, DC 20210. Comments should also be sent to the OMB reviewer, Nancy Wentzler, Telephone 202 395–6880, Office of Information and Regulatory Affairs, Office of Management and Budget, Room 3208, Washington, DC 20503.

Any member of the public who wants to comment on a recordkeeping/reporting requirement which has been submitted to OMB should advise Mr. Larson of this intent at the earliest possible date.

Extension

Employment Standards Administration
Requests for Medical Reports
1215–0106; LS–158, LS–415, LS–525
On occasion
Businesses or other for-profit; Small businesses or organizations
2,520 responses; 1,260 hours; 3 forms.

These medical reports are used by the Longshore and Harbor Workers' Compensation Act program to support injured worker's claims for Compensation benefits under section 7 of the Longshore and Harbor Workers' compensation Act (33 U.S.C. 901 et seq) as amended and extended.

Mine Safety and Health Administration
Mine Operator Dust Data Card
1219–0011
Bimonthly
Businesses and other for profit; small businesses or organizations
5,000 respondents; 114,808 burden hours.

Coal Mine Operators are required to collect and submit respirable dust samples to MSHA for analysis. Pertinent information associated with identifying and analyzing these samples is submitted on the dust data cards that accompanies the samples.

Signed at Washington, DC this 24th day of September, 1985.

Paul E. Larson,

Department Clearance Officer.

[FR Doc. 85–23169 Filed 9–26–85; 8:45 am]

BILLING CODE 4510–27–M

Employment and Training Administration

[TA–W–16,191]

Aeolian Corp., Memphis, TN; Termination of Investigation

Pursuant to section 221 of the Trade Act of 1974, an investigation was initiated on July 29, 1985 in response to a worker petition filed by the United Furniture Workers of America, Local 282, on behalf of workers at Aeolian Corporation, Piano Manufacturing Plant, Memphis, Tennessee.

A certification applicable to the petitioning group of workers was issued on July 24, 1985 (TA–W–15,810A). Consequently, further investigation in this case would serve no purpose; and the investigation has been terminated.

Signed at Washington, D.C., this 18th day of September 1985.

Marvin M. Fooks,

Director, Office of Trade Adjustment Assistance.

[FR Doc. 85–23159 Filed 9–26–85; 8:45 am]

BILLING CODE 4510–30–M

[TA–W–16,200]

Ely Group, Inc., Rockford Textile Mills, McMinnville, TN; Termination of Investigation

Pursuant to Section 221 of the Trade Act of 1974, an investigation was initiated on July 29, 1985 in response to a worker petition received on July 22, 1985 which was filed on behalf of workers at the Ely Group, Incorporated, Rockford Textile Mills, McMinnville, Tennessee.

The petitioning group of workers are subject to an ongoing investigation for which a determination has not yet been issued (TA–W–16,013). Consequently further investigation in this case would serve no purpose and the investigation has been terminated.

Signed at Washington, D.C. this 18th day of September 1985.

Marvin M. Fooks,

Director, Office of Trade Adjustment Assistance.

[FR Doc. 85–23160 Filed 9–26–85; 8:45 am]

BILLING CODE 4510–30–M

[TA–W–16,369]

Prestige Sportswear, Inc., Boston, MA; Termination of Investigation

Pursuant to section 221 of the Trade Act of 1974, an investigation was initiated on August 30, 1985 in response to a worker petition which was filed by the International Ladies Garment Workers' Union on behalf of workers at Prestige Sportswear, Incorporated, Boston, Massachusetts.

A certification applicable to the petitioning group of workers was issued on April 25, 1985 (TA–W–15,737). Consequently, further investigation in this case would serve no purpose; and the investigation has been terminated.

Signed at Washington, DC, this 19th day of September 1985.

Marvin M. Fooks,

Director, Office of Trade Adjustment Assistance.

[FR Doc. 85–23161 Filed 9–26–85; 8:45 am]

BILLING CODE 4510–30–M

[TA–W–15,772]

United Technologies Corp. Diesel Systems, Springfield, MA; Affirmative Determination Regarding Application for Reconsideration

By an application dated August 21, 1985, the International Union of Electrical Workers requested administrative reconsideration of the Department of Labor's Notice of Determination Regarding Eligibility to Apply for Worker Adjustment Assistance on behalf of workers and former workers producing diesel systems at United Technologies Corporation, Springfield, Massachusetts. The notice of determinations was published in the **Federal Register** on August 13, 1985 (50 FR 32654).

The application claims, among other things, that United Technologies' market share in the diesel pump business for trucks and farm vehicles has declined and that their leading competitors are foreign. The union lists seven foreign competitors of United Technologies Corporation.

Conclusion

After careful review of the application, I conclude that the claim is of sufficient weight to justify reconsideration of the Department of Labor's prior decision. The application is therefore, granted.

Signed at Washington, DC, this 18th day of September 1985.

Stephen A. Wandner,

Deputy Director, Office of Legislauion and Actuarial Services, UIS.

[FR Doc. 85–23162 Filed 9–26–85; 8:45 am]

BILLING CODE 4510–30–M

[TA–W–16,135]

West Orange Manufacturing Co., West Orange, NJ; Termination of Investigation

Pursuant to section 221 of the Trade Act of 1974, an investigation was initiated on June 28, 1985 in response to a worker petition received on June 7, 1985 which was filed by the International Ladies' Garment Workers Union on behalf of workers at West Orange Manufacturing Company/Lady

Gilda, Incorporated, West Orange, New Jersey. The petitioner has requested that the petition be withdrawn. Consequently, further investigation in this case would serve no purpose, and the investigation has been terminated.

Signed at Washington, DC, this 19th day of September 1985.

Marvin M. Fooks,

Director, Office of Trade Adjustment Assistance.

[FR Doc. 85-23163 Filed 9-26-85; 8:45 am]

BILLING CODE 4510-30-M

Mine Safety and Health Administration

[Docket No. M-85-18-M]

Hydrocarbon Resources Co.; Petition for Modification of Application of Mandatory Safety Standard

Hydrocarbon Resources Company, Star Route 2, Box 192, Randlett, Utah 84063 has filed a petition to modify the application of 30 CFR 57.19102 (shafts) to its Wild Horse Mine (I.D. No. 4201744) located in Uintah County, Utah. The petition is filed under section 101(c) of the Federal Mine Safety and Health Act of 1977.

A summary of the petitioner's statements follows:

1. The petition concerns the requirement that a means be provided to guide the movement of a shaft conveyance.

2. As an alternate method, petitioner proposes to use glancing boards on the shafts ends and the walls of the shaft for the sides.

3. Due to the narrowness of the vein, the shaft walls can be cut to accommodate the conveyance. The interface between the Gilsonite and the rock walls is smooth, which allows the conveyance to move freely in the shaft.

4. Because the vein is narrow, petitioner states that a guide rail would restrict the ingress and egress from the conveyance, creating a potential hazard.

5. For these reasons, petitioner requests a modification of the standard.

Request for Comments

Persons interested in this petition may furnish written comments. These comments must be filed with the Office of Standards, Regulations and Variances, Mine Safety and Health Administration, Room 627, 4015 Wilson Boulevard, Arlington, Virginia 22203. All comments must be postmarked or received in that office on or before October 28, 1985. Copies of the petition are available for inspection at that address.

Dated: September 23, 1984.

Patricia W. Silvey,

Director, Office of Standards, Regulations and Variances.

[FR Doc. 85-23170 Filed 9-26-85; 8:45 am]

BILLING CODE 4510-43-M

[Docket No. M-85-111-C]

Kanawha Coal Co.; Petition for Modification of Application of Mandatory Safety Standard

Kanawha Coal Company, Route 1, Box 420, Ashford, West Virginia 25009 has filed a petition to modify the application of 30 CFR 75.1710 (cabs and canopies) to its Madison No. 2 Mine (I.D. No. 46-02844) located in Boone County, West Virginia. The petition is filed under section 101(c) of the Federal Mine Safety and Health Act of 1977.

A summary of the petitioner's statements follows:

1. The petition concerns the requirement that cabs or canopies be installed on the mine's electric face equipment.

2. Petitioner states that the use of canopies would result in a diminution of safety for the miners affected because the canopies could rub the roof and damage roof bolts. The canopy legs are being knocked loose to the extent that the canopy has hit the equipment operator. In addition, the canopies decrease the equipment operator's visibility, increasing the chances of an accident.

3. For these reasons, petitioner requests a modification of the standard for mining machines in mining heights lower than 48 inches.

Request for Comments

Persons interested in this petition may furnish written comments. These comments must be filed with the Office of Standards, Regulations and Variances, Mine Safety and Health Administration, Room 627, 4015 Wilson Boulevard, Arlington, Virginia 22203. All comments must be postmarked or received in that office on or before October 28, 1985. Copies of the petition are available for inspection at that address.

Dated: September 23, 1985.

Patricia W. Silvey,

Director, Office of Standards, Regulations and Variances.

[FR Doc. 85-23171 Filed 9-26-85; 8:45 am]

BILLING CODE 4510-43-M

[Docket No. M-85-112-C]

Magnum Quality Coal Co., Inc.; Petition for Modification of Application of Mandatory Safety Standard

Magnum Quality Coal Company, Inc., HCR 85, Box 1166, Whitesburg, Kentucky 41858 has filed a petition to modify the application of 30 CFR 75.1710 (cabs and canopies) to its No. 6 Mine (I.D. No. 15-07154) located in Letcher County, Kentucky. The petition is filed under section 101(c) of the Federal Mine Safety and Health Act of 1977.

A summary of the petitioner's statements follows:

1. The petition concerns the requirement that cabs or canopies be installed on the mine's electric face equipment.

2. Petitioner states that the use of a canopy on the mine's equipment would result in a diminution of safety for the miners affected because it could strike and dislodge roof support due to uneven roof and soft and uneven bottom. In addition the canopy would limit the equipment operator's visibility, increasing the chances of an accident.

3. For these reasons, petitioner requests a modification of the standard.

Request for Comments

Persons interested in this petition may furnish written comments. These comments must be filed with the Office of Standards, Regulations and Variances, Mine Safety and Health Administration, Room 627, 4015 Wilson Boulevard, Arlington, Virginia 22203. All comments must be postmarked or received in that office on or before October 28, 1985. Copies of the petition are available for inspection at that address.

Dated: September 23, 1985.

Patricia W. Silvey,

Director, Office of Standards, Regulations and Variances.

[FR Doc. 85-23172 Filed 9-26-85; 8:45 am]

BILLING CODE 4510-43-M

[Docket No. M-85-105-C]

Mettiki Coal Corp.; Petition for Modification of Application of Mandatory Safety Standard

Mettiki Coal Corporation, Route 3, Box 124A, Deer Park, Maryland 21550 has filed petition to modify the application of 30 CFR 75.1400 (hoisting equipment; general) to its C Mine (I.D. No. 18-00655) located in Garrett County, Maryland. The petition is filed under section 101(c) of the Federal Mine Safety and Health Act of 1977.

A summary of the petitioner's statements follows:

1. The petition concerns the requirement that a qualified hoisting engineer be on duty while any person is underground.

2. As an alternate method, petitioner proposes to allow a fireboss and pumper to travel into and out of the mine on a diesel powered 955 Eimco Mine Tender utility vehicle on weekends and holidays without having a hoisting engineer on duty. The mine has a roadway continuous from all sections to the surface which is maintained in a safe condition and is always available for travel. The mine phones provide the fireboss and pumper with continual contact with surface guards who could summon additional help immediately, if necessary.

3. For these reasons, petitioner requests a modification of the standard.

Request for Comments

Persons interested in this petition may furnish written comments. These comments must be filed with the Office of Standards, Regulations and Variances, Mine Safety and Health Administration, Room 627, 4015 Wilson Boulevard, Arlington, Virginia 22203. All comments must be postmarked or received in that office on or before October 28, 1985. Copies of the petition are available for inspection at that address.

Dated: September 23, 1985.

Patricia W. Silvey,

Director, Office of Standards, Regulations and Variances.

[FR Doc. 85-23173 Filed 9-26-85; 8:45 am]

BILLING CODE 4510-43-M

[Docket No. M-85-103-C]

Windsor Power House Coal Co.; Petition for Modification of Application of Mandatory Safety Standard

Windsor Power House Coal Company, Windsor Heights, West Virginia 26075 has filed a petition to modify the application of 30 CFR 75.503 (permissible electric face equipment; maintenance) to its Beech Bottom Mine (I.D. No. 46-01286) located in Brooke Country, West Virginia. The petition is filed under section 101(c) of the Federal Mine Safety and Health Act of 1977.

A summary of the petitioner's statements follows:

1. The petition concerns the use of a located padlock to secure battery plugs to machine-mounted battery receptacles on permissible, mobile, battery-powered machines.

2. As an alternate method, petitioner proposes to use metal locking devices, each consisting of a fabricated metal bracket in lieu of padlocks to secure battery plugs to machine mounted battery receptacles on permissible, mobile, battery-powered machines. The metal locking device will be designed, installed and used to prevent the threaded rings securing the battery plugs to the battery receptacles from unintentionally loosening. The metal locking devices will be securely attached to the battery receptacles to prevent accidental loss of the devices.

3. Petitioner states that the locking device will be easier to maintian than padlocks because there are no keys to be lost and dirt can't interfere with the working as with a padlock.

4. Operators of permissible, mobile, battery-powered machines affected by this modification will be trained in the proper use of the locking device, trained in the hazards of breaking battery-plug connnections under load, and trained in the hazards of breaking battery-plug connections in areas of the mine where electric equipment is required to be permissible.

5 For these reasons, petitioner requests a modification of the standard.

Request for Comments

Persons interested in this petition may furnish written comments. These comments must be filed with the Office of Standards, Regulations and Variances, Mine Safety and Health Administration, Room 627, 4015 Wilson Boulevard, Arlington, Virginia 22203. All comments must be postmarked or received in that office on or before October 8, 1985. Copies of the petition are available for inspection at that address.

Dated: September 23, 1985.

Patricia W. Silvey,

Director, Office of Standards, Regulations and Variances.

[FR Doc. 85-23174 File 9-26-85; 8:45 am]

BILLING CODE 4510-43-M

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

[Notice 85-61]

NASA Advisory Council, Space Systems and Technology Advisory Committee (SSTAC); Meeting

AGENCY: National Aeronautics and Space Administration.

ACTION: Notice of meeting.

SUMMARY: In accordance with the Federal Advisory Committee Act, Pub. L. 92-463, as amended, the National Aeronautics and Space Administration announces a forthcoming meeting of the NASA Advisory Council, Space Systems and Technology Advisory Committee, Informal Exectutive Subcommittee.

DATES AND TIMES: October 17, 1985, 8:30 a.m. to 5:30 p.m.; October 18, 1985, 8:30 a.m. to 1 p.m.

ADDRESS: National Aeronautics and Space Administration, 600 Independence Avenue, SW, Room 625, Washington, DC.

FOR FURTHER INFORMATION CONTACT: Mr. John Clark, Office of Aeronautics & Space Technology; Telephone: Area Code 202/453-2703

SUPPLEMENTARY INFORMATION: The Space Systems and Technology Advisory Committee was established to provide overall guidance and direction to the space research and technology activities in the Office of Aeronautics and Space Technology (OAST). The Committee, chaired by Mr. Norman Augustine, is comprised of 8 members. The meeting will be open to the public up to the seating capacity of the room (approximately 50 persons including the Subcommittee members and other participants).

Type of Meeting: Open.

Agenda:

October 17, 1985

8:30 a.m.—Chairperson's Remarks.
9 a.m.—Review of Space Systems Technology Committee (SSTAC) Charter; Review of SSTAC and Aerospace Research & Technology Subcommittee (ARTS) Membership.
9:30 a.m.—Review of FY 86 Study Topics.
10 a.m.—Review of Automation and Robotics Program.
1 p.m.—Review of OAST Mission Drivers.
5:30 p.m.—Adjourn.

October 18, 1985

8:30 a.m.—OAST Mission Drivers: summary of recommendations.
10:30 a.m.—OAST's Role as Catalyst to Build a National Constituency for In-Space Technolgy Experiments.
12:30 p.m.—Chairperson's Closing Comments.
1 p.m.—Adjourn.

Richard L. Daniels,

Deputy Director, Logistics Management and Information Programs Division, Office of Management.

September 20, 1985.

[FR Doc. 85-23093 Filed 9-26-85; 8:45 am]

BILLING CODE 75-10-01-M

[Notice 85-62]

NASA Advisory Council (NAC), Space and Earth Science Advisory Committee (SESAC); Meeting

AGENCY: National Aeronautics and Space Administration.

ACTION: Notice of meeting.

SUMMARY: In accordance with the Federal Advisory Committee Act, Pub. L. 92-463, as amended, the National Aeronautics and Space Administration announces a forthcoming meeting of the NASA Advisory Council, Space and Earth Science Advisory Committee.

DATE AND TIME: October 21, 1985, 9:30 a.m. to 5:30 p.m.; October 22, 1985, 8:30 a.m. to 5:30 p.m.; October 23, 1985, 8:30 a.m. to 12:30 p.m.

ADDRESS: National Aeronautics and Space Administration, FOB 10-B, Room 226-A, 600 Independence Avenue, S.W., Washington, DC 20546.

FOR FURTHER INFORMATION CONTACT: Dr. Jeffrey D. Rosendhal, Code E, National Aeronautics and Space Administration, Washington, DC 20546 (202/453-1410).

SUPPLEMENTARY INFORMATION: The NAC Space and Earth Science Advisory Committee consults with and advises the Council as a whole and NASA on plans for, work in progress on, and accomplishments of NASA's Space and Earth Science programs. The Committee is chaired by Louis Lanzerotti and is composed of 30 members.

Type of Meeting: Open.

Agenda: Full Committee—Room 226-A

October 21, 1985

9:30 a.m.—Introduction of New Members, Announcements, Review of Agenda.
9:45 a.m.—Status of FY 1986 Budget in Congress and Office of Space Science and Applications (OSSA) Major Projects.
10:15 a.m.—Shuttle/Spacelab Payloads: A Status Report.
11:15 a.m.—Task Force on the Scientific Utilization of the Space Station Activities.
1:30 p.m.—SESAC Study Planning/Review of Progress to Date and Report on Planning Group Activities.
2:30 p.m.—Organization of Study Activities; Splinter Meetings as Appropriate.
4:30 p.m.—Plenary Session to Discuss Study Planning.
5:30 p.m.—Adjourn.

October 22, 1985

8:30 a.m.—European Space Agency (ESA) Program Planning.
9:30 a.m.—An Overview of the Microgravity Science and Applications Program.
11:00 a.m.—Continuation of SESAC Study Planning; Splinter Meetings as Appropriate
1:30 p.m.—Continuation of SESAC Study Planning.
4:00 p.m.—Plenary Session to Review Study Activities.
5:30 p.m.—Adjourn.

October 23, 1985

8:30 a.m.—The Physics Survey Report.
9:30 a.m.—Earth System Science Committee (ESSC)/A Status Report.
10:45 a.m.—Progress of the National Commission on Space, Role of Various Federal Agencies in the Earth Sciences, and Other Office of Science Technology Policy (OSTP) Activities.
11:45 a.m.—Meeting Summary, Future Plans.
12:30 p.m.—Adjourn.

Richard L. Daniels,

Deputy Director, Logistics Management and Information Programs Division, Office of Management.

[FR Doc. 85-23094 Filed 9-26-85; 8:45 am]

BILLING CODE 7510-01-M

[Notice 85-63]

National Commission on Space; Meeting

AGENCY: National Aeronautics and Space Administration.

ACTION: Notice of meeting.

SUMMARY: In accordance with the Federal Advisory Committee Act, Pub. L. 92-463, as amended, the National Aeronautics and Space Administration announces a forthcoming meeting of the National Commission on Space (NCS).

DATE AND TIME: October 22, 1985, 8:30 a.m. to 5 p.m.; October 23, 1985, 8:45 a.m. to 4 p.m.

ADDRESS: Massachusetts Institute of Technology, Building 34, Room 401 (Greer Room), Cambridge, MA 02139.

FOR FURTHER INFORMATION CONTACT: Mrs. Mechthild E. "Mitzi" Peterson, National Commission on Space, Suite 3212, 490 L'Enfant Plaza East, SW, Washington, DC 20024 (202/453-8685).

SUPPLEMENTARY INFORMATION: The National Commission on Space was established to study existing and proposed U.S. space activities; formulate an agenda for the U.S. civilian space program; and identify long-range goals, opportunities, and policy options for civilian space activity for the next 20 years. The Commission, chaired by Dr. Thomas O. Paine, consists of 15 voting members. The meeting will be open to the public up to the seating capacity of the room (approximately 75 persons including Commission members and other participants).

Type of Meeting: Open.

Agenda

October 22, 1985

8:30 a.m.—Opening remarks

Space Sciences

8:45 a.m.—Evolution of the Universe
9:30 a.m.—Space-Based Observatories
10:30 a.m.—Solar and Space Plasma Physics
11:15 a.m.—Fundamental Physics and Chemistry
1 p.m.—Life Sciences
2 p.m.—Discussion
5 p.m.—Adjourn

October 23, 1985

8:45 a.m.—Opening Remarks

Space Technology

9 a.m.—Space Research and Technology
10 a.m.—Technology for Manned Space Flight
11 a.m.—Technology for Space Science
1 p.m.—Technology for Space Transportation
2 p.m.—Discussion
4 p.m.—Adjourn

Richard L. Daniels,

Deputy Director, Logistics Management and Information Programs Division, Office of Management.

NATIONAL SCIENCE FOUNDATION

Privacy Act of 1974; Alteration to System of Records

Notice is hereby given of a proposed amendment to the NSF System of Records No. 29 entitled "Principal Investigator/Project Director Subsystem," as published in the Federal Register, "Privacy Act Issuances, 1984 Compilation," Volume V, Page 209. The purpose of this amendment is to provide a better description of the categories of records covered by the "system."

NSF-29

SYSTEM NAME:

Principal Investigator/Project Director Subsystem.

SYSTEM LOCATION:

National Science Foundation, Office of Information Systems, 1800 G. Street, NW, Washington, D.C. 20550.

CATEGORIES OF INDIVIDUALS COVERED BY THE SYSTEM:

Each individual that requests support from the National Science Foundation, and Principal Investigators or Project Directors from institutions requesting NSF support.

CATEGORIES OF RECORDS IN THE SYSTEM:

Personal data as well as data on the disposition of each application of proposal submitted to the National Science Foundation.

ROUTINE USES OF RECORDS MAINTAINED IN THE SYSTEM, INCLUDING CATEGORIES OR USERS AND THE PURPOSES OF SUCH USES:

Information, other than personal information of a sensitive nature, may be released to other government agencies, which often receive proposals from the same Principal Investigator/ Project Director.

POLICIES AND PRACTICES FOR STORING, RETRIEVING, ACCESSING, RETAINING, AND DISPOSING OF RECORDS IN THE SYSTEM:

STORAGE:

Information is stored in an automated data base on disk and magnetic tapes.

RETRIEVABILITY:

Information can be retrieved by name or Social Security Number of applicant.

SAFEGUARDS:

Authorized Users: Employees who maintain records in this system are instructed to grant access to other employees on a need-to-know basis as specifically authorized by the Privacy Act Officer.

Physical Safeguards: Building employs security guards. Building is locked during non-business hours when guard is not on duty. Room in which records are kept is locked during non-business hours.

Procedural Safeguards: Access to computer files is controlled by the use of passwords. Access to source data files is strictly controlled by program staff.

RETENTION AND DISPOSAL:

Computer files are cumulative and maintained indefinitely.

SYSTEM MANAGER(S) AND ADDRESS:

(Computer files), Chief, Administrative Systems Branch, Office of Information Systems, National Science Foundation, Washington, D.C. 20550.

NOTIFICATION PROCEDURES:

To determine if a record exists write to: NSF Privacy Act Officer, National Science Foundation, Washington, D.C. 20550.

The National Science Foundation reserves the right to require sufficient identification to positively identify the individual making the request. Complete procedures are found at 45 CFR Part 613.

RECORDS ACCESS PROCEDURES:

See "Notification Procedures" above. Requester should reasonably specify the record contents being sought.

CONTESTING RECORDS PROCEDURES:

See "Notification Procedures" above. Requester should reasonably identify the record and specify the information to be contested, and state the corrective action sought and the reasons for the correction, with supporting justification.

RECORD SOURCE CATEGORIES:

Information is taken from materials submitted by the applicants.

SYSTEM EXEMPTED FROM CERTAIN PROVISIONS OF THE ACT:

None.

Dated: September 23, 1985.

Herman G. Fleming,

NSF Privacy Act Officer.

[FR Doc. 85-23079 Filed 9-26-85; 8:45 am]

BILLING CODE 7555-01-M

NUCLEAR REGULATORY COMMISSION

Regulatory Guides; Issuance and Availability

The Nuclear Regulatory Commission has issued revisions to three guides in its Regulatory Guide Series. This series has been developed to describe and make available to the public methods acceptable to the NRC staff of implementing specific parts of the Commission's regulations and, in some cases, to delineate techniques used by the staff in evaluating specific problems or postulated accidents and to provide guidance to applicants concerning certain of the information needed by the staff in its review of applications for permits and licenses.

Regulatory Guide 1.84, Revision 23, "Design and Fabrication Code Case Acceptability, ASME Section III, Division 1," and Regulatory Guide 1.85, Revision 23, "Materials Code Case Acceptability, ASME Section III, Division 1," list those code cases that are generally acceptable to the NRC staff for implementation in the licensing of light-water-cooled nuclear power plants. Revision 4 to Regulatory Guide 1.147, "Inservice Inspection Code Case Acceptability, ASME Section XI, Division 1," lists those code cases that are generally acceptable to the NRC staff for implementation in the inservice inspection of light-water-cooled nuclear power plants. These three guides are periodically revised to update the listings of acceptable code cases and to include the results of public comment and additional staff review.

Comments and suggestions in connection with (1) items for inclusion in guides currently being developed or (2) improvements in all published guides are encouraged at any time. Written comments may be submitted to the Rules and Procedures Branch, DRR, ADM, U.S. Nuclear Regulatory Commission, Washington, DC 20555. Comments may also be delivered to Room 4000, Maryland National Bank Building, 7735 Old Georgetown Road, Bethesda, Maryland, from 8:15 a.m. to 5:00 p.m. Copies of comments received may be examined at the NRC Public Document Room, 1717 H Street NW., Washington, DC.

Regulatory guides are available for inspection at the Commission's Public Document Room, 1717 H Street NW., Washington, DC. Copies of active guides may be purchased at the current Government Printing Office price. A subscription service for future guides in specific divisions is available through the Government Printing Office. Information on the subscription service and current prices may be obtained by writing to the Superintendent of Documents, U.S. Government Printing Office, Post Office Box 37082, Washington, DC 20013-7082.

(5 U.S.C. 552(a))

Dated at Silver Spring, Maryland this 23rd day of September 1985.

For the Nuclear Regulatory Commission.

Robert B. Minogue,

Director, Office of Nuclear Regulatory Research.

[FR Doc. 85-23184 Filed 9-26-85; 8:45 am]

BILLING CODE 7590-01-M

Advisory Committee on Reactor Safeguards; Meeting Agenda

In accordance with the purpose of section 29 and 182b. of the Atomic Energy Act (42 U.S.C. 2039, 2232b), the Advisory Committee on Reactor Safeguards will hold a meeting on October 10-12, 1985, in Room 1046, 1717 H Street, NW, Washington, DC. Notice of this meeting was published in the **Federal Register** on August 21, 1985.

The agenda for the subject meeting will be as follows:

Thursday, October 10, 1985

8:30 A.M.-8:45 A.M.: Report of ACRS Chairman (Open)—The ACRS Chairman will report briefly regarding items of current interest to the Committee.

8:45 A.M.-10:00 A.M.: Advanced Reactors (Open)—The members will discuss proposed ACRS comments to the NRC regarding the proposed Commission policy statement on advanced reactors. Members of the NRC Staff will participate, as appropriate.

10:00 A.M.-10:30 A.M.: Topics for Meeting with NRC Commissioners (Open)—The members will discuss ACRS recommendations to the NRC regarding EPA standards for high level waste disposal.

10:30 A.M.-11:30 A.M.: Meeting with NRC Commissioners (Open)—The members will meet with the NRC Commissioners to discuss ACRS comments on the EPA standards for high level waste disposal.

11:30 A.M.–12:45 P.M.: TVA Organizational Changes (Open)—Representatives of the NRC Staff will brief the Committee regarding proposed changes in the TVA organization to correct deficiencies in the construction and operation of TVA plants.

1:45 P.M.–3:45 P.M.: Davis-Besse Nuclear Power Plant (Open)—The members will hear and discuss a report by representatives of the NRC Staff regarding the results of the start-up test program for Unit 1 of this nuclear station. Representatives of the licensee will participate, as appropriate.

3:45 P.M.–5:45 P.M.: General Electric Standard Safety Analysis Report (GESSAR II) (Open/Closed)—The Committee will discuss the proposed ACRS report regarding the FDA for this type nuclear station.

Portions of this session will be closed as needed to discuss Proprietary Information applicable to this plant and detailed security arrangements for this type of facility.

5:45 P.M.–6:15 P.M.: Regulatory Activities (Open)—The members will hear and discuss the reports of its subcommittee on proposed changes to NRC Regulatory Guides, including meteorological measurement programs; criteria for power, instrumentation, and control portions of safety systems; and instrument setpoints for safety-related systems.

Friday, October 11, 1985

8:30 A.M.–11:30 A.M.: Source Terms for Nuclear Power Plant Accidents (Open)—The members will hear the report of its subcommittee and discuss proposed NUREG–0956, Reassessment of Source Term. Members of the NRC Staff will participate as appropriate.

11:30 A.M.–1:30 P.M.: Emergency Planning (Open)—The Committee will hear and discuss the report of its subcommittee on consideration of extreme external phenomena in emergency planning. Representatives of the NRC Staff and invited experts will participate, as appropriate.

2:30 P.M.–4:30 P.M.: General Electric Standard Safety Analysis Report (GESSAR II) (Open/Closed)—The members will continue discussion of the Committee's report to the NRC regarding an FDA for this type of nuclear plant.

Portions of this session will be closed as necessary to discuss Proprietary Information applicable to this matter and details of security arrangements for this type of plant.

4:30 P.M.–5:30 P.M. Indian Point Nuclear Generating Station, Units 2 and 3 (Open)—The members will discuss proposed ACRS comments regarding application of the PRA for these units to this nuclear station.

5:30 P.M.–6:30 P.M.: Nuclear Power Plant Safety-Related Issues (Open)—The members will discuss a proposed ACRS report on the state of nuclear power safety. Members of the NRC Staff will participate, as appropriate.

Saturday, October 12, 1985

8:30 A.M.–12:30 P.M.: Preparation of ACRS Reports (Open/Closed)—The members of the Committee will discuss proposed reports to the NRC regarding items considered during this meeting. In addition, proposed ACRS comments regarding the use of natural ability testing of nuclear power plant operators will also be considered.

1:30 P.M.–2:30 P.M.: Quantitative Safety Goals (Open)—The members will hear and discuss the report of its subcommittee on proposed quantitative safety goals for nuclear power plants.

2:30 P.M.–3:00 P.M.: ACRS Procedures and Practices (Open)—The Committee will hear and discuss the report of its subcommittee on proposed changes in ACRS procedures and practices based on meetings of its subcommittee and the report of its Panel on ACRS Effectiveness.

3:00 P.M.–4:00 P.M.: ACRS Annual Report to the U.S. Congress on the Proposed NRC Safety Research Program and Budget (Open)—The members will discuss the proposed scope and content of its annual reports to the U.S. Congress on proposed NRC safety research programs and budgets.

Procedures for the conduct of and participation in ACRS meetings were published in the **Federal Register** on October 3, 1984 (49 FR 193). In accordance with these procedures, oral or written statements may be presented by members of the public, recordings will be permitted only during those portions of the meeting when a transcript is being kept, and questions may be asked only by members of the Committee, its consultants, and Staff. Persons desiring to make oral statements should notify the ACRS Executive Director as far in advance as practicable so that appropriate arrangements can be made to allow the necessary time during the meeting for such statements. Use of still, motion picture and television cameras during this meeting may be limited to selected portions of the meeting as determined by the Chairman. Information regarding the time to be set aside for this purpose may be obtained by a prepaid telephone call to the ACRS Executive Director, R. F. Fraley, prior to the meeting. In view of the possibility that the schedule for ACRS meetings may be adjusted by the Chairman as necessary to facilitate the conduct of the meeting, persons planning to attend should check with the ACRS Executive Director if such rescheduling would result in major inconvenience.

I have determined in accordance with subsection 10(d) Pub. L. 92–463 that it is necessary to close portions of this meeting as noted above to discuss Proprietary Information [5 U.S.C. 552b(c)(4)], and detailed security information [5 U.S.C. 552b(c)(3)].

Further information regarding topics to be discussed, whether the meeting has been cancelled or rescheduled, the Chairman's ruling on requests for the opportunity to present oral statements and the time allotted can be obtained by a prepaid telephone call to the ACRS Executive Director, Mr. Raymond F. Fraley (telephone 202/634–3265), between 8:15 A.M. and 5:00 P.M. EDT.

Dated: September 23, 1985.

John C. Hoyle,

Advisory Committee Management Officer.

[FR Doc. 85–23216 Filed 9–26–85; 8:45 am]

BILLING CODE 7590-01-M

Advisory Committee on Reactor Safeguards; Combined Site Evaluation and Extreme External Phenomena; Meeting

The ACRS Subcommittees on Site Evaluation and Extreme External Phenomena will hold a combined meeting on October 9, 1985, Room 1046, 1717 H Street, NW, Washington, DC.

The entire meeting will be open to public attendance.

The agenda for the subject meeting shall be as follows:

Wednesday, October 9, 1985—8:30 a.m. until the conclusion of business.

The Subcommittees will: (1) Evaluate, from a probabilistic approach, the relative importance of various natural phenomena, and prioritize them in terms of their potential impact on offsite emergency planning, considering the likelihood that such phenomena might cause an accident that would require the implementation of offsite emergency plans, and (2) review the proposed final amendments to 10 CFR Part Part 50, Appendix E, Consideration of Earthquakes in Emergency Planning, SECY–85–283, dated August 21, 1985, and develop an ACRS consensus on this issue for the Commission.

Oral statements may be presented by members of the public with the concurrence of the Subcommittee Chairman; written statements will be accepted and made available to the

Committee. Recordings will be permitted only during those portions of the meeting when a transcript is being kept, and questions may be asked only by members of the Subcommittee, its consultants, and Staff. Persons desiring to make oral statements should notify the ACRS staff member named below as far in advance as is practicable so that appropriate arrangements can be made.

During the initial portion of the meeting, the Subcommittees, along with any of its consultants who may be present, may exchange preliminary views regarding matters to be considered during the balance of the meeting.

The Subcommittees will then hear presentations by and hold discussions with respresentatives of the NRC Staff, its consultants, and other interested persons regarding this review.

Further information regarding topics to be discussed, whether the meeting has been cancelled or rescheduled, the Chairman's ruling on requests for the opportunity to present oral statements and the time allotted therefor can be obtained by a prepaid telephone call to the cognizant ACRS staff member, Mr. Owen Merrill (telephone 202/634-1413) between 8:15 a.m. and 5:00 p.m. Persons planning to attend this meeting are urged to contact the above named individual one or two days before the scheduled meeting to be advised of any changes in schedule, etc., which may have occurred.

Dated: September 24, 1985.

Morton W. Libarkin,

Assistant Executive Director for Project Review.

[FR Doc. 85-23217 Filed 9-26-85; 8:45 am]

BILLING CODE 7590-01-M

[Docket No. 50-289 SP (Management Phase)]

Metropolitan Edison Co., et al. (Three Mile Island Nuclear Station, Unit No. 1); Oral Argument

Notice is hereby given that, in accordance with the Appeal Board's order of September 5, 1985, oral argument on the appeals of Three Mile Island Alert, Inc., and the Union of Concerned Scientists from the Licensing Board's May 3, 1985, partial initial decision on licensed operator training (LBP-85-15) will be heard at *2:00 p.m. on Thursday, October 24, 1985, in the NRC Public Hearing Room, Fifth Floor, East-West Towers Building, 4350 East-West Highway, Bethesda, Maryland.*

Dated: September 23, 1985.

For the Appeal Board.

Barbara A. Tompkins,

Secretary to the Appeal Board.

[FR Doc. 85-23218 Filed 9-26-85; 8:45 am]

BILLING CODE 7590-01-M

OFFICE OF THE UNITED STATES TRADE REPRESENTATIVE

Advisory Committee for Trade Negotiations; Meeting and Determination of Closing

The meeting of the Advisory Committee for Trade Negotiations (the Advisory Committee) to be held Wednesday, October 23, 1985 from 1:00 p.m. to 4:00 p.m. in Washington, D.C., will involve a review and discussion of the current issues involving the trade policy of the United States. Pursuant to section 2155(f)(2) of Title 19 of the United States Code, I have determined that this meeting will be concerned with matters the disclosure of which would seriously compromise the Government's negotiating objectives or bargaining positions.

More detailed information can be obtained by contacting Phyllis O. Bonanno, Director, Office of Private Sector Liaison, Office of the United States Trade Representative, Executive Office of the President, Washington, D.C. 20506.

[FR Doc. 85-23099, Filed 9-26-85; 8:45 am]

BILLING CODE 3190-01-M

SECURITIES AND EXCHANGE COMMISSION

[Release No. 22439; File No. SR-NASD-80-10]

Statement of Commission Views on Side-by-Side Market Making Pilot; Denial of NASD Petition for Reconsideration of Securities Exchange Act Releases No. 22026

I. Background

On May 8, 1985, the Commission issued a release that, among other things, approved in concept a pilot program in side-by-side market making involving the six most active National Market System ("NMS") Securities, subject to Commission determinations that grants of unlisted trading privileges ("UTP") and exchange side-by-side trading in the pilot stocks would be consistent with the Act and the creation of adequate equity and options audit trails.[1] In that Release the Commission solicited comment on exchange participation in the pilot. As discussed in a separate release,[2] the Commission is now prepared to grant exchanges UTP in certain NMS Securities, subject to certain conditions. For the reasons discussed below, the Commission continues to believe that the side-by-side pilot is appropriate, and also believes that the exchange participation in the pilot appears appropriate. In addition, the Commission announces that this pilot will begin on January 20, 1986.

On June 17, 1985, the NASD filed with the Commission a Petition for Reconsideration and Statement of Comments regarding the OTC Options Release.[3] For the reasons discussed below, the Commission denies the Petition for Reconsideration; the Commission, however, has carefully considered the Statement of Comments, along with those of other commentators, in addressing the question of exchange participation in the side-by-side market making pilot.

II. The Side-by-Side Market Making Pilot

A. Comments

The Commission received comments on the side-by-side market making pilot from the NASD[4] CBOE,[5] NYSE[6] PSE[7] and nineteen members[8] of the NASD.

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[1] Securities Exchange Act Release No. 22026, May 8, 1985, 50 FR 20310 ("OTC Options Release").

[2] Securities Exchange Act Release No. 22412, September 16, 1985 ("UTP Release").

[3] NASD Petition for Reconsideration and Statement of Comments on Securities Exchange Act Release No. 22026 ("Petition"), dated June 17, 1985.

[4] These comments are contained in the NASD's Petition *supra* note 3. In its Petition, and NASD also questions the Commission's April 16 deliberations for reasons other than the design of the pilot program. For example, the NASD complains that the April 16th meeting was affected by procedural irregularities. In addition, the NASD complains that it is competitively unfair not to allow the NASD to trade options on listed stocks and requests that the Commission's policy of allowing the multiple trading of index options should either be rescinded or clarified. There portions of the NASD Petition are discussed in Section III of this release.

[5] Letter from Walter E. Auch, Chairman and Chief Executive Officer, CBOE, to John Wheeler, Secretary, SEC, dated June 14, 1985 ("CBOE Letter").

[6] Letter from James E. Buck, Secretary, NYSE, to John Wheeler, Secretary, SEC, dated June 17, 1985 ("NYSE Letter").

[7] Letter from Jim Gallagher, President, PSE, to John Wheeler, Secretary, SEC, dated June 10, 1985 ("PSE Letter").

[8] Thomas J. Asher, Executive Vice President, Robinson Humphrey Company, Inc. to John S.R. Shad, Chairman, SEC, dated May 24, 1985; Jerry Williams, Williams Securities Group, Inc., to Commissioner Charles C. Cox, dated June 6, 1985; from C. Edward Fisher, Van Kampen Merritt, Inc., to SEC, dated May 31, 1985; Craig L. Beach, Vice President, George K. Baum & Company, to Commissioner Charles C. Cox, dated June 14, 1985; L. Massey Clarkson, Jr., Former Vice Chairman,

Continued

The NYSE and PSE comments restate the objections of those exchanges to the NASD's proposal on side-by-side market making; the PSE also asserts that approving the NASD proposal without permitting side-by-side market making on exchanges would subject the exchanges to an unfair competitive disadvantage; neither the PSE or NYSE discuss any issues peculiar to side-by-side market making on exchanges.

The NASD questions the design of the pilot for a variety of reasons. The NASD argues that (1) conditioning the pilot on participation of applicant exchanges is unnecessary competitively; (2) the pilot contains too few stocks; (3) the delay in the commencement of the pilot is competitively unfair to the NASD; and (4) the pilot should be limited to NASDAQ market makers [9] or if exchanges are allowed to participate in the pilot, "premium" listed stocks should be included in the pilot. Two broker-dealers also suggest that exchanges be excluded from the pilot.[10]

The CBOE states that the opportunity for exchanges to participate in the pilot would be "illusory," at least for CBOE, because CBOE, as an options only exchange, cannot obtain stock order flow in the six pilot stocks and would not have a realistic opportunity to compete for such order flow until systems and rules are in place that would provide CBOE market makers a fair opportunity to compete as a stock exchange. This problem is made especially difficult, according to CBOE, because of the ability of OTC market maker firms with retail orders to execute internally a substantial portion of their order flow. CBOE argues that it is unrealistic for CBOE to be ready to compete on a fair basis by October 1.[11]

Third, the CBOE argues that it is unfair for the Commission, "by inventing the pilot," to "impose on every OTC options market that wishes to avoid the unnecessary and unfair competitive burdens of NASD integrated trading a new trading environment" it has not sought and the terms of which have not yet been articulated. In this connection, the CBOE criticizes the absence of any criteria for determining whether market making in a pilot stock remains "competitive" and "dispersed," and argues that no single measure of "dominance" or "dispersion" can be applied in a uniform manner to both the OTC market and the securities exchanges.[12]

B. Discussion

(1) Competition Issues

(a) *Competitive Unfairness of Excluding Exchanges.* The NASD asserts that approval of side-by-side market making in NASDAQ but not on exchanges would not impose an unfair competitive burden on exchanges.[13] First, the NASD argues that exchanges already have an unfair competitive advantage over the NASD market due to their monopoly in listed options. The Commission continues to believe, however, that it would be competitively unfair to allow one group of market makers to make side-by-side markets in OTC options and stocks but to forbid another group of market makers from doing the same. The exchanges' existing franchises in options on certain listed stocks does not offset or somehow justify this competitive unfairness.

The NASD also argues that excluding exchanges from the pilot will not subject them to an unfair competitive disadvantage because exchange market makers may become NASDAQ market makers. Such an approach, however, ignores an essential difference between exchange trading and upstairs market making—the presence of a trading crowd on the floor of an exchange. While the NASD's alternative would permit the exchange specialist, or individual competing market maker, to enter quotations and compete with upstairs market makers, it would not provide a similar opportunity for other members of the exchange trading crowd. Thus, while the NASD's alternative permits competition between individual market makers, it does not provide for fair competition between marketplaces. Therefore, Commission continues to believe that allowing side-by-side market making in the OTC market but not on exchanges—if exchanges desire side-by-side market making—would subject exchanges to unfair competitive disadvantages.[14]

(b) *Competitive Fairness of Including Exchanges.* The NASD argues that allowing side-by-side market making on exchanges would be inappropriate because exchange specialists operating on exchange floors have unfair advantages in attracting order flow. According to the NASD, the absence of a "fair and neutral intermarket linkage in a multiple market environment would substantially impair the ability of broker-dealers to provide customers with the best execution of orders." Requiring exchange specialists and market makers to participate via NASDAQ, would, according to the NASD, avoid this problem. The NASD also argues that limiting the pilot to NASDAQ represents a preferable alternative with regard to "surveillance, regulatory concerns and the

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NASD District 7, to Chairman Shad, dated May 24, 1985; Maxwell Benson Jr., Vice President, Equitable Securities Corporation, John S.R. Shad, Chairman, SEC, dated May 30, 1985; John E. Herzog, President, Herzog, Heine Geduld, dated June 5, 1985; Nicholas Kelne, Vice President, Options, R.H. Dickinson & Co., to SEC, dated June 5, 1985; Edward T. Borer, President, Hopper Soliday & Co., Inc., to John S.R. Shad, Chairman, SEC, dated June 3, 1985; J. Stephen Putman, Robert Thomas Securities, Inc. dated May 25, 1985; Don D. Strand, President, Blakely, Strand & Williams, Inc. dated May 21, 1985; Thomas J. McAllister, CFP, McAllister Financial Planning Corp., dated June 7, 1985; Peter B. Madoff, Bernard L. Madoff, to John Wheeler, Secretary, SEC, dated June 27, 1985; Douglas A. Campbell, D.A. Campbell Company, Inc., to John S.R. Shad, Chairman, SEC, dated June 10, 1985; Murray L. Finebaum, Executive Vice President, Cantor, Fitzgerald & Co., Inc. to John S.R. Shad, Chairman, SEC, dated June 13, 1985; Alan S. Pareira, President, First Equity Corporation of Florida to John S.R. Shad, Chairman, SEC, dated June 12, 1985; H. Michael Collins, President, San Diego Securities Incorporated to John S.R. Shad, Chairman, SEC, dated, June 14, 1985; and Arnold Seidel, President, Morton Seidel & Co., Inc. to John S.R. Shad, Chairman, SEC, *et al.*, dated June 17, 1985; and Michael Silvestri, President, M.H. Myerson & Co., Inc. to Alden Adkins, SEC, dated June 26, 1985.

[9] The NASD points out that there is no restriction in its rules on exchange specialists acting as NASDAQ market makers. Thus, while this NASD alternative would exclude "exchanges" from participation in the pilot, it would not, according to the NASD, exclude exchange market makers or specialists. *See* Petition, *supra* note 3, at 31.

[10] Letters from Nicholas Kelne, Vice President, Options, R.H. Dickinson & Co., and letter from Michael Silvestri, M.H. Myerson & Co., Inc., *supra*, note 8. The other broker-dealers who commended suggested that the number of options on NMS Securities in the pilot be increased and that options on listed stocks should be included as well.

[11] The CBOE also indicates that approval of side-by-side market making OTC but not on exchanges would be inconsistent with section 15A(b)(9) of the Act by imposing a burden on competition not necessary or appropriate in furtherance of the Act's purposes.

[12] CBOE letter, *supra* note 5, at 6. The CBOE also raised additional concerns regarding the effects of UTP, as such, on the structure of the securities markets and the incentives for listing. In this regard, CBOE suggests that if the Commission nonetheless determines to proceed with the pilot and grant exchanges UTP in certain NMS Securities: (1) A uniform short sale rule should apply to the pilot stocks; (2) transaction and quotation information should be collected and disseminated on a consolidated basis; (3) pilot stocks should be deemed qualified for trading through ITS and CAES and subject to applicable trade-through rules; (4) the Commission should consider adoption of all-markers order exposure and uniform priority rules for these stocks; (5) the governance provision of the intermarket trading linkage and information systems must be modified to provide CBOE (and other new markets) an appropriate voice thereunder; and (6) regulatory disparities in trading requirements between the OTC and exchange markets should be eliminated. These issues are discussed in the UTP Release, *supra* note 2.

[13] NASD Petition, *supra* note 3, at 29. As indicated above, the NYSE, PSE and CBOE all argued that allowing side-by-side market making in the OTC markets but not on exchanges would result in competitive unfairness to the exchanges.

[14] The NASD also argues that if exchanges are not excluded from the pilot "premium" listed stocks should be included. This comment is discussed in *infra* in section II B.1.c.

Commission's need for data to . . . evaluate the success of the pilot."[15]

As discussed in the OTC Options Release, the Commission does not believe that the market linkage facilities the NASD describes are necessary conditions to multiple trading because the benefits that multiple trading provides outweigh the short-term inefficiencies that may result from the absence of such facilities.[16] The Commission also found that such facilities should not be mandated, at least until it was clear that such a linkage was economically justified. These findings are equally applicable to multiple trading between the OTC market and exchanges as for multiple trading among exchanges.[17] In addition, side-by-side market making both in the OTC market and on exchanges appears likely to provide more, not less, data with which to evaluate the trading experience during the pilot. Finally, the Commission believes that, with the equity audit trail being developed by the NASD and the options audit trails possessed by each of the options exchanges, surveillance of trading under the pilot will be satisfactory, whether such trading occurs OTC or on an exchange.[18]

As discussed above, the CBOE, on the other hand, argues that exchanges participating in the pilot, particularly the CBOE, would be at an unfair competitive disadvantage. The fact that, in order to participate in the pilot, CBOE would have to become a new entrant to the stock market, however, is not a "burden on competition" imposed upon CBOE by the Commission or by the Commission's rules or regulations. Indeed, the purpose of allowing CBOE and other exchanges to participate in the pilot is to remove obstacles that otherwise might exist and allow the exchanges an opportunity to compete. The Commission, in prividing this opportunity, cannot assure that all competitors will be on an absolutely equal footing; rather, the Commission has an obligation to ensure that the competition that results is not restricted in a manner that is not necessary or appropriate in furtherance of the purposes of the Act. In this connection, the Commission notes that the Commission allowed trading in options on OTC stocks on a non side-by-side basis pending its decision on exchange participation in the pilot, thus permitting CBOE and other established options exchanges to trade options in OTC stocks before the NASD, a new options market entrant, was ready to commence trading, despite claims by the NASD that this later start was competitively unfair.[19] The Commission also permitted established options exchanges to multiply trade these options, despite claims by new entrants, NASD and BSE, that this would be competitively unfair.[20] In making these determinations, the Commission decided that it could not, and would not, attempt—in the name of competitive fairness—to ameliorate the results of business decisions made by the various competitors in creating and operating their respective markets. Instead, the Commission consistently sought to ensure only that there are no significant regulatory disparities among those competitors. Up until now, the CBOE has made a conscious business decision to remain an options-only exchange. The Commission does not believe it would be appropriate to delay the introduction of new trading programs for other marketplaces solely to allow the CBOE to reposition itself as a stock exchange.

Second, the competitive unfairness CBOE alleges all exchanges will confront in the side-by-side pilot—essentially that upstairs firms can internalize order flow—is an allegation that exchanges have made before in the proceedings leading to the adoption of Rule 19c-3 under the Act.[21] As discussed in the OTC Options Release, exchanges have maintained their market share in Rule 19c-3 stocks despite initial active market making by many OTC firms. In that context, most firms continued to send their order flow to exchanges because (1) they did not find it profitable to make markets in Rule 19c-3 stocks, (2) they did not wish to send order flow to competitors in that stock, or (3) they preferred an auction market environment. The Commission recognizes that because the six pilot stocks are now solely traded in the NASDAQ market, brokerage firms may make different decisions as to where to allocate their customer order flow in the stock and options than they did for Rule 19c-3 securities. The Commission believes, however, that the Rule 19c-3 experience indicates that these decisions are not preordained simply by the firm's ability to internalize customer order flow.[22] Indeed, the NASD believes that the Rule 19c-3 experience indicates that upstairs firms are at an unfair disadvantage in competing with exchange specialists and market makers.[23]

In approving, in concept, the NASD's proposal to trade options OTC and the side-by-side pilot, but only if it determined to permit exchange participation, the Commission believed that such a pilot would be consistent with the Act. The Commission believes that the pilot, by allowing exchange and OTC market makers to make side-by-side markets in the same stocks and their related options, subject to comparable regulatory restrictions (giving due consideration to structural differences in these two trading systems), is structured to provide exchange and OTC market makers an opportunity to compete fairly. Accordingly, rather than either speculate on which system—exchange or OTC—will be more successful competitively or attempt to require that each system operate in an identical fashion, the Commission believes that the market place should determine which system, if any, is more successful in attracting order flow.[24] As the Congress noted in connection with the 1975 Amendments,[25] while "the goals of the [NMS] . . . are nearly universal in scope . . . [t]his is not to say that the goal of the legislation is to eliminate distinctions between the exchange market and [OTC] markets . . . some present distinctions may tend to disappear in a [NMS] but it is not the intention of the bill to force all markets

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[15] Petition, *supra* note 3, at 32.

[16] OTC Options Release, *supra* note 1, 50 FR at 20332.

[17] As discussed in section II B.1.a., the Commission does not believe that allowing exchange specialists and market makers to participate in the pilot as NASDAQ market makers is a feasible alternative. For the reasons discussed in the text above, the Commission believes that competition under the pilot between OTC and exchange market makers will occur on fair terms; thus, the Commission also finds that this proposed alternative is unnecessary.

[18] *See* UTP Release, *supra* note 2.

[19] OTC Options Release, *supra* note 1, 50 FR at 20333. *See* also Securities Exchange Act Release No. 22044, May 12, 1985, 50 FR 21532 (approving Phlx National OTC Index Option).

[20] OTC Options Release, *supra* note 1, 50 FR at 20329 n.137 and 191; and 20331–20332.

[21] 17 CFR 240.19c-3 (1985).

[22] OTC Options Release, *supra* note 1, 50 FR at 20332 n.221.

[23] NASD Petition, *supra* note 3, at 20–21.

[24] In adopting Rule 19c-3, the Commission concluded that, at least in that limited context, the competitive benefits offered by the Rule, combined with the experiential benefits to the Commission and the industry of observing the trading that would occur if the Rule were enacted, outweighed the potential risks that might result from enacting the Rule. Securities Exchange Act Release No. 16888, June 11, 1980, 45 FR 41125 at 41129–30. In coming to this conclusion, the Commission fully considered internalization concerns identified by some commentators, including adverse competitive effects on exchange market makers. *Id.*, 45 FR at 41128. The Commission believes this reasoning is equally applicable in the context of the limited six stock side-by-side market making pilot.

[25] Securities Acts Amendments of 1975 (Pub. L. No. 94–29, June 4, 1975).

for all securities into a single mold." [26] The Commission will, of course, monitor the operation of both systems and watch for opportunities whereby either system can be improved in a manner which furthers investor protection or the integrity of and confidence in the trading markets.[27]

(c) *Inclusion of Listed Stocks.* As an alternative to the exclusion of exchanges from the pilot, the NASD suggests that "premium" listed stocks be included in the pilot. The NASD argues that it would be competitively unfair to allow side-by-side market making in OTC stocks and their related options while not simultaneously allowing side-by-side trading in listed stocks and their related options. The Commission disagrees.

First, with respect to the six stocks included in the pilot, all exchange specialists and OTC market makers would have an opportunity to participate. So, as these six stocks a competitively fair environment would exist.

Second, the NASD's proposed alternative is premature. The NASD has not previously sought to trade options on "premium" listed stocks.[28] In the event the NASD submits such a filing, the Commission, of course, would review it under the standards and procedures set forth in the Act and Commission rules. However, the Commission does not believe, nor has the NASD offered, any persuasive reason why Commission action on side-by-side trading of OTC stocks and options on those stocks pursuant to the pilot must be delayed pending Commission substantive review of and action on an integrated market making pilot involving listed stocks that no market has yet requested.

In this regard, without seeking to address on the merits the question of a side-by-side pilot involving listed stocks, the Commission notes that such a proposal would raise issues distinct from those raised by the integrated market making pilot in OTC stocks and their related options. Historically, the Commission and commentators have found that side-by-side market making by exchange specialists, [29] who dominate order flow in listed stocks, [30] always has raised regulatory concerns of a different degree than side-by-side market making in more dispersed and competitive markets.[31] Extension of a side-by-side pilot in premium listed stocks to NASDAQ market makers also would require that exchange off-board trading rules be lifted in these stocks. While the Commission notes the relationship asserted by the NASD and others between granting exchanges UTPs (to allow exchange market makers to trade OTC stocks and their related options side-by-side) and the removal of off-board trading restrictions (to allow NASDAQ market makers to trade listed stocks and their related options side-by-side), as the Commission has made clear in the UTP release it views these matters as district, and believes each must be addressed separately on its own merits.[32]

(2) *Number of Stocks in the Pilot.* The NASD argues that the OTC Options Release both fails to explain why these six stocks are appropriate and fails to explain why more stocks would not be appropriate. The Commission believes that the OTC Options Release adequately explains both the basis for including the six pilot stocks chosen and the basis for not approving the NASD's proposal as submitted.

In summary, the NASD proposal—which essentially would have allowed side-by-side market making in all options eligible OTC stocks with at least 15 market makers—raised concerns that side-by-side market making could occur in markets that are not both highly competitive and characterized by dispersal of order flow among market makers. The Commission concluded that, because of the potential regulatory risks that might arise in such markets, side-by-side market making should occur initially only in clearly competitive, dispersed markets. Moreover, the OTC Options Release noted that the six stocks chosen have sufficient depth, liquidity and dispersion of order flow to reduce these concerns to manageable proportions, so long as comprehensive equity and options audit trails are in place.[33]

While the NASD suggests that the number of pilot stocks should be increased to at least 20, and should include stocks in a greater variety of industries, it fails to justify these suggestions in terms of the regulatory concerns that underlie the pilot's more limited approach.

Thus, the NASD attempts to justify the inclusion of more stocks in the pilot on the ground that six stocks and the one year duration of the pilot "mute" the economic incentives for NASDAQ market makers to commit the resources necessary to participate in the pilot. The NASD also argues that the inclusion of a larger number of NMS Securities (and listed stocks) from a broader cross-section of industries would make it possible to gather more useful empirical data during the pilot. In the absence of any justification for concluding that such economic incentive concerns should override the Commission's regulatory concerns, and given the significance of these concerns, the Commission concludes that the number of stocks in the pilot should not be increased.[34] Moreover, the Act does not

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[26] Senate Comm. on Banking, Housing & Urb. Affs., *Report to Accompany S. 249: Securities Acts Amendments of 1975*, S. Rep. No. 94-75, 94th Cong., 1st Sess. 7 (Comm. Print 1975).

[27] The CBOE also argues that, in the long term, competition in the stock market will be adversely affected by the side-by-side pilot. The Commission does not believe that a limited, six stock pilot is likely to have the "profound" effects CBOE alleges. Indeed, whether side-by-side trading will have structural consequences on the securities markets is one purpose of commencing such trading on a limited pilot basis. Accordingly, the Commission finds it unnecessary at this time to seek to determine with finality whether side-by-side trading will have the long-term impact the CBOE asserts.

[28] The NASD originally included in its filing a request to trade options on Rule 19c-3 stocks. While the term "premium" is not defined in the NASD Petition, we read the NASD's request as extending primarily, or perhaps exclusively, to listed stocks that are not covered by Rule 19c-3. Any such pilot would raise substantial new questions regarding the further removal of exchange off-board trading restrictions and the expansion of options multiple trading.

[29] Because it would raise competitive concerns if OTC market makers were allowed to make side-by-side markets in listed stocks and their related options, but exchange specialists, and market makers were not allowed to do so, we assume, for purposes of discussion, that exchange specialists also would be permitted to participate in any extension of the pilot to listed stocks.

[30] The NYSE accounts for approximately 85% of volume in consolidated share volume (*i.e.*, volume on all exchanges) in its listed stocks.

[31] *See e.g.*, SEC, *Report of the Special Study of the Options Markets*, H.R. Rep. No. IFC 3, 96th Cong. 1st sess. (Comm. Print 1978), at 870-926; and Securities Exchange Act Release No. 21710, February 4, 1985, 50 FR 5708 (approving NYSE specialists use of options for hedging purposes).

[32] A listed stock side-by-side pilot would also raise discrete questions regarding the expansion of multiple trading for options on listed stocks. OTC Options Release, *supra* note 1, 50 FR 20330-20333.

[33] *Id.*, 50 FR at 20333.

[34] Moreover, the Commission in approving in concept a side-by-side trading pilot carefully considered these issues and indicated that the economic incentives associated with the pilot are adequate. The NYSE, for example, commenced an options program with only one index option. Subsequently the NYSE began an individual options program with only three individual stock options. Thus, at least one SRO, and its membership, has been able to begin options trading with a smaller number of products. Indeed, although the NASD contends that six stocks are too few to create sufficient economic incentives for participation, it also argues that the six pilot stocks represent the "significant portion of those securities believed likely candidates for application for exchange UTP." NASD Petition, *supra* note 3, at 18-19. Indeed, the NASD provides data in its Petition showing that the six pilot stocks represent 5.8% of the total volume of trading in NASDAQ securities, 9.3% of total volume of NMS Securities and 20.3% of total capitalization of the top 100 NMS Securities. *Id.* Finally, the Commission notes that it has recently approved a

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contemplate the approval of a trading environment simply to obtain more interesting research data. Rather, the foremost concern of the Commission is, and must be, that the proposal ensures the protection of investors and is consistent with the other purposes of the Act. Thus, even if an expanded pilot might provide interesting information, because the Commission cannot conclude, at this time, that the regulatory risks associated with such an expansion are minimal, the Commission continues to believe, for the reasons stated in the OTC Options Release, that a six stock pilot is the appropriate starting point.[35]

(3) *Exchange Participation.* As discussed in the OTC Options Release, a premise of the side-by-side pilot is that the markets for the six pilot stocks are sufficiently deep, liquid and dispersed that the regulatory concerns raised by side-by-side market making can be addressed by appropriate surveillance systems so long as equity and options audit trails are in place. While, as discussed above, the Commission cannot predict the extent to which exchanges will attract orders in the six pilot stocks, it believes it unlikely, in the absence of an issuer listing decision, that any exchange will initially receive a dominant market share in any of those stocks. Therefore, the Commission sees no reason why the same premise underlying approval of OTC side-by-side trading should not be applicable to exchange specialists and market markers, who would initially, at least, constitute additional competitors in these deep, liquid and dispersed markets.[36] The Commission also believes that the increased efficiency and market liquidity which should be obtained from the side-by-side pilot can also be anticipated from exchange participation in that pilot.[37] The Commission agrees with CBOE, however, that, to participate in the pilot, an exchange would of course have to file an appropriate submission pursuant to Rule 19b-4 under the Act. Subject to an exchange making the necessary Rule 19b–4 filing and surveillance enhancements, the Commission believes that exchange participation in the pilot would be appropriate.[38]

The Commission believes that the markets for the six pilot stocks are such that side-by-side trading in the stocks and their related options will not give rise to unmanageable surveillance problems during the one-year duration of the pilot.[39] At the conclusion of the one year period, the Commission believes it will be in a better position to determine the standards that might be appropriate in order to allow side-by-side trading in any additional stocks, or indeed, to allow side-by-side trading to continue in the six pilot stocks.[40]

(4) *Commencement Date.* The NASD also suggests that it is unfair not to have required the exchanges to wait to commence trading options on NMS stocks until the NASD is ready, but to require the NASD to wait to commence trading side-by-side until the exchanges are provided an opportunity to participate in the side-by-side pilot.

In brief, the Commission allowed the exchanges to commerce trading when they were operationally ready to do so, and determined that it would be unfair to delay their programs because the NASD had chosen not to prepare itself for a start up of trading the same time.[41] The Commission also made clear that, aside from the question of side-by-side trading, the NASD proposal appeared consistent with the Act and could be approved whenever the NASD so requested.[42]

In contrast, the Commission delayed the side-by-side pilot for two reasons. First, the Commission determined that the side-by-side pilot would only be fair if the exchanges were given an opportunity to participate, a matter that was not before the Commission at the April 16th meeting. In addition, exchange participation was contingent on the Commission's decision of whether to grant the exchanges UTP in the six pilot stocks, a matter that was under review by the Commission at the time of the April 16th meeting. Thus, the Commission was unable to determine whether exchanges would be able to participate in the pilot. Hence, some period of delay was needed to enable the Commission to deliberate on the questions of extending to exchanges OTC/UTP and exchange participation in the side-by-side pilot.

Second, the target start-up date of the pilot—October 1—was designed to coincide with the earliest time that the NASD had indicated it could have in place the surveillance systems (equity and options audit trails) necessary to support side-by-side market making in the pilot program. The NASD had informed the Commission staff that it now estimates that it will not have an equity audit trail in place until January 1, 1986, at the earliest. Similarly, the implementation of transaction and quotation reporting plans for OTC securities which was set as a condition for the grant of UTP will take several months.[43]

While no one has requested a postponement of the Commencement of the pilot, because it is now clear that the NASD's best, *bona fide* efforts will result in an equity audit trail no sooner than January 1, 1986, the Commission believes that it is appropriate to postpone the commencement of the pilot to January 20, 1986, to coincide with the first Monday after the January expiration date.[44]

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proposal for the NASD to commence trading two OTC index options. Securities Exchange Act Release No. 22404 (September 13, 1985). The NASD has indicated it will begin index options trading on September 27, 1985, notwithstanding the availability of any other OTC options products.

[35] The Commission does not believe the number of industries represented in the pilot is relevant. The principal purpose of the pilot is to determine whether particular stocks with specific trading characteristics (*i.e.*, market maker participation, dispersion of order flow, firm trade and quote information, among other things) can be traded side-by-side with their related options without raising undue regulatory concerns. Accordingly, the fact that the six stocks with the characteristics desired fall into but two industry groups (Computer technology and communications) in no way impairs the validity of the pilot. Nor has the NASD suggested that there are particular characteristics of these industries, or the stocks of companies in these industries that would somehow render integrated market making in these stocks *per se* inappropriate or non-comparable with trading of stocks of companies in other industries.

[36] It is possible that, during the pilot, an exchange may establish a significant market share. The Commission does not believe, however, that it is necessary to establish standards for the pilot which would limit a market's share of volume or otherwise inhibit such a development. In designing the pilot, and particularly in limiting it to six stocks, the Commission has allowed for the possibility of market concentration and believes that the surveillance concerns would be manageable. Nevertheless, the Commission will, of course, monitor trading during the pilot to determine if the market share of any participant raises any regulatory concerns.

[37] OTC Options Release, *supra* note 1, 50 FR at 20321–22.

[38] In this connection, the Commission emphasizes that, once equity and options audit trails and other necessary surveillance enhancements are in place, the pilot may commence. Should the exchanges decide not to avail themselves of the opportunity to participate in the pilot, the NASD can proceed at such time by itself.

[39] Should manipulation or other concerns arise during the pilot period in any of the six stocks or their related options, the Commission, of course, would be prepared to take action promptly to address the concerns.

[40] In that regard, the Commission might also determine that it was inappropriate to allow the pilot to continue under any circumstances.

[41] OTC Options Release, *supra* note 1, 50 FR at 20333.

[42] *Id.*, 50 FR at 20321.

[43] *See* UTP Release, *supra* note 2.

[44] January 20 is the first trading day in January after the expiration of January series of options. The OTC Options Release also made clear that October 1, 1985, was a tentative date.

III. The Other Portions of the NASD Petition

As indicated above,[45] the NASD commented on the design of the side-by-side pilot program and the timing of commencement of trading of options on OTC stocks. In its Petition, the NASD also questions other aspects of the April 16th meeting and OTC Options Release. The NASD argues that (1) certain procedural irregularities taint the Commission's decisions, particularly as to multiple trading of OTC options and the side-by-side pilot; (2) it is competitively unfair to allow the exchanges to trade options on OTC stocks but not to allow the NASD to trade options on listed stocks; (3) the Commission should make clear that its decision to approve the multiple trading of index options does not affect any legitimate proprietary rights in such indexes. Even assuming that the Commission's procedures regarding reconsideration are applicable here,[46] we believe the NASD's arguments are unpersuasive.

A. Procedural Irregularities

The NASD claims that, in connection with its April 16th meeting, the Commission did not comply with the applicable public notice requirements.[47] The NASD states that the notice of the Commission's April 16th meeting, issued pursuant to the Government in the Sunshine Act, was not exhaustive in listing the issues to be discussed. The NASD also argues that the Commission failed to comply with the notice requirements of the Exchange Act and the rules thereunder. These failures, the NASD claims, deprived it of due process and equal protection of the law.

The NASD was accorded every procedural protection to which it was entitled. The Commission's Sunshine Act notice fully complied with the statutory requirements. The purpose of that notice, like other similar notices of upcoming meetings, is merely to announce the date of an upcoming meeting and the subjects to be discussed therein. It is issued to give interested persons an opportunity to attend the meeting (and representatives from the NASD were at the April 16th meeting), not for the purpose of providing an opportunity to submit comments.[48]

Further, the Commission provided the NASD with an opportunity to comment on the subject matter of the meeting. In April 1984, the Commission published a release which framed the issues to be considered in these proceedings, indicated certain preliminary views and specifically solicited comments.[49] The NASD, like many other commentators, availed itself of this opportunity by submitting comments.[50] Thus, the NASD not only had adequate notice of the issues to be discussed at the April 16th meeting, but the NASD also actually commented on these issues (as did numerous other commentators). The Commission noted and addressed these comments in the OTC Options Release.[51]

B. Competitive Fairness

The gravamen of the NASD's Petition seems to be that it is competitively unfair to permit the multiple trading of options on OTC stocks while (1) not approving, and indeed discouraging, the NASD proposal to trade options on exchange listed stocks which are not covered securities under Rule 19c-3 under the Act ("19c-3 stocks")[52] and (2) not allowing the multiple trading of options on all eligible listed stocks.

The NASD correctly notes that it agreed to deferral of Commission action on the listed options proposal at the request of the Commission staff.[53] Once the NASD made its decision to defer, the issue of multiple trading of listed stocks was not before the Commission at the April 16th meeting.[54]

Moreover, the Commission notes that the NASD has not in any way been actually prejudiced by and Commission action or inaction as to options on 19c-3 stocks, for the simple reason that the NASD is apparently still not ready operationally to trade any options.[55] Further, the NASD has not explained why, nor does the Commission perceive a clear basis for concluding that, the NASD would be in a better position to compete in the market for options in OTC stocks if it also could trade options on all or certain listed stocks.

Nevertheless, the Commission believes that the OTC Options Release adequately explains why it is not competitively unfair to permit the multiple trading of options on OTC stocks but not allow the multiple trading of options on listed stocks. The OTC Options Release explains the reasons why the Commission does not currently approve the multiple trading of listed stocks (essentially the potential disruption of existing market structures); and why multiple trading of options on OTC stocks does not raise these concerns. The OTC Options Release also explains why the "unfair advantage" that the NASD and others[56] argue will result is, in fact, simply the consequence of competition between new entrants and established market participation.[57] For the reasons stated

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[45] *Supra* note 4.

[46] *See* Commission Rule of Practice 21(e), 17 CFR 201.21(e).

[47] NASD Petition, *supra* note 3, at 4. The NASD also claims that it was inappropriate for the Commission to rely upon non-public staff memoranda at the April 16th meeting. Subsequent to the April 16th meeting the NASD sought these memoranda pursuant to the Freedom of Information Act. Letters from John J. Flood, Senior Attorney, NASD, to Edward A. Wilson, FOIA Officer, SEC, dated May 24, 1985. The Commission denied that this request in reliance upon 5 U.S.C. 552(b)(5). Letter from Edward A. Wilson, FOIA Officer, SEC, to John Flood, Senior Attorney, NASD, dated June 11, 1985.

[48] *See* Senate Committee on Governmental Operations, *Report to Accompany S. 5: Government in the Sunshine Act*, S. Rep. No. 94-354, 94th Cong., 1st Sess. 1-2 (Comm. Print 1975). Indeed, the remedy for violations of the Government in the Sunshine Act is not setting aside action taken at meetings improperly noticed or closed, but rather is to provide transcripts and minutes of meetings. 5 U.S.C. 552b(h) (1) and (2). Although the Commission does not believe the Sunshine Act notice was defective, the NASD has been provided transcripts of the Commission's discussion that occurred during the April 16th meeting and the resulting Commission minute.

[49] Securities Exchange Act Release No. 20853, April 12, 1984, 49 FR 45291.

[50] Among other things, the NASD stated that options on OTC stocks initially should be traded exclusively in NASDAQ and that the multiple trading of options on OTC stocks should not be allowed unless the Commission also allowed the NASD to multiply trade options on listed stocks. Letter from Gordon Macklin, President, NASD to George A. Fitzsimmons, Secretary, SEC, dated June 15, 1984. The Commission notes that the NASD's comments on the multiple trading of options on OTC stocks contained in its Petition for Reconsideration are substantially similar to its comments submitted previously and discusssed in the OTC Options Release.

[51] OTC Options Release, *supra* n.1, 50 FR at 20329 n.192 and 20331-32.

[52] The term "19c-3 stocks" refers to stocks listed on an exchange after April 26, 1979. These stocks are not subject to exchange off-board trading restrictions. *See* Rule 19c-3 under the Act, 17 CFR 240.19c-3 (1984).

[53] Letter from John J. Flood, Attorney, NASD to Alden Adkins, Division of Market Regulation, SEC, dated April 8, 1985, and OTC Options Release, *supra* note 1, 50 FR at 20316, n.62.

[54] Subsequent to the April 10th meeting, the NASD did withdraw this consent. Letter from John S. Flood, Attorney, NASD, to Alden Adkins, Attorney, Division of Market Regulation, dated June 17, 1985.

[55] The Commission notes that in the OTC Options Release it specifically approved the continued multiple trading of options on any OTC stock that lists on an exchange after multiple trading of options on the stock commences. OTC Options Release, *supra* note 1, 50 FR at 20331, n.214. In other words, the Commission already has approved, in part, the multiple trading of options on stocks that become 19c-3 stocks after standardized options are multiply traded on the stocks. If the NASD obtains approval of its propsoal to trade options on 19c-3 stocks, it could of course multiply trade such options, just as the exchanges can.

[56] The Boston Stock Exchange, Inc., another potential new options entrant, made essentially the same comment.

[57] OTC Options Release, *supra* note 1, 50 FR 20331-20332. *See also* Section II B 1.b., *supra*.

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in the OTC Options Release, the Commission does not believe that as a matter of logic or competitive fairness it is necessary that the Commission couple its consideration of issues related to options on OTC stocks with its review of those same matters in connection with options on listed stocks.

C. Multiple Trading of Index Options

As discussed above, the NASD also suggests that the Commission should not have approved the multiple trading of index options. It requests that this approval either be reconsidered or that the Commission make clear that the approval of multiple trading of index options does not represent "a legal determination by the Commission with respect to the validity of any copyright, trademark, service mark or related claims" with respect to the index.[58]

The Commission believes that the issue of the multiple trading of index options was properly before the Commission at its April 16th meeting and continues to believe that its approval of the multiple trading of index options was appropriate and consistent with its previous decisions to allow the multiple trading of index options among exchanges.[59] The Commission recognizes that the primary effect of this policy is to allow more than one SRO to trade options on similar indexes.[60] Indeed, the Commission in announcing its decision on the multiple trading of index options has not been faced either with proposals to trade options on identical indexes or with any claims of infringement of copyright or other proprietary rights.[61]

IV. Conclusions

For the reasons discussed in the OTC Options Release, the Commission continues to believe that side-by-side market making in the six pilot stocks should offer substantial market benefits and, with equity and options audit trails in place, also should reduce to surveillable levels the regulatory concerns raised by side-by-side market making. The Commission also believes that the inclusion of exchange specialists and market makers does not appear to create any additional or unique regulatory problems and provides all relevant markets a fair competitive opportunity. The Commission believes that the pilot can commence on January 20, 1986, if equity and options audit trails and other necessary surveillance enhancements are in place. For the reason discussed above, and in the OTC Options Release, the Commission also denies the NASD's Petition.

Dated: September 20, 1985.

By the Commission.

John Wheeler,

Secretary.

[FR Doc. 85-23137 Filed 9-26-85; 8:45 am]

BILLING CODE 8010-01-M

Issuer Delisting; Notice of Application To Withdraw From Listing and Registration; Getty Petroleum Corp

September 19, 1985.

The above name issuer has filed an application with the Securities and Exchange Commission pursuant to section 12(d) of the Securities Exchange Act of 1934 ("Act") and Rule 12d2-2(d) promulgated thereunder, to withdraw the Common Stock, $.10 Par Value, of Getty Petroleum Corporation ("Company"), from listing and registration on the American Stock Exchange, Inc. ("Exchange").

The reasons alleged in the application for withdrawing this security from listing and registration include the following:

Getty Petroleum Corporation has considered the direct and indirect costs and expenses attendant with maintaining the dual listing of the Common Stock on the New York Stock Exchange. The Company does not see any particular advantage in the dual trading of the stock and believes that dual listing would fragment the market for the Common stock.

Any interested person may, on or before October 10, 1985 submit by letter to the Secretary of the Securities and Exchange Commission, Washington, DC 20549, facts bearing upon whether the application has been made in accordance with the rules of the Exchange and what terms, if any, should be imposed by the Commission for the protection of investors. The Commission, based on the information submitted to it, will issue an order granting the application after the date mentioned, above, unless the Commission determines to order a hearing on the matter.

For the Commission, by the Division of Market Regulation, pursuant to delegated authority.

John Wheeler,

Secretary.

[FR Doc. 85-23134 Filed 9-26-85; 8:45 am]

BILLING CODE 8010-01-M

[Release No. IC-14730; File No. 812-6091]

Application and Opportunity for Hearing; The Northwestern Mutual Life Insurance Co., et al.

September 20, 1985.

Notice is hereby given that the Northwestern Mutual Life Insurance Company ("Northwestern"), a Wisconsin mutual life insurance company with its executive offices at 720 East Wisconsin Avenue, Milwaukee, Wisconsin 53202; Northwestern Mutual Variable Life Account (the "Account"), registered under the Investment Company Act of 1940, (the "Act") as a unit investment trust, and established by Northwestern in connection with the issuance and funding of certain single premium variable life contracts ("contracts"); and NML Equity Services, Inc., the principal underwriter for the contracts, (collectively, "Applicants"), filed an application on April 12, 1985 and amendments thereto on July 22, September 3, and September 13, 1985, for an order of the Commission pursuant to section 6(c) of the Act exempting Applicants from section 2(a)(32), 2(a)(35), 22(c) 22(d), and (27(c)(1) of the Act and Rules 6e-2(b)(1), (b)(12), (b)(13)(iv), (c)(1)(i), (c)(4), and 22c-1 thereunder, in connection with the issuance and funding of the contracts. All interested persons are referred to the application on file with the Commission for a statement of the representation made therein, which are summarized below, and are referred to the Act and rule thereunder for a statement of the relevant provisions.

Applicants represent that the Account is a separate account to which assets are allocated from time to time to support benefits payable under Northwestern's variable life insurance

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discussing competition between the OTC and exchange markets under the side-by-side pilot.

[58] NASD Petition, *supra* note 3, at p. 35.

[59] *See* Securities Exchange Act Release No. 19264 and 20075, November 22, 1982 and August 12, 1983, 47 FR 53981 and 48 FR 37556.

[60] *Id.* For example, the Commission recently approved the NASD's proposals to trade options on two indexes comprised of 100 NMS stocks each (Securities Exchange Act Release No. 22404, September 13, 1985); these contracts will trade simultaneously with Phlx's existing but not identical 100 stock NMS index option contract, the "National Over-the-Counter Stock Index."

[61] The NASD also suggests that the Phlx's amendment to its off-board trading rules was inadequate because it covers only options on indexes composed "entirely" of OTC stocks, thus not covering an index containing, for example, 99 OTC stocks out of 100. The Commission can address the NASD's concerns if the Phlx (or anyone else) proposes such an index option. Such a proposal would have to be made under Rule 19b-4, and thus the Commission would be able to impose appropriate conditions (including amendments of off-board trading rules) on approval of such a proposal.

contracts. Applicants presently are offering certain periodic premium variable life insurance contracts, which are subject to a front-end sales load, and intend to offer a new single premium variable life insurance contract to be funded through the Account. The single premium contract will differ from Northwestern's existing periodic premium contracts in certain respects, including the fact that no front-end sales charge will be deducted from the single premium payment and that the surrender values are structured so as to impose what may be considered to be the imposition of a contingent deferred sales load ("CDSL"). Applicants state that the surrender values under the contract will be adjusted to reflect a charge of not more than 9% of the contract's tabular cash value at the beginning of the first contract year, declining over time thereafter until the charge reaches 0% at the end of the tenth contract year. Applicants represent that shares of the fund in which the assets of the Account are invested are voted pursuant to the instructions of the contractowners. The number of shares for which the owner of a single premium contract may give instructions will not be reduced during the first ten contract years by reason of the deduction for sales expenses which would be made if the contract were surrendered. Applicants represent that the deferred sales charge will never exceed 9% of the single premium, excluding the administrative charge of $150 which is deducted from the premium. Applicants further represent that in no sense will Northwestern make any deduction for sales expenses except when a single premium contract is actually surrendered. In the event of a partial surrender of a single premium contract, the surrender deduction will be made only on that portion of the contract which is surrendered and not on the entire tabular cash value. In the event of a conversion of a single premium contract into a fixed contract within the first 24 months after issuance, as permitted by Rule 6e–2(b)(13)(v), the equitable adjustment of cash values will result in the collection of a surrender deduction by Northwestern, but there will be no duplication of sales loads in connection with the conversions.

Applicants assert that their deferred sales load will benefit the public because, among other things, it will permit the purchaser to participate in the investment experience of a larger amount than would be the case with a traditional deduction from the purchase price. Applicants also state that the deferred sales charge is consistent with the policies of the Act as applied under Rule 6e–2 to variable life insurance. Applicants acknowledge, however, that certain provisions of Rule 6e–2 are, by their literal terms, arguably inconsistent with a deferred charge for sales expenses. Applicants therefore request relief in order to eliminate any questions regarding full compliance with the Act and the rules thereunder.

Applicants make various specific arguments in support of the requested relief. Regarding section 2(a)(35) and Rules 6e–2(b)(1) and (c)(4), Applicants assert that although these provisions reflect an assumption that any charges for distribution expenses will be taken as deductions from premiums, the purpose of these provisions is to define the amounts which are subject to the sales load limitations of the Act and the rules thereunder, not to limit the timing of the sales load charge.

Applicants also seek exemptive relief from sections 2(a)(32) and 27(c)(1) of the Act and Rules 6e–2(b)(12) and (13)(iv) thereunder, to the extent that such provisions do not contemplate the imposition of a sales charge at the time of redemption. Applicants assert that because the deferral of a sales charge does not affect the purpose of the charge, the sale of investment company securities with a deferred charge for distribution costs is not fundamentally inconsistent with the redeemability requirement of section 2(a)(32). Applicants also assert that the deferral of sales charges is not unreasonable, unfair or discriminatory within the meaning of Rule 6e–2(b)(12).

With respect to section 22(c) of the Act and Rule 22c–1, Applicants assert that Rule 6e–2(b)(12) affords exemptive relief from these provisions with respect to certain variable life insurance "redemption procedures". Applicants acknowledge that because these provisions are drawn in terms of a mechanism for determination of surrender values, the imposition of a charge at surrender arguably raises some question. Applicants assert, however, that the deferred sales charge has no impact on security holders who do not redeem, and therefore does not result in the dilution of values which Rule 22c–1 was designed to prohibit; and that the proposed single premium policies will have cash surrender values determined on the basis of forward pricing, meeting any such requirement in Rules 6e–2(b)(12) and 22c–1. Applicants also request exemption from Rule 6e–2(c)(1)(i), which defines "variable life insurance contract" in terms of a cash surrender value that varies to reflect the investment experience of a separate account, to the extent necessary to reconcile this provision with the cash surrender values under the Applicants' contracts.

Finally, Applicants requet exemption from section 22(d) and Rule 6e–2(b)(12)(ii), the latter of which grants exemption from the uniform offering price requirements of the former. Applicants submit that the use of a single separate account for both periodic premium contracts with a front-end sales load and single premium contracts with a surrender charge does not raise any significant issue in the context of the Act's requirement for a uniform offering price because Rule 6e–2(b)(12)(ii) expressly contemplates separate accounts funding more than one "class or series" of contracts. Applicants also assert that Rule 6e–2(b)(12)(ii) contemplates that prices will differ depending on differences in the securities being offered and that there is no violation of the Rule so long as premiums are reasonable, fair and not discriminatory to the interests of contract owners of the same class or series.

Notice is further given that any interested person wishing to request a hearing on the application may, not later than October 15, 1985, at 5:30 p.m., do so by submitting a written request setting forth the nature of his interest, the reasons for the request, and the specific issues, if any, of fact or law that are disputed, to the Secretary, Securities and Exchange Commission, Washington, DC 20549. A copy of the request shall be served personally or by mail on Applicants at the address stated above. Proof of service (by affidavit or, in the case of an attorney at law, by certificate) shall be filed with the request. After said date an order disposing of the application will be issued unless the Commission orders a hearing upon request or upon its own motion.

For the Commission, by the Division of Investment Management, pursuant to delegated authority.

John Wheeler,

Secretary.

[FR Doc. 85–23135 Filed 9–26–85; 8:45 am]

BILLING CODE 8010-01-M

[Release No. 14735 (File No. 812-6171)]

Prudential-Bache Government Plus Fund, Inc.; Application for an Order Permitting Quarterly Distributions of Long-Term Capital Gains

September 23, 1985.

Notice is hereby given that Prudential-Bache Government Plus Fund, Inc. ("Applicant"), One Seaport Plaza, New York, NY 10292, filed an application on August 5, 1985, requesting an order of the Commission, pursuant to Section 6(c) of the Investment Company Act of 1940 ("Act"), exempting Applicant from the provisions of section 19(b) of the Act and Rule 19b-1 thereunder to the extent necessary to permit quarterly distributions of long-term capital gains on options and futures transactions. All interested persons are referred to the application on file with the Commission for a statement of the representations therein, and to the Act and the rules thereunder for the text of their relevant provisions.

Applicant is a diversified open-end management investment company whose investment objective is to seek a high current return by investing primarily in United States Government securities and obligations issued or guaranteed by United States Government agencies or instrumentalities, writing exchange-traded covered put and call options ("Treasury Options") on such securities and entering into closing purchase and sale transactions with respect to certain of such options. Applicant states that it also enters into purchase and sale transactions of interest rate futures contracts ("Future Contracts") and options thereon ("Futures Options") in order to hedge its portfolio against a decline in value resulting from an adverse change in interest rates.

Applicant states that it declares daily dividends, payable monthly, of its net investment income from interest on debt securities. Applicant also states that it declares and distributes quarterly its net short-term capital gains, consisting of the short-term portion of net gains from transactions in options on United States Government securities, transactions in Futures Contracts and Futures Options and of net gains from the sales of portfolio securities held for less than six months. Any long-term capital gains are currently distributed annually.

Applicant represents that under the Internal Revenue Code of 1954 (the "Code"), Treasury Options, Futures Contracts and Futures Options are all considered "Section 1256 Contracts". In general, sixty percent of the realized gain or loss with respect to Section 1256 Contracts are treated as long-term capital gain or loss, and forty percent are treated as short-term capital gain or loss ("60/40 rule"). Applicant further represents that this 60/40 rule was devised to prevent possible tax abuses and not to limit the frequency with which registered investment companies may distribute capital gains from transactions in section 1256 Contracts.

Applicant asserts that the realization of gains from premiums on Treasury Options is an integral part of the Applicant's investment objective of seeking a high current return. Notwithstanding the characterization under the Code of a portion of gains from options as long-term, the use of Treasury Options transactions by the Fund is primarily an income rather than a capital gains strategy. Similarly, Applicant asserts that its use of Futures Contracts and Futures Options (and the purchase of treasury put options) constitutes a hedging rather than a capital gains strategy. Applicant alleges that these hedging transactions are not entered into for the purpose of generating long-term capital gains, and any such gains resulting from the application of the 60/40 rule are purely incidental.

Applicant states that none of the purposes of section 19(b) and Rule 19b-1 are applicable to the quarterly distribution of long-term capital gains from transactions in Section 1256 Contracts. Applicant believes that, in light of its investment objective of seeking a high current return and the importance of income from Treasury Options and hedging through the use of Futures Contracts and Futures Options to this investment objective, it is appropriate that it distribute quarterly all gains from transactions in Section 1256 Contracts.

Applicant submits that quarterly distribution of gains from transactions in section 1256 Contracts, regardless of their characterization under the Code, will not cause stockholders of the Fund to confuse long-term capital gains with dividends out of new interest income. Applicant believes that all net gains from Treasury Options transactions, regardless of their characterization under the technical rules of Code Section 1256, constitute part of the current return contemplated by the Applicant's investment objective. Similarly, net gains from Futures Contracts and Futures Options are viewed by Applicant and its stockholders not as a source of long-term capital gains, but as an incidental effect of Applicant's hedging strategies. Applicant further represents that it will clearly distinguish any distribution of capital gains from distribution out of net interest income in a notice from Applicant to its stockholders.

Applicant also submits that permitting quarterly distributions of capital gains from Section 1256 transactions will not encourage more frequent trading in Applicant's portfolio than what is appropriate in light of the Applicant's investment objective. Applicant's state that its objective of a high current return clearly contemplates the seeking of net gains from Treasury Options and attempting to hedge Applicant's portfolio with Futures Contracts and Futures Options. Therefore, Applicant contends that whether the entire amount of these gains, as opposed to only the short-term portion, is distributed quarterly will not affect the investment decisions of Applicant's investment adviser.

Finally, Applicant submits that quarterly distributions of all of its net gains from transactions in Section 1256 Contracts will not increase administrative expenses because it already makes, and expects to continue to make, quarterly distributions of the short-term portion of such gains.

Notice is further given that any interested person wishing to request a hearing on the application may, not later than October 18, 1985, at 5:30 p.m., do so by submitting a written request setting forth the nature of his interest, the reasons for his request, and the specific issues, if any, of fact or law that are disputed, to the Secretary, Securities and Exchange Commission, Washington, DC 20549. A copy of the request should be served personally or by mail upon Applicants at the address stated above. Proof of service (by affidavit or, in the case of an attorney-at-law, by certificate) shall be filed with the request. After said date an order disposing of the application will be issued unless the Commission orders a hearing upon request or upon its own motion.

For the Commission, by the Division of Investment Management, pursuant to delegated authority.

John Wheeler,

Secretary.

[FR Doc. 85-23136 Filed 9-26-85; 8:45 am]

BILLING CODE 8010-01-M

[Release No. 34-22429; File No. SR-Amex-85-34]

Self-Regulatory Organizations; Filing of Proposed Rule Change by the American Stock Exchange, Inc., Relating to Amendments to Amex Company Guide; Sections 103 and 104

Pursuant to section 19(b)(1) of the Securities Exchange Act of 1934, 15 U.S.C. 78s(b)(1), notice is hereby given that on September 16, 1985, the American Stock Exchange, Inc. filed with the Securities and Exchange Commission the proposed rule change as described in Items I, II, and III below, which Items have been prepared by the self-regulatory organization. The Commission is publishing this notice to solicit comments on the proposed rule change from interested persons.

I. Self-Regulatory Organization's Statement of the Terms of Substance of the Proposed Rule Change

The American Stock Exchange, Inc. ("Amex" or "the Exchange") is proposing to amend Amex Company Guide, Sections 103 and 104, to permit the acceptance of convertible debt securities of non-listed issuers which are subject to last sale reporting and to modify the existing prohibition against accepting convertible securities for listing which contain a provision allowing the issuer the right to adjust the conversion price.

II. Self-Regulatory Organization's Statement of the Purpose of, and Statutory Basis for, the Proposed Rule Change

In its filing with the Commission, the self-regulatory organization included statements concerning the purpose of and basis for the proposed rule change and discussed any comments it received on the proposed rule change. The text of these statements may be examined at the places specified in Item IV below. The self-regulatory organization has prepared summaries, set forth in sections (A), (B), and (C) below, of the most significant aspects of such statements.

A. Self-Regulatory Organization's Statement of the Purpose of, and the Statutory Basis for, the Proposed Rule Change

(1) Purpose

Convertible Debt Securities. Section 104 of the Amex Company Guide sets forth as a general guideline that the Exchange will not accept a convertible bond (or debenture) for listing unless the underlying stock is traded on either the Amex or NYSE. Since the price of the underlying issue into which a security is convertible will influence the market price of the convertible instrument, the historical inability to obtain ongoing price information in over-the-counter issues was considered sufficient reason to deny the Amex marketplace to such convertible issues. Last sale reporting for NASDAQ/NMS securities, which commenced in 1982, is believed adequate to permit removal of the present restriction.

This change will enhance the Exchange's bond listing program. Underwriters are often anxious to have their clients list corporate debt, even though the issuer's common stock may not be listed on one of the principal securities exchanges, since many foreign and institutional investors are reluctant or precluded from investing in unlisted debt securities. Prints in exchange-listed debt are also viewed as important for retail investors.

Conversion Provisions. Sections 103 and 104 of the Amex Company Guide set forth guidelines which prohibit the listing of convertible debt and convertible preferred stock containing provisions which grant the issuer discretion to temporarily adjust the conversion price unless the issuer undertakes not to exercise such rights while the security remains listed on the Amex. Many companies today publicly issue securities which permit their management to alter the conversion price for such periods as management may determine throughout the life of the instrument. These so-called "flush-out" provisions are viewed by many as necessary to provide corporate management with the flexibility to encourage conversions of outstanding securities into permanent capital.

The Exchange is of the view that the existing prohibitions against temporary price reductions are not well founded and interfere with legitimate corporate planning. Therefore, it is proposed that Sections 103 and 104 of the Company Guide be amended to permit the listing of convertible securities which contain "flush-out" provisions. However, to ensure that investors have sufficient time to evaluate temporary reductions in the conversion price of their securities, the proposed rule changes provide that the Exchange will not list an issue containing such a provision, unless the issuer establishes a minimum "window period" of not less than ten business days within which investors may determine whether to exercise their conversion privileges.

(2) Basis

The proposed amendments are consistent with Section 6(b) of the Exchange Act, in general, in that they are designed to ensure that the Exchange's rules remain up-to-date and are consistent with section 6(b)(5), in particular, in that they eliminate regulation not related to the purposes of this Act.

B. Self-Regulatory Organization's Statement on Burden on Competition

The proposed amendments create no new regulations and will not impose a burden on competition.

C. Self-Regulatory Organization's Statement on Comments on the Proposed Rule Change Received From Members, Participants or Others

No written comments were solicited or received with respect to the proposed rule change.

III. Date of Effectiveness of the Proposed Rule Change and timing for Commission Action

Within 35 days of the date of publication of this notice in the Federal Register or within such longer period (i) as the Commission may designate up to 90 days of such date if it finds such longer period to be appropriate and publishes its reasons for so finding or (ii) as to which the self-regulatory organization consents, the Commission will:

(A) By order approve such proposed rule change, or

(B) Institute proceedings to determine whether the proposed rule change should be disapproved.

IV. Solicitation of Comments

Interested persons are invited to submit written data, views and arguments concerning the foregoing. Persons making written submissions should file six copies thereof with the Secretary, Securities and Exchange Commission, 450 5th Street, NW, Washington 20549. Copies of the submission, all subsequent amendments, all written statements with respect to the proposed rule change that are filed with the Commission, and all written communications relating to the proposed rule change between the Commission and any person, other than those that may be withheld from the public in accordance with the provisions of 5 U.S.C. 552, will be available for inspection and copying in the Commission's Public Reference Section, 450 5th Street, NW, Washington, DC. Copies of such filing will also be available for inspection and copying at the principal office of the above-mentioned self-regulatory organization. All submissions should refer to the file number in the caption above and should be submited by October 18, 1985.

For the Commission by the Division of Market Regulation, pursuant to delegated authority.

John Wheeler,

Secretary.

September 19, 1985.

[FR Doc. 85-23140 Filed 9-26-28; 8:45 am]

BILLING CODE 8010-01-M

[Rel. No. 34-22442; SR-Amex-85-8]

Self-Regulatory Organization; American Stock Exchange, Inc., Order Approving Proposed Rule Change

I. Introduction and Background

Pursuant to Section 19(b) of the Securities Exchange Act of 1934 ("Act") [1] and Rule 19b-4 thereunder,[2] the American Stock Exchange ("Amex"), submitted on April 4, 1985, copies of a proposed rule change to request Commission approval of a joint plan, and accompanying rule changes, implementing a linkage between the Amex and the Toronto Stock Exchange ("TSE").[3] The linkage would permit order flow in securities dually listed on the Amex and TSE to be routed between the two exchanges. The Amex-TSE linkge will be the first linkage of a primary market in the United States with a primary market in a foreign jurisdiction. Accordingly, it represents a significant step in the increasing internationalization of the securities markets. By integrating the Amex and TSE's market making capabilities, the linkage aims to enhance liquidity in both markets, thereby benefiting Canadian and American investors by making available to them the best available price on either exchange.

Previously, the Commission approved, on November 1, 1984, a similar linkage between the Boston Stock Exchange, Inc. ("BSE") and the Montreal Exchange, Inc. ("Montreal" or "ME").[4] Through this linkage, orders can be routed from Montreal to Boston in approximately 200 U.S. stocks which are eligible for trading through the Intermarket Trading System ("ITS"), as well as in forty U.S.-listed Canadian stocks. In reviewing the BSE-ME linkage, the Commission was satisfied that there existed satisfactory trading and clearance and settlement procedures as well as surveillance and information sharing mechanisms between the two exchanges, and their respective regulatory agencies, the SEC and the Quebec Securities Commission.

The Amex-TSE proposed linkage differs from the ME-BSE linkage in that the Amex-TSE linkage involves two primary markets [5] and will provide for orders to be sent from the Amex to the TSE (*i.e.*, northbound) as well as for orders to be sent from the TSE to the Amex (*i.e.*, southbound).[6] Thus, An Amex specialist or Amex member will have the ability to route an order in a dually listed stock directly to Toronto.

The Amex and TSE joint Trading Linkage Plan ("Plan") reflects their agreement with respect to all facets of the linkage, including transmission of orders, execution, clearance and settlement of transactions, implementation of a surveillance program, conflict resolution and arbtration proceedings.[7] The Amex also has developed a new series of rules (Rules 240 to 244) that are designed to implement the Plan and assure the applicability of Exchange rules to orders received from Toronto and executed on the Amex.[8]

The exchanges expect to commence trading on a pilot basis on September 24 with seven dually listed securities [9] and will later expand the list of securities to include all dually-listed issues. The Amex and TSE will each display on their trading floors the quotes distributed by the other exchange in linkage stocks.[10] In addition, each exchange will display the best U.S. dollar/Canadian dollar exchange rates quoted by currency dealers on the TSE. This will enable traders to determine which market has a more favorable price at any given time.

The Amex and the TSE are prepared to commence linkage trading immediately on both a northbound and southbound basis. Thus, TSE members will be able to direct orders from the TSE trading floor to the Amex trading floor for execution, and to receive orders from Amex for execution. Likewise, Amex members will have the ability to direct orders from the Amex to the TSE trading floor for execution and to execute orders sent from the TSE. Quotes and orders will be forwarded between the exchanges using their existing automated routing systems.[11] In

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[1] 15 U.S.C. 78(c)(b) (1982).

[2] 17 CFR 240.19b-4 (1985).

[3] The proposal was published for comment in Securities Exchange Act Release No. 22001 (April 30, 1985), 50 FR 19503 (May 8, 1985). The Midwest Stock Exchange, Inc. ("MSE") also has proposed a similar linkage with the TSE. Although this order focuses on the Amex-TSE linkage, the proposed MSE-TSE linkage will raise similar concerns. *See* File No. SR-MSE-85-4, Securities Exchange Act Release No. 22156 (June 12, 1985), 50 FR 25501 (July 19, 1985).

[4] Phase I of that linkage was established solely for the purpose of routing orders from the ME to the BSE in 40 dually listed stocks. *See* Securities Exchange Act 21449 (November 1, 1984), 49 FR 44575, November 7, 1984 (File No. SR-BSE-84-5). Phase II expanded the list of securities eligibile to trade through the linkage to include U.S.-listed securities eligible to trade through ITS. *See* Securities Exchange Act Release No. 21925, April 8, 1985, 50 FR 14480, April 12, 1985.

[5] The TSE is Canada's largest public securities market, capturing approximately 74% of the dollar value of all shares traded in Canada. The Amex is the primary U.S. market in all of the equity securities traded on the exchange.

[6] Currently, the BSE-ME linkage only allows southbound traffic from ME to the BSE, although Phase III of that linkage contemplates northbound traffic.

[7] The Commission description of the Plan herein is based on the following documents, as well as conversations with the staff of the Amex, TSE and Ontario Securities Commission ("OSC"): (1) Amex's proposed rule change relating to the linkage, (File No. SR-Amex-85-8), published for notice and public comment in Securities Exchange Act Release No. 22001, April 30, 1985; 50 FR 19503, May 8, 1985; (2) letter from Dr. Stephen Williams, Senior Vice President, Amex, to Michael Cavalier, Branch Chief, Branch of Exchange Regulation, SEC, dated April 4, 1985; (3) letter from Dr. Stephen Williams, Amex, to Brandon Becker, Assistant Director, Office of Exchange and Options Regulation, dated June 21, 1985; (4) letter from Gordon Nash, Lord, Day and Lord (counsel to Amex), to Brandon Becker, dated June 27, 1985, which includes a copy of an opinion letter from the law firm of Tory, Tory, Deslauriers and Binnington, to the TSE dated June 18, 1985; (5) letter from Gordon Nash to Brandon Becker, dated August 23, 1985; and (6) letter from Blake, Cassels & Graydon, counsel to TSE, dated August 27, 1985.

[8] Commentary to Amex Rule 244 also makes certain Amex rules applicable to orders sent from Amex to TSE when deemed appropriate. The remaining rule amendments make conforming changes to existing Amex rules to accommodate linkage orders.

[9] The six pilot stocks are expected to be Asamera Inc., Canadian Marconi Co., Echo Bay Mines Ltd., Gulf Canada Ltd., Husky Oil Ltd., and Imperial Oil Ltd.

[10] Quotes distributed by the Amex for any linked stock which also is traded through ITS, shall reflect the national best bid and offer distributed by the consolidated quotation system. However, the price quoted on the TSE would be the best published quote on the TSE at the time the order is received on the exchange, though not necessarily the best Canadian price available at that time.

[11] All marketable orders sent through the linkage to the Amex will be routed through the Amex's automatic routing system, the Post Execution Reporting System ("PER") to the AUTOPER terminal (incorporating touchscreen technology to increase efficiency of the routing system) at the appropriate specialist's location on the Amex floor. Orders sent to the TSE will be entered into the TSE's Market Order System of Trading ("MOST") and will print out at the appropriate TSE trading post for manual execution on the TSE floor. Orders may not be entered through the linkage automatically by Canadian brokers from terminals located away from the TSE floor. All linkage orders must be directed through the linkage by TSE members from terminals located on the floor of the TSE.

The MOST system was introduced on the TSE in 1984, and permits the TSE to route small orders directly from member offices across Canada to the post on the floor of the exchange where the stock is traded. The system also enables members to notify clients promptly after their trade has been

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addition, the exchanges intend to implement in the hear future a mechanism for immediate currency conversion, so that all U.S. dollar and Canadian dollar settlement obligations will be known at the time a trade is executed.[12]

Initially, the linkage only will provide for the execution of marketable limit orders.[13] In other words, an order received from either exchange will only be executed by the receiving exchange if the order is at a price which is equal to or better than the quote then being distributed on the receiving exchange. Such orders are guaranteed an execution at the best available quote on the receiving exchange for up to a 1,000 shares.[14] It is anticipated that "away from the market" orders will be permitted to be sent through the linkage as soon as sufficient experience is gained with two-way trading and the execution, clearance and settlement of "at the market" orders. The Plan also provides that at some future date the linkage may be expanded to include other securities traded on either exchange. Agency orders will be subject to the normal priority rules on each exchange, while professional orders will be on parity with those of the market makers on each floor. Furthermore, all linkage orders will be subject to the rules of the receiving exchange.

The Plan provides that the linkage will not operate during a trading halt on either exchange with respect to securities subject to the trading halt. In the case of a trading halt based on regulatory concerns, the market surveillance units of both exchanges will undertake to contact each other as quickly as possible and exchange information concerning matters giving rise to the trading halt. This will enable the Amex and TSE to coordinate actions regarding trading halts in dually listed securities.

With respect to the administration of the linkage, the Plan provides that a six member joint operating committee will be responsible for administering the linkage and will meet periodically to oversee implementation of the linkage, review operational concerns and advise with respect to enhancement or expansion of the linkage.[15] In addition, the Operation Committee is authorized under the Plan to consider and evaluate complaints against individual members or member organizations of either exchange which have engaged in conduct which constitutes an abuse of the linkage procedures or a pattern of violating the provisions of the Plan. [16]

The Amex/TSE Plan also provides for on-floor dispute resolution. Article 5, section 2(a) of the Plan ("On-Floor Dispute Resolution") provides that disputes and/or questions relating to the linkage are to be resolved in accordance with procedures and policies of the receiving exchange, including any appeals process by the exchange. The Plan also provides that determinations by those persons delegated the authority to resolve disputes and questions on the receiving exchange also will be binding upon members of the originating exchange with respect to transactions effected on the receiving exchange.[17] In addition, both exchanges indicated that they have the authority to enforce their rules governing trades sent by their respective members through the linkage for execution.[18] The Plan also provides for abbreviated arbitration proceedings, permitting Amex or TSE members asserting a claim against a member of the other exchange, arising out of a linkage transaction, to resort to arbitration, notwithstanding any

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completed. Trade confirmations are transmitted via MOST to the place of order origination.

[12] Amex has stated that this will minimize U.S./Canadian dollar exchange rate risk from the time of execution to the time of settlement, and, more importantly, will permit the settlement of such transactions to be effected in the U.S. in U.S. currency. As initially planned, however, the linkage will not contain an automatic conversion mechanism. Thus, the Amex specialist will receive the Canadian quotations from the TSE in Canadian dollars only. The currency conversion rate will also be displayed and the Amex specialist will have to convert the Canadian price into U.S. dollars. For northbound orders, the Amex specialist will send orders to the TSE in U.S. dollars and specify the conversion rate that applies to the transaction, based on the TSE rate displayed on the Amex. The TSE specialist will first execute the Amex order in Canadian dollars, then convert Canadian dollars into U.S. dollars, and finally report the execution to Amex in U.S. dollars. In the near future, TSE intends to provide for automatic currency conversion, eliminating any separate manual currency conversion. In addition, Amex has indicated that, within six months, the Amex specialist will be able to receive the Canadian price simultaneously in U.S. and Canadian dollars. Such a currency conversion system, of course, would have to be filed with the Commission as a proposed rule change under section 19(b)(1) of the Act.

[13] A "marketable limit order" is an order which may be executed at the current market quote, but at no worse price. For example, if the current bid for XYZ was $20.00 for 100 shares, an order to sell 100 shares at $20.00 but not at a price less than $20.00 would be a marketable limit order. The mechanism for excution of marketable limit orders is as follows: A member of the originating exchange may send through the linkage an order at a price which is equal to the quote then being distributed on the receiving exchange. The order will be treated as an "immediate or cancel" order, so that it will be cancelled if, when received by the market maker on the receiving exchange, it is not a marketable order (*e.g.*, the order is mispriced or the market has moved between the time it was priced on one exchange and received on another). If, however, it is a marketable order when received by the market maker price or a more favorable price.

Orders larger than the displayed quotation will be subject to partial excution. Although the market maker will not be required to provide an excution in excess of the applicable guarantee, he may purchase or sell for his account to fill an order which cannot be filed from the floor of the receiving exchange.

[14] The Plan provides that this minimum guarantee may be different for specific stocks as they are added to the linkage in the future. An order, however, may not be divided into several 1,000 share lots to take advantage of the guarantee. If several orders are received from one member for the account of the same customer, the guarantee will apply only to the first 1,000 shares. Professional orders (*i.e.*, an order for the account of a market maker or a broker-dealer's firm proprietary account) will not be entitled to a guarantee but will otherwise be handled in the same manner.

In addition to providing a minimum size guarantee for orders flowing through the linkage, the Amex also will ensure the same minimum size guarantee for all public orders received through the PER system. This guarantee, however, would be limited to those stocks which are included in the linkage.

[15] *See* Article V, Section 1 of the Plan.

[16] If the Committee determines that a complaint appears to have merit, it will refer the complaint to the member's exchange and an investigation will be conducted. If it is determined by the Exchange that there may, in fact, have been abuses or repeated violations, that Exchange will be responsible for taking appropriate disciplinary action through its regular disciplinary procedures. Sanctions may include the denial of use of the linkage facility to the member or member firm.

[17] The Amex has indicated that this provision is intended to make clear that the decision of floor officials on the floor of the receiving exchange where the order is executed would be binding upon the member of the originating exchange who initiated the order, to the same extent as such decisions are binding upon the members of the receiving exchange. The Amex, however, noted that the provision was not intended to expand in any way the authority of floor officials on either exchange. *See* letter from Gordon Nash, Director, Division of Market Regulation, dated August 23, 1985.

[18] *See* letter from Gordon Nash, Lord Day & Lord, to Brandon Becker, dated August 23, 1985. *See also* letter from Gordon Nash, Lord Day & Lord, to Kenneth Leibler, Senior Executive Vice President, Amex, dated August 23, 1985. This letter sets forth the opinion of Amex's counsel that the Exchange has the clear authority to discipline an Amex member who engages in linkage trading activity which would subject him to disciplinary action if engaged in on the floor of the Amex. In this regard, the letter noted that the new Amex Rule 244, adopted as part of the linkage rules, requires that "each tranaction effected through a linkage shall be subject to the rules of the Exchange, applicable to trading on the Exchange, except to the extent such rues are inconsistent with provisions of this Section 8 (the Linkage rules) or the relevant Linkage Plan." In addition, the letter notes that Sections 9 and 10 of the Securities Exchange Act, which proscribe manipulation and fraud, are applicable to "any facility of any national securities exchange," *i.e.*, the Amex-TSE linkage, and that these sections provide an additional legal basis for Amex disciplinary action and other legal sanctions (*i.e.*, possible Commission action) against a member of the Amex who engages in such activity through use of the linkage.

The TSE also provided a letter from TSE's outside counsel which made similar representations with respect to TSE's authority to take disciplinary actions against its members in connection with trading activities conducted through the linkage. *See* letter from the law firm of Blake, Cassels and Graydon, to Keith E. Boast, Esq., TSE, dated August 27, 1985.

determination by the on-floor dispute resolution process.[19]

A. Regulatory Provisions

The Commission notes that TSE's rules regulating trading and dispute resolution, as well as the Ontario and federal antifraud provisions under which it operates, are markedly similar to the Amex's own rules and to the U.S. federal securities laws' antifraud provisions.[20] For example, TSE's rules include provisions, comparable to Amex rules, relating to manipulative or abusive trading practices, such as rules regarding suitability, short sales,[21] net capital [22] and best execution.

In addition, TSE rules contain a number of provisions which augment and assist its surveillance program.[23] For example, TSE recordkeeping rules include the requirement that TSE members maintain floor tickets and other records of customer orders, confirmations of purchases or sales and written records of customer accounts and approval for a period of five years.[24] Furthermore, TSE, like the Amex and other U.S. national securities exchanges, imposes specific "know your customer" requirements on its members and requires a member to maintain records regarding the identity of customers.[25]

TSE rules specifically prohibit manipulative and deceptive methods of trading and the transaction of business which is not in accordance with just and equitable principles of trade.[26] Both the Criminal Code of Canada and the Ontario Securities Act contain general anti-fraud provisions.[27] Insider trading, for example, is strictly regulated under the Ontario Securities Act.

In addition, the TSE's dispute resolution system is analogous to the Amex's own procedures. Under Section 10.03 of the TSE's by-laws, the Floor Procedure Committee is authorized to govern all sessions of the Exchange, and has jurisdiction over trading, conduct and discipline of all members.[28] The TSE also has provisions for compulsory arbitration in the event of any dispute arising between members, regarding a TSE contract which has not been settled.[29] Finally, the TSE, like the Amex, has detailed procedures in its by-laws for procecution of violations.[30]

B. Clearing Procedures

Clearance and settlement of linkage-trades will follow the same procedures established for the BSE–ME link. Amex will be responsible for submitting trades executed on either market to NSCC for clearance and settlement. Specifically, the Amex will confirm each trade by sending a report to the TSE trading party on the same electronic terminals used to place the order. Amex then will submit all trades to NSCC as compared trades expressed in U.S. dollars. The Canadian Depository for Securities Limited ("CDS") is a member of NSCC and clearance and settlement will occur through the facilities of NSCC.[31]

All linkage trades, whether executed in New York or Toronto, will be cleared and settled through NSCC in U.S. funds. Although northbound trades will be executed on the TSE floor in Canadian dollars, a simultaneous currency transaction will be made with a currency dealer on the TSE floor to convert the trade amount into U.S. dollars. This will allow Amex members to settle their side of the trade with NSCC in U.S. currency and TSE members to settle the other side with CDS in Canadian currency, without being subject to the risk of currency fluctuations during the settlement process.[32] CDS will settle all NSCC obligations in U.S. funds.

II. Discussion

The Commission believes that a linkage of two primary markets with two-way order flow could benefit investors by providing greater liquidity and increasing market competition in dually listed issues traded through the linkage. At the same time, however, the Comission recognizes that such a linkage may encourage U.S. investors to trade on a foreign exchange where the Commission does not have oversight responsibility regarding that exchange's trading and dispute resolution rules. In addition, such international trading could limit the Commission's access to information relating to trades through the linkage by Canadian brokers on behalf of Canadian customers because foreign broker-dealers and investors may not be under the Commission's jurisdiction. Accordingly, the Amex's and Commission's ability to monitor transactions effected through the linkage depends, to a large extent, on the mutual cooperation and surveillance of both the SEC and the OSC as well as the TSE and Amex. In this regard, the Commission is especially concerned that its ability to monitor linkage transactions could be adversely affected by a recently enacted Canadian statute,

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[19] *See* Article 5, section 2(b).

[20] The Ontario Securities Act gives the OSC broad powers over the TSE, including jurisdiction over the manner in which the Exchange conducts its business or trading through its facilities. While somewhat different than the SEC's authority over SRO rulemaking, the OSC also has authority to review any by-law, ruling or other regulation by the Exchange. The OSC also has power to review decisions of the TSE and persons affected by TSE decisions have a right of appeal to the OSC.

[21] *See* TSE by-laws, Section 11.27[1].

[22] *See* TSE by-laws, Section 16.13 (capital and margin requirements).

[23] *See* Notes 34 and 35 and accompanying text, *infra* for discussion of TSE's surveillance mechanisms.

[24] *See* Sections 16.02, 16.03, 16.07, 16.09 and 16.36 of TSE's by-laws for record retention procedures. In addition, TSE maintains records of all trades generated on and through its floor for comparable periods.

[25] *See* Section 16.01 of TSE by-laws ("Supervision of Accounts"). This requirement is also set forth in the Ontario Securities Act, R.S.O. 1980, c. 466 as amended, under Section 102 of the regulations under the Act.

[26] *See*, for example, Section 11.17 of TSE by-laws, providing that "no fictitious sale or contract shall be made and each member shall transact his business openly and fairly and in accordance with just and equitable principles of trade." *See also* Section 11.26 of by-laws ("Manipulative or Deceptive Methods of Trading") which provides a list of examples of activities deemed to constitute manipulative or deceptive activity.

[27] *See* Criminal Code, RSC, 1980 section 338(2), section 340, and section 341. *See also*, Ontario Securities Act, Part XXII ("Civil Liability"). Insider trading, for example is strictly regulated by Part XX and Sections 75 and 131 of the Ontario Securities Act and the regulations under that Act.

[28] Section 10.03 outlines the authority by which the Floor Procedure Committee may discipline and impose appropriate penalties on a member or employee for trading violations.

[29] *See* Section 16.26 of TSE by-laws.

[30] *See* in general, Part XVII of TSE's by-laws ("Hearing Procedures and Discipline"). The Hearing Committee has been delegated authority by the Board of Governors to commence disciplinary proceedings and to revoke, suspend or amend the rights or privileges of a member. Like the Amex disciplinary proceedings, the TSE rules contain detailed provisions to ensure the procedural due process rights of the alleged violator.

[31] As an NSCC member, CDS clears and settles securities transactions within NSCC's automated system, including its continuous net settlement system. CDS, like any other NSCC member, is liable as a principal for all trades submitted to NSCC, is subject to NSCC's comprehensive safeguarding mechanisms and must comply with all other NSCC rules and procedures.

NSCC has requested that the Division of Market Regulation ("Division") extend a previous no-action position, regarding CDS compliance with the clearing agency registration requirements of Section 17A of the Act in connection with the BSE–ME linkage, to cover CDS' similar role in connection with the Amex-TSE linkage. The Division is granting the no-action request concurrent with this release. *See* letter from Jonathan Kallman, Assistant Director, Division of Market Regulation, to Karen L. Saperstein, Assistant General Counsel, NSCC, dated September 20, 1985.

[32] The Amex has informed the Commission that when an Amex member initiates a trade through the linkage, he will specify a limit price in U.S. dollars equal to the Canadian dollar amount of the trade. If the currency conversion transaction cannot be executed at better than that limit, the order will be rejected and sent back to the Amex unexecuted. NSCC will not be involved in the currency conversion transaction. Rather, the compared trade reported to NSCC will specify a dollar value of the trade expressed in U.S. dollars reflecting the combined securities transaction and related currency conversion transaction. *See* letter from Gordon Nash, to Brandon Becker, page 3, dated August 23, 1985.

which enables Canadian officials to limit the transfer of information and documents between Canada and a foreign country under certain enumerated circumstances.[33]

The Commission has sought to address these concerns in detail with the exchanges and the OSC and is satisfied that the appropriate channels for information sharing and cooperation between the exchanges and the two agencies have been established.[34] As discussed below, the Commission believes these issues have been adequately resolved through (1) the TSE-Amex's own mechanism for facilitating surveillance and information exchange under the terms of the Plan, (2) the channels developed by the SEC and the OSC to provide for sharing information and investigative resources, and (3) representations by OSC and TSE regarding the probable usage of the Canadian blocking statute.

A. TSE-Amex Surveillance Procedures

Both the Amex and TSE have active surveillance and record retention procedures to monitor trading through the linkage. The Amex has informed the Commission that linkage trades sent by Amex to the TSE for execution will be subject to both Amex's and Toronto's routine market surveillance procedures. TSE's rules and surveillance procedures appear to be comparable to the rules and surveillance capabilities of most U.S. exchanges.[35] Toronto, like the Amex, maintains an audit trail of all securities transactions that occur on its floor, permitting it to reconstruct the market for any particular stock and identify the time, price, size and participants of each trade in the stock.[36]

B. Amex-TSE Information Sharing Provisions

Under the Plan, the Amex and TSE have created mechanisms for the routine exchange of information [37] as well as for the exchange of additional documents and record, if requested by either exchange.[38] The Plan also provides that both exchanges will "cooperate fully" in any investigation relating to linkage transactions.[39] In this regard, each exchange has agreed to use its "best efforts" to obtain relevant information from its membership in connection with an investigation of linkage-related trading, and, to the extent not inconsistent with applicable law, to provide the other exchange with information tending to resolve any linkage-related questions or compliants.[40]

In this regard, the Amex has stated that it views the terms "cooperate fully" and "best efforts" to require the Amex and TSE each "to obtain and make accessible to the other pertinent information with with the same degree of diligence, the same allocation of resources, and the same attention to regulatory concerns as it would deem appropriate in an investigation that was solely within its own area of regulatory responsibility."[41]

C. SEC-OSC Cooperative Efforts

While most routine inquiries will be resolved though Amex's and TSE's own surveillance and information sharing procedures under the Plan, the Commission anticipates thaf some matters will require SEC and OSC participation, as, for example, when an investigation raises questions regarding the action of a person who is not a member of the Amex or TSE. In other cases, the SEC may find that it needs to obtain access to information possessed by or accessible to the OSC to determine whether, for example, to issue a formal order of investigation. The Commission also can foresee situations where both the SEC and OSC may be interested in investigation a linkage related transaction or where a joint SEC/OSC investigation may be undertaken.

The Commission and OSC have a long-standing history of cooperative efforts concerning regulatory matters originating either in the United States or Canada. Because the linkage will present both Commissions with the potential of increased integration of the U.S. and Canadian markets, the Commission has focused its efforts on information, as well as to provide assistance for any investigation and related subpoena enforcement actions. In this context, the Commission has sought to ensure, prior to approval of the linkage, that the Canadian blocking statute would not be used to impede the exchange of information so as to call into question the continued viability of the linkage.

The Canadian Foreign Extraterritorial Measures act ("FEMA") authorizes the Attorney General of Canada to issue orders to prevent the production or use of records or information in Canada in connection with foreign laws and proceedings and to issue orders to prevent persons in Canada from complying with foreign laws and orders. Under Section 3 of that Act, for example, the Attorney General may make the determination that a foreign tribunal is exercising or is likely to exercise jurisdiction in a manner that is likely to adversely affect significant Canadian interests in relation to commerce carried on in Canada or has

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[33] Foreign extraterritorial Measures Act, Stat. Can. c.49. (1985), *see* text accompanying notes 41 to 45, *infra.*

[34] The comparability of the trading and dispute resolution rules of Amex and TSE as well as the similarity of the U.S. and Ontario securities laws also ensures that U.S. investors engaging in transactions through the linkage will be appropriately protected.

[35] Surveillance efforts are conducted by two separate offices. the Market Surveillance Department monitors trading activity in all listed securities continuously from the opening of the market until the close, and maintains a comprehensive file on every listed company. If, for example, the Surveillance Department becomes aware of unusual trades, it will call a senior officer of the company and, in certain instances, ask for a statement to be issued. If the Department sees evidence of, for example, insider trading, wash trading, or other forms of market manipulation, the matter is forwarded to the TSE Division of Investigative Services and the OSC for follow-up. the TSE's Division of Investigative Services uses a specially designed computer program called TRACE to identify the clients behind trades made on the exchange when an in-depth investigation into trading in a particular stock appears necessary.

[36] TSE also has an on-line display of trades and quotes which identifies the most active securities, and securities with the largest price movement, and identifies instances of unusual volume or price movement for further investigation by the TSE staff.

[37] Under Article IV, Section 1, of the Plan, the exchanges have agreed to exchange on a regular basis trade documentation including, but not limited to, market surveillance reports and market data necessary to enable each exchange to carry out its respective market surveillance programs relating to linkage transactions. The Amex has informed us, however, that, at this time, the only information exchanged on a daily basis will be the TSE and Amex equity audit trail reports in linkage securities. These reports specify for every transaction the time, number of shares, price, clearing number of the two participants and dollar value of the transaction. The Amex also will receive the Toronto Daily Record which in part contains closing trade and quote information for all equity securities listed on the TSE.

[38] Article IV, Section 1, of the Plan provides that the exchanges shall furnish additional documents and records regarding linkage transactions as the exchanges shall "reasonably request" from time to time. According to the Amex, "reasonably requested" information would include such items as individual customer account information, firm proprietary positions or documentation concerning specific trades. The Amex also has indicated that, as more experience is gained with the linkage, the exchanges may agree to the exchange of additional information on a routine basis.

[39] Article IV, Section 2, of the Plan.

[40] Article IV, Section 2, also states that "any such information forwarded to the other exchange is to be kept confidential except to the extent that disclosure is required in connection with a regulatory proceeding of such exchange or pursuant to any obligation of such exchange to disclose information for surveillance purposes to any other self-regulatory organization or to the SEC or the OSC."

[41] *See* letter from Stephen Williams, Senior Vice President, Amex, to Brandon Becker, Assistant Director, Division of Market Regulation ("Division") dated June 21, 1985 at 17-18.

otherwise infringed upon Canadian sovereignty. If such a determination is made, the Attorney General may, by order, prohibit or restrict the production of Canadian records or information to a foreign tribunal, or the doing of any act in Canada which might cause the records tobe produced before a foreign tribunal.[42]

The OSC and counsel to TSE have represented that it is highly unlikely the Canadian blocking statute would be invoked to impede the flow of information regarding linkage transaction.[43] According to counsel for TSE, the legislative history of FEMA makes clear that the statute is to be invoked only as a "mechanism of last resort [to be relied upon] only if problems arise with respect to extraterritorial application of U.S. laws which U.S. and Canadian officials cannot resolve satisfactorily."[44] For example, before the Attorney General may issue an order under Section 3 of FEMA, he must reasonably form the opinion that a foreign tribunal, such as the Amex or SEC, is exercising jurisdiction of a kind that is likely to adversely affect significant Canadian interests or infringe upon Canadian sovereignty, or that the manner in which it is to be exercised is likely to adversely affect significant Canadian interests or infringe or Canadian sovereignty.[45]

Counsel to the TSE emphasized that, in light of the similar policy objectives of the U.S. and Canadian securities laws, as well as the agreements to cooperate between Amex and TSE under the Plan, "it would be difficult [to] conceive of a plausible scenario whereby the Attorney General might form the option necessary to interfere with an exchange of information pursuant to the Plan, or in investigation by Amex, the SEC or the TSE, carried out in a reasonable manner, into trading in securities undertaken in a manner contemplated in the Plan."[46] Accordingly, counsel concluded that the procedures for information sharing and surveillance are "reasonable and necessary" and are unlikely to be seen as conflicting with significant Canadian interests but rather as a framework for cooperation and consultation which will be respected by the Attorney General.[47]

The OSC confirmed TSE counsel's opinion, in a letter which stated that it is "extremely unlikely" the blocking statute would be invoked to impede the flow of information or assistance, particularly in light of the pledge of cooperation embodied in the Mutual Legal Assistance Treaty[48] which aims to improve the effectiveness of Canada and the U.S. in the investigation, prosecution and suppression of securities offenses.[49]

The SEC and OSC also have exchanged letters confirming their mutual commitment to sharing both their investigatory resources and any relevant information obtained pertaining to linkage-related transactions. The Commission's letter described the assistance it might provide the OSC under the broad statutory mandate of the U.S. securities laws.[50] In this regard, the Commission noted that such cooperation would be an extension of its long-standing policy of encouraging cooperation with foreign government authorities regarding investigations and enforcement proceedings.[51] In its letter, the Commission also noted that its investigatory authority was limited to cases involving potential violations of the U.S. securities laws.

The OSC also confirmed its commitment to cooperate with the SEC to the "fullest extent" possible in any investigation.[52] In addition, the OSC noted that its assistance also could extend to obtaining documents located in other provinces or territories of Canada, notwithstanding the fact its investigatory authority only extends to compelling attendance of witnesses and

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[42] Section 5 of FEMA similarly provides that where the Attorney General has formed the opinion that a foreign state or tribunal has taken, is taking or will take measures that adversely affect significant Canadian interests, he may, with the concurrence of the Secretary of State for External Affairs, by order, require a person in Canada to give him notice of any such directives, measures or communications, and may prohibit any person in Canada from complying with them.

[43] *See* letter from Gordon Nash, Lord Day and Lord (counsel to Amex), to Brandon Becker, dated June 27, 1985, which includes a copy of an opinion letter from the law firm of Tory, Tory, Deslauriers and Binnington, to the TSE, dated June 18, 1985 ("Tory, Tory letter"). *See* also letter from Ermanno Pascutto, Director, Ontario Securities Commission, to Richard Ketchum, Director, Division of Market Regulation, SEC and Gary Lynch, Director, Division of Enforcement, SEC, dated September 24, 1985.

[44] *See* Tory, Tory letter, page 3 and citing the Minister of Justice, John Crosbie, in the House of Commons. In the Senate debetes, the Honorable Nathan Nurgitz, sponsor of the bill, emphasized that the act is "clearly designed to protect national sovereignty in exceptional cases, after diplomatic efforts have been exhausted" and that "cooperation and consultation" will be the preferred routes of resolving any extraterritorial disputes. *Id.* at page 4.

[45] *Id.* at p. 3. Counsel's letter notes that when a particular Minister such as the Attorney General, has a statutory responsibility to carry out a duty, albough he may act in his own name, the Government is responsible, *i.e.*, a so-called "responsible form of government."

[46] Tory, Tory letter, *supra* note 42, at 4.

[47] *Id.* at 5. The TSE's opinion letter also discussed the impact on the linkage of two other Ontario laws which, like the federal blocking statute, also may grant governmental officials or agencies the right to interfere with the exchange of information or enforcement proceedings envisioned by the Plan. The Ontario Securities Act (R.S.O 1980, c.466) grants the OSC the authority to make any dicision in the public interest with respect to the manner in which any stock exhange carries on its business. This jurisdiction extends to permitting the OSC to direct the TSE as to the manner in which it conducts its business in implementing the Amex-TSE Plan. In the opinion of TSE counsel, because the Plan has been approved by the OSC, it is unlikely that the OSC would intervene in the implementation of the Plan to prevent an exchange of information, market surveillance or any investigation carried out pursuant to the Plan, absent strong public policy reasons. This is strongly reinforced by the letter the OSC has sent the Commission regarding the linkage. *See* text at note 48, *infra*. The second relevant law, the Business Records Act, R.S.O. 1980, c.56, was passed in 1947, to prevent forced or involuntary taking of records out of Canada. TSE counsel noted that there are no reported cases under the Business Records Act, and, in its opinion, it would be inapplicable to linkage related activity because, while the statute appears to be aimed at a forced or involuntary taking of records out of Canada, the plan provides for the voluntary submission of information to the TSE pursuant to Amex requests for information.

[48] The as yet unretified Treaty Between the Government of Canad and the Government of the United States of America on Mutual Legal Assistance in Criminal Matters (March 18, 1985) provides, pursuant to Article II of the Treaty, for mutual legal assistance in, among other things, (1) exchanging information and objects; (2) locating or identifying persons; (3) serving documents, (4) taking the evidence of persons; (5) providing documents and records; and (6) executing requests for searches and seizures.

[49] *See* letter from Ermanno Pascutto, Director, OSC, to Richard G. Ketchum, Director, Division of Market Regulation and Gary Lynch, Director, Division of Enforcement, page 3, dated September 24, 1985.

[50] *See* letter from Richard G. Ketchum and Gary Lynch, to Ermanno Pascutto at page 4–6, dated September 24, 1985. We noted that if the OSC requested the SEC's assistance in connection with a northbound transaction, or a request to obtain information in furtherance of an OSC inquiry, the SEC would assist the OSC to the fullest extent possible. In its letter, the Commission also noted that its investigatory authority is limited to cases involving potential violations of the U.S. securities laws.

[51] The United States, as a rule, has offered its resources in assisting foreign discovery efforts. Under 28 U.S.C. § 1782, the United States federal district courts are authorized to lend assistance to foreign and international tribunals and to litigants before such tribunals, by ordering a person to give testimony or to produce documents for use in a proceeding of that tribunal. Such an order generally is made pursuant to a letter rogatory issued, or a request made, by a foreign or international tribunal, or upon the application of any interested person, directing that the testimony or statement be given or document produced before a person appointed by the court. The person appointed has the power to administer any necessary oath, and take the testimony or statement. The statute also provides that the order may set out the practices and procedures to be used which may be, in whole or part, the practices and procedures of the foreign country.

[52] The OSC also has investigatory authority similar to the Commission's which may be exercised through informal and, where appropriate, formal procedures.

production of documents by persons within Ontario. The OSC stated that it has a close working relationship with securities administrators in other provinces and information is obtained and shared interprovincially and on an informal or formal basis.[53]

III. Conclusion

The Commission is satisfied that the Linkage Plan adequately addresses the key issues relating to the effective operation of the Amex-TSE linkage. The Commission believes that adequate surveillance and information sharing, and procedures between both the Amex and TSE and the SEC and OSC, are in place at this time. In this regard, the Commission obtained firm representations from the OSC that it is "extremely unlikely" the Canadian federal blocking statute will, in any way, hamper the Amex's or Commission's surveillance efforts.[54] Thus, the Commission believes that the exchanges, and the SEC and OSC, have provided for effective avenues for cooperation that will ensure the integrity of the linkage and the protection of investors participating in and affected by the linkage.

It is therefore ordered, pursuant to section 19(b)(2) of the Act, that the proposed rule changed, be, and hereby, are approved.

By the Commission.

Dated: September 20, 1985.

John Wheeler,

Secretary.

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[53] The OSC also stated that OSC staff have participated in investigations authorized by a formal order issued by an extra-provincial securities administration.

[54] As noted above, however, the Commission believes that in the event FEMA were invoked regarding linkage related trading, the Commission would have to consider under the circumstances whether, or in what form, it would be appropriate to permit continued operation of the linkage.

[Rel. No. 34-22443; File No. SR-Amex-85-15]

Self-Regulatory Organizations; American Stock Exchange, Inc.; Order Approving Proposed Rule Change

The American Stock Exchange, Inc. ("Amex") submitted, on April 29, 1985, copies of a proposed rule change (SR-Amex-85-15) pursuant to Section 19(b) of the Securities Exchange Act of 1934 ("Act") and Rule 19b-4 thereunder, to amend section 140 of the Amex *Company Guide* to provide for a reduction in the Exchange's original listing fee schedule for Canadian companies listed on the Toronto Stock Exchange ("TSE") that seek to list on the Amex.

The Exchange determined to retain its current rate structure, which is based on the number of shares to be listed but will apply a fixed percentage reduction.[1] The impetus for the amendment is the proposed rule change submitted by the Amex which would establish an electronic linkage between the Amex and the TSE to allow orders in securities dually-listed on both exchanges to be routed between the two exchanges.[2] The linkage is expected to commence on a pilot basis in six of the most actively traded dual issues and eventually will be expanded to include all dually-listed securities.

The Amex anticipates that the Amex-TSE linkage will provide an incentive for Canadian companies listed on the TSE and also traded over-the-counter in the United States, to list on the Amex, in order to obtain the benefits of the linkage for their shareholders. As an additional incentive to listing, the Amex believes that it is appropriate to offer a reduced original listing fee to all Canadian companies who list on the TSE or any other Canadian stock exchange. The Amex has stated that a reduced fee is warranted because these issuers already have paid an original listing fee to a Canadian market to trade their shares. Although these companies may desire to expand the market for their issues, the Amex believes that, without the listing fee reduction, Canadian issuers may hesitate to pay a substantial additional fee to list on the Amex, particularly because the Canadian market generally remains the primary market in inter-listed securities.[3]

Notice of the proposed rule change together with the terms of substance of the proposed rule change was given by the issuance of a Commission release (Securities Exchange Act Release No. 22184, June 28, 1985) and by publication in the **Federal Register** (50 FR 27873, July 8, 1985). No comments were received regarding the proposed rule change.

The Commission finds that the proposed rule change is consistent with the requirements of the Act and the rules and regulations thereunder applicable to a national securities exchange and, in particular, the requirements of Section 6, as appropriate, and the rules and the regulations thereunder. In particular, the Commission finds the proposed rule change is consistent with section 6(b)(4) of the Act which requires that the rules of an exchange provide for the equitable allocation of reasonable dues, fees and other charges among its members and issuers and other persons using its facilities. In addition, the Commission believes that the reduced listing fees do not unfairly discriminate among issuers as proscribed by section 6(b)(5) of the Act. The Commission agrees with the Amex that a reduced fee for Canadian companies is warranted because these companies already have listed on a Canadian stock exchange, and, in addition, the Canadian market generally has been the dominant market in the majority of the inter-listed securities. Moreover, the Canadian issuers would be required to pay the same annual fee for continued Amex listing paid by all other Amex listed companies. Accordingly, the Commission views as appropriate Amex's reduction in initial listing fees for Canadian issuers that have incurred initial and continuing listing fees on a Canadian exchange that is often the primary market for their stock. Finally, the Commission notes that, to test the impact of the reduced listing fees, the reductions will be implemented on a one year pilot basis.

It is therefore ordered, pursuant to section 19(b)(2) of the Act, that the proposed rule change be, and hereby is, approved.

For the Commission, by the Division of Market Regulation pursuant to delegated authority.

John Wheeler,

Secretary.

September 20, 1985.

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[1] The Amex will be applying a 50% reduction of the current rates to the Canadian companies, subject to a $30,000 overall maximum. For example, the total fee for listing 10 million shares currently would be $60,000. Under the 50% reduction for Canadian companies, the total fee for listing would be $30,000.

[2] The proposal was submitted by the Amex on April 4, 1985 and published for comment in Securities Exchange Act Release No. 22001 (April 30, 1985) 50 FR 19504 (May 8, 1985) (See File No. SR-Amex-85-8). The Commission approved the proposal on September 20, 1985. Securities Exchange Act Release No. 22442, September 20, 1985.

[3] The Amex has stated that for the year up to March 29, 1985, the Canadian market has been the dominant market for 23 out of 37 inter-listed securities on the Amex.

[Rel. No. 34-22438; File Nos. SR-Amex-85-19 and CBOE-85-38]

Self-Regulatory Organizations; Proposed Rule Changes by the American Stock Exchange, Inc., and Chicago Board Options Exchange, Inc.; Relating to the Options Allocation Plan

Pursuant to section 19(b)(1) of the Securities Exchange Act of 1934, 15 U.S.C. 78s(b)(1), notice is hereby given that on May 16 and August 29, 1985, the American Stock Exchange, Inc. ("Amex") and Chicago Board Options Exchange, Inc. ("CBOE") filed with the Securities and Exchange Commission the proposed rule changes as described in Items I, II, and III below, which Items have been prepared by the self-regulatory organizations. The Commission is publishing this notice to solicit comments on the proposed rule changes from interested persons.

I. Self-Regulatory Orgainzations' Statement of the Terms of Substance of the Proposed Rule Changes

Amex and CBOE propose to amend the Options Allocation Plan ("Plan") to explicitly exclude from coverage of the Plan options on Over-the-counter stocks designated as National Market System securities in Rule 11Aa2-1 under the Securities Exchange Act of 1934 ("NMS securities"). *Italics* indicates material proposed to be added to the plan.

Options Allocation Plan

(A)-(I) No change.

(J) This Option Allocation Plan shall not be applicable to the listing or delisting of any option on a stock which is traded primarily through the facilities of the National Association of Securities Dealers automated Quotation System at the time said option is to be admitted to trading on the Exchange.

II. Self-Regulatory Organizations' Statement of the Purpose of, and Statutory Basis for, the Proposed Rule Changes

In their filings with the Commission, the self-regulatory organizations included statements concerning the purpose of and basis for the proposed rule changes and discussed any comments they received on the proposed rule changes. The text of these statements may be examined at the places specified in Item IV below. The self-regulatory organizations have prepared summaries, set forth in sections (A), (B), and (C) below, of the most significant aspects of such statements.

A. Self-Regulatory Organizations' Statement of the Purpose of, and Statutory Basis for, the Proposed Rule Changes

On May 8, 1985, the Commission issued Release No. 34-22026 ("Release") regarding proposals to trade standardized options on NMS securities designated as such pursuant to Rule 11Aa2-1(b)(1) of the Securities Exchange Act of 1934. Among other things, the Release stated that the Exchange's proposal to trade options on NMS securities is conditioned upon the elimination of certain existing barriers to the multiple trading of these options.

The Commission found that the Options Allocation Plan is one such barrier to multiple trading. Thus, the Commission is requiring the participants in the Plan to amend the Plan to specifically exclude options on NMS securities from the coverage of the Plan.[1] The Plan will exclude from coverage such options irrespective of whether or not the stock is subsequently listed on an exchange. Accordingly, any NMS security which has been selected for standardized options trading by one or more exchanges will, even upon its subsequent listing on an exchange, continue to be eligible for selection by any other marketplace.

The proposed rule change is consistent with sections 6(b) and 11A of the Act in general and furthers the objectives of section 6(b)(5) in particular in that it will remove barriers to multiple trading, thereby permitting exchange members, member organizations and affiliated persons to freely transact business in standardized options on NMS Securities either on an exchange or through the NASDAQ system.

B. Self-Regulatory Organizations' Statement on Burden on Compeition

The Amex and CBOE believe that the proposed rule changes will not impose a burden on competition.

C. Self-Regulatory Organizations' Statement on Comments on the Proposed Rule Changes Received From Members, Participants, or Others

No written comments were either solicited or received.

III. Date of Effectiveness of the Proposed Rule Changes and Timing for Commission Action

Within 35 days of the date of publication of this notice in the **Federal Register** or within such longer period (i) as the Commission may designate up to 90 days of such date if it finds such longer period to be appropriate and publishes its reasons for so finding or (ii) as to which the self-regulatory organization consents, the Commission will:

(A) By order approve such proposed rule changes, or

(B) Institute proceedings to determine whether the proposed rule changes should be disapproved.

IV. Solicitation of Comments

Interested persons are invited to submit written data, views, and arguments concerning the foregoing. Persons making written submissions should file six copies thereof with the Secretary, Securities and Exchange Commission, 450 Fifth Street, NW., Washington, D.C. 20549. Copies of the submission, all subsequent amendments, all written statements with respect to the proposed rule change that are filed with the Commission, and all written communications relating to the proposed rule changes between the Commission and any person, other than those that may be withheld from the public in accordance with the provisions of 5 U.S.C. 552, will be available for inspection and copying in the Commission's Public Reference Section, 450 Fifth Street, NW., Washington, D.C. Copies of such filings will also be available for inspection and copying at the principal offices of the Amex and CBOE. All submissions should refer to the file numbers in the caption above and should be submitted by October 18, 1985.

For the Commission by the Division of Market Regulation, pursuant to delegated authority.

Dated: September 20, 1985.

John Wheeler,

Secretary.

[FR Doc. 85-23143 Filed 9-26-85; 8:45 am]

BILLING CODE 8010-01-M

[Rel. No. 34-22425; File No. SR-CBOE-85-39]

Self-Regulatory Organizations; Proposed Rule Change by Chicago Board Options Exchange, Inc.; Relating to Crossing Orders for Foreign Currency Options

Pursuant to section 19(b)(1) of the Securities Exchange Act of 1934, 15 U.S.C. 78s(b)(1), notice is hereby given that on August 29, 1985, the Chicago

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[1] Amex and CBOE, as well as the New York, Pacific and Philadelphia Stock Exchanges, previously have agreed not to apply the existing Options Allocation Plan to options on NMS stocks and to submit rule filings formally amending the Plan to this effect. *See e.g.*, Securities Exchange Act Release No. 22094, May 31, 1985, 50 FR 23859 (approval of Amex's proposal to trade options on NMS stocks).

Board Options Exchange, Incorporated filed with the Securities and Exchange Commission the proposed rule change as described in Items I, II and III below, which Items have been prepared by the self-regulatory organization. The Commission is publishing this notice to solicit comments on the proposed rule change from interested persons.

I. Text of the Proposed Rule Change

This rule change adds rule 22.14 to chapter XXII concerning currency options.

Crossing Orders

Rule 22.14. Exchange Rule 6.74 shall apply to the trading of currency options, except that when a facilitation order is from a firm's proprietary account or from a public customer who is not a broker-dealer, the facilitation order shall have the right to facilitate up to a minimum of 20 percent of the order being facilitated.

II. Self-Regulatory Organization's Statement of the Purpose of, and Statutory Basis for, the Proposed Rule Change

In its filing with the Commission, the self-regulatory organization included statements concerning the purpose of and basis for the proposed rule change and discussed any comments it received on the proposed rule change. The text of these statements may be examined at the places specified in Item IV below and is set forth in sections (A), (B), and (C) below.

(A) Procedures of the Self-Regulatory Organization

The purpose of this proposed rule change is to encourage market participants to facilitate public customer orders by providing that they can participate in at least 20 percent of the transaction, while at the same time providing that trading crowd participants also have an opportunity to participate.

The statutory basis for the proposed rule change is section 6(b)(5) of the Securities Exchange Act of 1934 (the Act), in that it would protect investors and the public interest by enhancing the facilitation of public customer orders.

(B) Self-Regulatory Organization's Statement on Burden on Competition

The Exchange does not believe that this proposed rule change will impose any burden on competition.

(C) Self-Regulatory Organization's Statement on Comments on the Proposed Rule Change Received From Members, Participants or Others

Comments were neither solicited nor received.

III. Date of Effectiveness of the Proposed Rule Change and Timing for Commission Action

Within 35 days of the date of publication of this notice in the Federal Register or within such longer period (i) as the Commission may designate up to 90 days of such date if it finds such longer period to be appropriate and publishes its reasons for so finding or (ii) as to which the self-regulatory organization consents, the Commission will:

(A) By order approve such proposed rule change, or

(B) Institute proceedings to determine whether the proposed rule change should be disapproved.

IV. Solicitation of Comments

Interested persons are invited to submit written data, views and arguments concerning the foregoing. Persons making written submission should file six copies thereof with the Secretary, Securities and Exchange Commission, 450 Fifth Street, Washington, D.C. 20549. Copies of the submission, all subsequent amendments, all written statements with respect to the proposed rule change that are filed with the Commission, and all written communications relating to the proposed rule change between the Commission and any person, other than those that may be withheld from the public in accordance with the provisions of 5 U.S.C. 552, will be available for inspection and copying at the Commission's Public Reference Section, 450 Fifth Street, NW, Washington, D.C. Copies of such filing will also be available for inspection and copying at the principal office of the above-mentioned self-regulatory organization. All submissions should refer to the file number in the caption above and should be submitted by October 18, 1985.

For the Commission by the Division of Market Regulation, pursuant to delegated authority.

Dated: September 18, 1985.

John Wheeler,

Secretary.

[FR Doc. 85-23144 Filed 9-26-85; 8:45 am]

BILLING CODE 8010-01-M

[Rel. No. 34-22427; File No. SR-CBOE-85-30]

Self-Regulatory Organizations; Proposed Rule Change by Chicago Board Options Exchange, Inc., Relating to Disclosure of Orders

Pursuant to section 19(b)(1) of the Securities Exchange Act of 1934, 15 U.S.C. 78s(b)(1), notice is hereby given that on July 25, 1985, the Chicago Board Options Exchange, Incorporated filed with the Securities and Exchange Commission the proposed rule change as described in Items I, II and III below, which Items have been prepared by the self-regulatory organization. The Commission is publishing this notice to solicit comments on the proposed rule change from interested persons.

I. Text of the Proposed Rule Change

Additions are italicized; deletions are bracketed.

Disclosure of Orders

Rule 7.8. [Except for the bids and offers that he displays or makes orally in accordance with Rule 7.7, no Board Broker or Order Book Official shall directly or indirectly disclose to any person, other than an official of the Exchange, any information in regard to the orders entrusted to him, unless, in his opinion and with the concurrence of a Floor Official, the interests of a fair and orderly market call for such disclosure.]

Equal Access to Book Depth and Size. Upon the request of a member, and so long as such request does not interfere with operation of the book, an Order Book Official, or such other person designated by the Exchange, may disclose the price and number contracts bid below or offered above the book information displayed pursuant to Rule 7.7. The Exchange may, in its discretion, from time to time, establish the depth to which such information may be disclosed.

II. Self-Regulatory Organization's Statement of the Purpose of, and Statutory Basis for, the Proposed Rule Change

In its filing with the Commission, the self-regulatory organization included statements concerning the purpose of and basis for the proposed rule change and discussed any comments it received on the proposed rule change. The text of these statements may be examined at the places specified in Item IV below and is set forth in sections (A), (B), and (C) below.

(A) Self-Regulatory Organization's Statement of the Purpose of, and the Statutory Basis for, the Proposed Rule Change

The purpose of this rule change is to permit access to book size and depth information below and above the best bid and offer on the book. The best bid and offer on the book are displayed under Rule 7.7. Currently, Rule 7.8 precludes disclosure of Book size and depth above the best offer and below the best bid, except on a discretionary basis, with concurrence of a floor official. The proposed rule change would allow all market participants to gain access, on an equal basis, to information concerning book size and depth. Based upon experience, the Exchange will determine from time to time the extent of access to be permitted. The Order Book Official is given the responsibility to disclose book depth and size. The Exchange may designate someone else to assume this function.

This rule change will provide information to crowd participants which will permit them to establish better the best available price for handling larger orders. A common practice on stock exchanges, for example, is for block positioners to learn how much of the book, and at what price, will need to be purchased or sold as part of a transaction. This is useful information in establishing the price for transactions, and should facilitate options trading. The rule change is consistent with the Securities Exchange Act of 1934 ("the Act") and in particular Section 6(b)(5) of the Act because the rule change will facilitate the fair and efficient pricing of securities transactions, and is in the public interest, by providing equal access to information concerning book depth and size.

(B) Self-Regulatory Organization's Statement on Burden on Competition

The Exchange does not believe that this proposed rule change will impose any burden on competition.

(C) Self-Regulatory Organization's Statement on Comments on the Proposed Rule Change Received From Members, Participants or Others

This proposed rule change is the implementation of one of the recommendations of the CMTF, which was endorsed by a membership vote concluded on November 20, 1984.

III. Date of Effectiveness of the Proposed Rule Change and Timing for Commission Action

Within 35 days of the date of publication of this notice in the Federal Register or within such longer period (i) as the Commission may designate up to 90 days of such date if it finds such longer period to be appropriate and publishes its reasons for so finding or (ii) as to which the self-regulatory organization consents, the Commission will:

(A) By order approve such proposed rule change, or

(B) Institute proceedings to determine whether the proposed rule change should be disapproved.

IV. Solicitation of Comments

Interested persons are invited to submit written data, views and arguments concerning the foregoing Persons making written submission should file six copies thereof with the Secretary, Securities and Exchange Commission, 450 Fifth Street, Washington, DC 20549. Copies of the submission, all subsequent amendments, all written statements with respect to the proposed rule change that are filed with the Commission, and all written communications relating to the proposed rule change between the Commission and any person, other than those that may be withheld from the public in accordance with the provisions of 5 U.S.C. 552, will be available for inspection and copying at the Commission's Public Reference Section, 450 Fifth Street, NW., Washington, DC. Copies of such filing will also be available for inspection and copying at the principal office of the above-mentioned self-regulatory organization. All submissions should refer to the file number in the caption above and should be submitted by October 18, 1985.

For the Commission by the Division of Market Regulation, pursuant to delegated authority.

Dated: September 19, 1985.

John Wheeler,

Secretary.

[FR Doc. 85-23145 Filed 9-26-85; 8:45 am]

BILLING CODE 8010-01-M

[Release No. 34-22430; File No. SR-CSE-85-4]

Self-Regulatory Organizations; Cincinnati Stock Exchange, Inc.; Order Approving Proposed Rule Change

The Cincinnati Stock Exchange, Inc. ("CSE") on August 2, 1985 submitted a proposed rule change pursuant to section 19(b)(1) of the Securities Exchange Act of 1934 ("Act") and Rule 19b-4 thereunder, to adopt certain stated policies, practices and interpretations describing in detail the CSE's National Securities Trading System ("NSTS") and to amend CSE Rule 11.9 to reflect recent changes in, and certain proposed changes to, the NSTS.

The CSE's NSTS is an electronic securities communication and execution system through which bids and offers of public orders and competing dealers are consolidated for review and execution. In addition to displaying limit orders and CSE and other market quotes, the NSTS matches orders and quotes at the same price in the system and executes them electronically based on programmed price/time and agency/principal priorities.[1] In addition to describing the NSTS more fully, the proposed rule change reflects recent changes made by the CSE to NSTS in order to provide automated guaranteed executions for public agency order up to 1099 shares at the best available quote displayed by all Intermarket Trading System ("ITS") participants. Under this guarantee, public agency market and marketable limit orders are priced at the best bid and offer of all ITS participants ("ITS/BBO"), and matched against any existing contra agency interest in the system at this price, and then against any similar principal interest. If less than 1099 shares of the order have been executed through this process, the system will execute the remainder of the order up to 1099 shares at the ITS/BBO on behalf of a dealer in the system designated as the Dealer of the day. The NSTS does not provide dealers the ability to improve the execution price received by the customer. If any of the order still remains, it will be flashed on NSTS terminals to approved dealers for 30 seconds to give each approved dealer an opportunity to fill the balance of the order at the ITS/BBO, before the order is formatted for potential entry into ITS.[2]

The proposed rule change also reflects changes to NSTS that make possible the automatic entry of NSTS orders into ITS. At present, orders are formatted and entered in ITS on a manual basis. The proposed rule change includes a detailed description of how the NSTS would process ITS activity through an automated interface.

Notice of the proposed rule change was given in Securities Exchange Act

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[1] The NSTS is described in greater detail in the release noticing this rule change, Securities Exchange Act Release No. 22330 (August 15, 1985) 50 FR 33880.

[2] Dealer orders entered into the system do not receive the 1099 share guaranteed execution; similarly, dealer orders, except for orders being handled by another dealer as agent, are not subject to the 30 second delay before being formatted for entry into ITS.

Release No. 22330 (August 15, 1985), and published in the **Federal Register** (50 FR 33880) on August 21, 1985. A comment letter was received from the New York Stock Exchange, Inc. ("NYSE"),[3] which noted that the CSE had already reflected several NYSE comments in the proposed rule change. The NYSE suggested, however, that because the terms of the automated CSE linkage with ITS are still under discussion among the CSE and other ITS participants, the Commission should refrain from approving the portion of the proposed rule change dealing with entry of orders into ITS until amendments to the ITS Plan "necessary to permit the automated linkage that the CSE seeks" have been prepared.[4]

The CSE proposed rule change should contribute to the efficiency of the securities markets, increased competition among markets, and promotion of the public interest. The Commission is concerned, however, that the CSE's NSTS system does not provide an opportunity for customer market orders executed automatically pursuant to the CSE guarantee to obtain an execution price between the ITS/BBO. Several other exchange small order system providing automated executions at the ITS/BBO, such as the Pacific Stock Exchange, Inc.'s SCOREX system and the Midwest Stock Exchange, Inc.'s MAX system, provide a brief period in which the order is displayed to the specialist in the stock; these systems differ from NSTS in that they enable the specialist to improve the price of the execution to reflect interest on the floor or trading patterns on another exchange. The CSE's NSTS does not at this point allow market makers to execute small customer market orders at prices other than the ITS/BBO. The Commission recognizes, however, that the CSE accounts at present for a very small share of the overall order flow in stocks traded through NSTS, and that the changes in the NSTS system are intended to be initial steps in improving the NSTS system. The Commission also has received assurances from members of the CSE governing bodies that the CSE is willing to consider modifying the NSTS in the near future to allow the execution price of customer orders to be improved in the system.

The elements of the proposed rule change designed to accommodate an automated interface between NSTS and ITS also provide a predicate for enhanced market linkages that could contribute substantially to the efficency of and competition in the market. When the Commission initially approved CSE's membership in ITS, which provided for a manual NSTS/ITS interface, it stated:

> The Commission is concerned . . . that a manual interface will provide too slow and cumbersome to provide an efficient mechanism for routing orders among market centers. Accordingly, the Commission expects that, if the CSE is willing to implement the necessary technical changes to affect an automated interface between the NSTS and ITS, the ITS participants will take necessary steps to implement such an interface.[5]

The CSE proposed rule change, by providing for the necessary changes to NSTS to accommodate an automated interface, is a necessary precondition to such action by ITS. Accordingly, the Commission finds that the CSE has set forth an approach in its rules and its stated policy, practice and interpretation concerning the interface of NSTS with ITS that, while not by any means the only possible approach to this interface, is consistent with the Act and the rules and regulations thereunder.[6]

It should be noted that the Commission, by taking this action, is not expressing any views with respect to any changes that may be made to the ITS Plan to accommodate an automated NSTS/ITS interface. The Commission recognizes that discussions are underway among the CSE and other ITS participants concerning the need for and terms of an amendment to the Plan governing the ITS to accommodate such an automated interface. In particular, the Commission understands that such discussions could result in an agreement among ITS participants that certain conditions be imposed on maintenance of this automated interface and that these discussions on Plan issues could result in changes in the description set forth in the CSE's proposed rule change. In the event changes in the CSE's proposed rules become necessary as the result of these discussions, these changes will be reviewed by the Commission in a subsequent rule filing, just as the Commission will review any ITS Plan amendments that may result from these discussions.

The Commission finds that the proposed rule change is consistent with the requirements of the Act and the rules and regulations thereunder applicable to a national securities exhange and, in particular, the requirements of Section 6 and the rules and regulations thereunder.

It is therefore ordered, pursuant to section 19(b)(2) of the Act, that the above-mentioned proposed rule change be, and hereby is approved.

For the Commission, by the Division of Market Regulation, pursuant to delegated authority.

Dated: September 20, 1985.

John Wheeler,

Secretary.

[FR Doc. 85-23146 Filed 9-26-85; 8:45 am]

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[3] Letter from James Buck, Secretary, NYSE, to John Wheeler, Secretary, SEC (September 10, 1985).

[4] *Id.* at 1.

[5] Securities Exchange Act Release No. 17532 (February 10, 1981), 46 FR 12919.

[6] For this reason, and because, as discussed below, the Commission is not in this order expressing any view with respect to any possible ITS Plan amendments, the Commission believes it is unnecessary to withhold action on those portions of the CSE proposed rule change relating to the NSTS/ITS interface.

[Release No. 34-22426; File No. SR-NASD-85-25]

Self-Regulatory Organizations; Filing and Order Granting Accelerated Approval of a Temporary Proposed Rule Change by National Association of Securities Dealers, Inc.; Relating to Modification of the Small Order Execution System for Transactions in Over-the-Counter Securities

Pursuant to section 19(b)(1) of the Securities Exchange of 1934 ("Act"), 15 U.S.C. 78s(b)(1), notice is hereby given that on September 13, 1985, the National Association of Securities Dealers, Inc. ("NASD") filed with the Securities and Exchange Commission the proposed rule change as described in Items I, II, and III below, which Items have been prepared by the NASD. The Commission is publishing this notice to solicit comment on the proposed rule change from interested persons.

I. Self-Regulatory Organization's Statement of Terms of Substance of the Proposed Rule Change

The following is the full text of a rule change by the NASD relating to modification of the facilities description of the Small Order Execution System ("SOES") for transactions in over-the-counter ("OTC") securities approved by the Commission in SR-NASD-84-26. The rule change shall be effective for a period of 90 days to permit consideration by the Commission of approval of the proposed modification on a permanent basis which is the subject of a separate rule filing. The text of the rule change is:

The NASD has filed as a stated policy, practice or interpretation, a description of a new facility which has been designed and developed by NASD Market Services, Inc. ("NASD market serviced") a subsidiary of the NASD.

NASD Market Services has developed SOES which is an order routing and execution system, specifically designed to execute limited size orders [(initially 500 shares)] (*currently 500 shares for NASDAQ and 1,000 shares for NASDAQ/NMS securities*) in OTC securities.

Remaining text is unchanged.

II. Self-Regulatory Organization's Statement of the Purpose of, and Statutory Basis for, the Proposed Rule Change

In its filing with the Commission, the NASD included statements concerning the purpose of and basis for the proposed rule change and discussed any comments it received on the proposed rule change. The text of these statements may be examined at the places specified in Item IV below. The NASD has prepared summaries, set forth in section (A), (B) and (C) below, of the most significant aspects of such statements.

A. Self-Regulatory Organization's Statement of the Purpose of, and Statutory Basis for, the Proposed Rule Change

The purpose of SOES is to improve the efficiency of execution of transactions in OTC securities through the use of new data processing and communications techniques. The entry and execution of up to 1,000 shares in NASDAQ/NMS issues is part of the increased efficiency and capability of the system.

The statutory basis for the development and implementation of SOES is found in section 11A(a)(1) (B) and (C)(i), 15A(b)(6), and 17A(a)(1) (B) and (C) of the Act. Section 11A(a)(1) (B) and (C)(i) set forth the Congressional goal for achieving more efficient and effective market operations and the economically efficient execution of transactions through new data processing and communications techniques. Section 15A(b)(6) requires that the rule of the NASD be designed "to foster cooperation and coordiantion with persons engaged in regulating, clearing, settling, and processing information with respect to, and facilitating transactions in securities, to remove impediments to and perfect the mechanism of a free and open market . . ." Section 17A(a)(1) (B) and (C) sets forth the Congressional goal of reducing costs involved in the clearance and settlement process through data processing and communications techniques. The NASD believes that the modification to SOES will further these ends by providing an enhanced mechanism for the efficient and economic execution and clearance of transactions in OTC securities.

B. Self-Regulatory Organization's Statement on Burden on Competition

SOES is a service to which participants subscribe on a voluntary basis and as such the NASD believes that it imposes no burden on competition. To the extent that any burden on competition may be found to exist, the NASD believes that the benefit of increased efficiency of SOES will outweigh any potential burden upon competition and materially advance the purposes to be served under the foregoing sections of the Act. It is also important to note that the transactional size provided for in SOES comports with transactional size in execution systems operated or under the control of other self-regulatory organizations.

C. Self-Regulatory Organization's Statement on Comments on the Proposed Rule Changes Received From Members, Participants, or Others

Comments were neither solicited nor received in connection with the proposed modification to SOES.

III. Solicitation of Comments

Interested persons are invited to submit written comments concerning the foregoing. Persons submitting comments should file six copies with John Wheeler, Secretary, Securities and Exchange Commission, 450 5th Street NW., Washington, DC 20549. Copies of the submission, and all related items other than those that may be withheld from the public in accordance with the provisions of 5 U.S.C. 552, will be available for inspection and copying at the principal office of the NASD, located at 1735 K Street NW., Washington, DC 20006. All submissions should refer to File No. SR-NASD-85-25 and should be submitted by October 21, 1985.

IV. Approval

The Commission finds that the proposed temporary rule change is consistent with the requirements of the Act and the rules and regulations thereunder applicable to the NASD and, in particular, the requirements of section 11A(a)(1)(B) and the rules and regulations thereunder.

The Commission finds good cause for approving the proposed rule change prior to the thirtieth day after the date of publication of notice of filing thereof in that accelerated approval and the implementation of the modification to SOES scheduled to occur on September 27, 1985, will benefit public investors by providing more rapid and cost-effective processing of transactions while assuring that such transactions are effected at the best quote available in the market at any particular point in time. The Commission recognizes that the NASD has prepared its system for implementation of the modification on that date. The Commission also notes that the issues involving SOES will be noticed for public comment in a permanent rule change filing and, as discussed above, the Commission believes that the benefits of approval of this temporary rule change outweigh any potential adverse effects to the commentators or other market participants during the short period of the rule change's effectiveness.

For the Commission, by the Division of Market Regulation, pursuant to delegated authority.

Dated: September 19, 1985.

John Wheeler,

Secretary.

[FR Doc. 85-23147 Filed 9-26-85; 8:45 am]

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[Release No. 34-22445; File No. SR-NYSE-85-34]

Self-Regulatory Organizations; Filing and Order Granting Accelerated Approval of Proposed Rule Change by New York Stock Exchange, Inc., Relating to the Capture and Submission of Specified Audit Trail Data

Pursuant to section 19(b)(1) of the Securities Exchange Act of 1934, 15 U.S.C. 78s(b)(1), notice is hereby given that on September 9, 1985, the New York Stock Exchange, Inc. filed with the Securities and Exchange Commission the proposed rule changes as described in Items I, II and III below, which Items have been prepared by the self-regulatory organization. The Commission is publishing this notice to solicit comments on the proposed rule changes from interested persons.

I. Self-Regulatory Organization's Statement of the Terms of Substance of the Proposed Rule Changes

The proposed rule changes consist of requirements in regard to the capture and submision of specified audit trail data to a clearing agency or the Exchange.

II. Self-Regulatory Organization's Statement of the Purpose of and Statutory Basis for, the Proposed Rule Changes

In its filing with the Commission, the self-regulatory organization included statements concerning the purpose of and basis for the proposed rule changes and discussed any comments it received on the proposed rule changes. The text of these statements may be examined at the places specified in Item IV below. The self-regulatory organization has prepared summaries, set forth in sections (A), (B), and (C) below, of the most significant aspects of such statements.

A. Self-Regulatory Organization's Statement of the Purpose of, and Statutory Basis for, the Proposed Rule Changes

(1) Purpose

The purpose of the proposed rule changes is to increase the accuracy and completeness of the Exchange's Audit Trail by:

(i) Requiring parties to round-lot regular-way transactions effected on the Exchange to submit specified audit trail data to a Clearing Agency that has agreed to supply the Exchange with such data;

(ii) Requiring parties to round-lot non-regular way transactions effected on the Exchange to submit specified audit trail data to the Exchange;

(iii) Requiring executing brokers to capture audit trail data at the point of trade.

(iv) Providing that the Exchange may impose fines under the expedited procedures of Rule 476A in any case where members and member organizations do not comply with audit trail requirements as to the capturing and submission of all specified audit trail data.[1]

The Genesis of the Audit Trail. The New York Stock Exchange Audit Trail is an automated surveillance tool that can be immediately accessed via computer terminals, which is used to rapidly reconstruct trades in order to identify rule violations and other intra and intermarket trading abuses. The SEC encouraged its development at least as far back as its 1963 *Special Study of the Securities Markets* and later also proposed to develop its own Market Oversight Surveillance System (MOSS).

Aided by the development of advanced computer systems technology, the Exchange also accelerated its efforts to develop its own audit trail system. It also joined with the other securities markets to form the Intermarket Surveillance Group (ISG). The audit trail's success so far, coupled with the work of the ISG, have resulted in the SEC's conclusion of MOSS as unnecessary.

The Audit Trail System. The audit trail system was efficiently designed by modifying Floor trade data collection and comparison systems that were already in place at the Exchange, National Securities Clearing Corporation (NSCC) and the member firms. Audit trail data elements received by a clearing agency for each trade from the clearing member firms are combined with other audit trail data elements and submitted to the Securities Industry Automation Corporation (SIAC). This data is matched to the tape print with corresponding audit trail data elements that are captured on the Floor to produce the audit trail. Audit trail data, however, is automatically obtained for orders processed by any one of the Exchange's automated trading support systems, which account for slightly more than one-half of all Exchange trades. This efficiently eliminates the need for the system user to have audit trail data in regard to his side of the trade sent to a clearing agency. At present, Exchange systems do not provide for distinguishing whether the account for which an order was executed was that of a member or member organization or of a non-member or non-member organization, as called for in subparagraph (9) of proposed Rule 132.30. Until such time as Exchange systems are programmed to provide this information, the Exchange will not require compliance with subparagraph (9) as to systematized orders, unless in the interim suitable alternative methods of collecting this information are identified and implemented.

The Need for an Audit Trail Rule. In order to ensure that the audit trail is as complete and accurate as possible, the proposed audit trail rule is felt to be necessary for two basic reasons. Primarily, the proposed rule change defines the audit trail data elements necessary for submission to a clearing agency and codifies the capture and submission of each data element as an Exchange rule. In appropriate cases where these requirements are not met, the Exchange intends to take appropriate disciplinary action, including the imposition of fines under the expedited procedures of Rule 476A.

Secondly, there are two types of transactions, namely cross transactions (i.e., transactions where a member is representing both orders to buy and orders to sell) and non-regular way trades, for which audit trail data is not currently gathered. Even though it is not mandatory to do so, the majority of regular way transactions are submitted to a clearing agency for comparison and/or settlement, with the exception of cross transactions. Therefore, audit trail data is not currently collected with respect to trades which are not submitted to a clearing agency, such as cross transactions.

In addition, the Exchange also believes that audit trail data should also be collected for the small number of non-regular way trades, which cannot be accepted by clearing agencies.

Therefore, the proposed rule change requires that all regular way trades effected on the Exchange, including crosses, be submitted to a clearing agency that has agreed to supply the Exchange with specified audit trail data. However, it would not be required that the clearing agency be used for the comparison and/or settlement of these trades. For non-regular way trades, members and member firms would be required to submit audit trail data to the Exchange.

The impact of the proposed rule change should not be significant for two reasons. As most regular way trades, which are the majority of trades executed on this Exchange, are already submitted to a clearing agency for comparison and/or settlement with audit trail data attached, no new requirements would be imposed for these trades. Secondly, the proposed rule change will not impose any new requirements that a clearing agency be actually used for the comparison and/or settlement of a trade, which may be more costly for members and member firms.

The proposed audit trail rule will benefit members and member organizations because audit trail information is often of financial value to them in assisting them to resolve uncompared trades, is a factor in the resolution of disputes between interested parties to a trade and provides a valuable information source to respond to inquiries from members, listed companies and public investors. Mandatory submission of all required audit trail data for all trades effected on the Exchange should significantly reduce "QTs" (questioned trades where trade comparison elements are missing), which are often costly and time-consuming for members and member organizations to resolve.

Statutory Basis for the Proposed Rule Changes. By imposing a requirement to help ensure that the audit trail is as complete and accurate as possible, the proposed rule changes will better enable the Exchange's regulatory and surveillance capabilities to keep pace with the complexity of trading in today's

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[1] The Exchange also requests an amendment to its minor disciplinary rule violation plan pursuant to Rule 19d-1(c) under the Securities Exchange Act of 1934. *See* Securities Exchange Act Release No. 22300 (August 8, 1985) 50 FR 32818 for the original notice of this plan.

sophisticated market environment, and are expected to remove impediments to and perfect the mechanism of a free and open market and a national market system, and this, in turn, will protect investors and the public interest, as called for in section 6(b)(5) of the Act. The proposed rule changes meet other requirements of section 6(b)(5) in that they will help prevent fraudulent and manipulative acts and practices, promote just and equitable principles of trade and foster cooperation and coordination with persons engaged in regulating, clearing, settling, processing information with respect to, and facilitating transactions in securities.

B. Self-Regulatory Organization's Statement on Burden on Competition

The Exchange does not believe that the proposed rule changes will impose any burden on competition.

C. Self-Regulatory Organization's Statement on Comments on the Proposed Rule Changes Received From Members, Participants or Others

The Exchange has neither solicited nor received written comments on the proposed rule changes.

III. Date of Effectiveness of the Proposed Rule Changes and Timing for Commission Action

The Exchange requests that the proposed rule changes be given accelerated effectiveness pursuant to Section 19(b)(2) of the Act. The Exchange believes that the proposed rule changes will enhance its ability to regulate and surveil trading in its market on a more comprehensive and timely basis. In view of the proposal's significant anticipated benefits and the fact that the Exchange is prepared to implement the proposed rule changes as soon as it receives approval to do so from the Commission, the Exchange requests that the Commission find good cause to approve the proposed rule changes on an accelerated basis.

The Commission finds that the proposed rule change is consistent with the requirements of the Act and the rules and regulations thereunder applicable to a national securities exchange and in particular, the requirements of section 6 and the rules and regulations thereunder.

The Commission finds good cause for approving the proposed rule change prior to the thirtieth day after the date of publication of notice of filing thereof, in that the rule change is intended to enhance existing audit trail procedures and will place no additional burden on member firms because the operational aspects of the proposed rule change have already been implemented.

IV. Solicitation of Comments

Interested persons are invited to submit written data, views and arguments concerning the foregoing. Persons making written submissions should file six copies thereof with the Secretary, Securities and Exchange Commission, 450 Fifth Street NW., Washington, DC 20549. Copies of the submission, all subsequent amendments, all written statements with respect to the proposed rule changes that are filed with the Commission, and all written communications relating to the proposed rule changes between the Commission and any person, other than those that may be withheld from the public in accordance with the provisions of 5 U.S.C. 522, will be available for inspection and copying in the Commission's Public Reference Section, 450 Fifth Street NW., Washington, DC. Copies of such filing will also be available for inspection and copying at the principal office of the above-mentioned self-regulatory organization. All submissions should refer to the file number in the capiton above and should be submitted by October 21, 1985.

It is therefore ordered, pursuant to section 19(b)(2) of the Act, that the above mentioned proposed rule change be, and hereby is, approved.

For the Commission, by the Division of Market Regulation pursuant to delegated authority.

Dated: September 20, 1985.

John Wheeler,

Secretary.

[FR Doc. 85-23148 Filed 9-26-85; 8:45 am]

BILLING CODE 8010-01-M

Self-Regulatory Organizations; Applications for Unlisted Trading Privileges and of Opportunity for Hearing; Philadelphia Stock Exchange, Inc.

September 19, 1985.

The above named national securities exchange has filed applications with the Securities and Exchange Commission pursuant to section 12(f)(1)(B) of the Securities Exchange Act of 1934 and Rule 12f-1 thereunder, for unlisted trading privileges in the following security:

Americus Shareowner Service Corp.
  Americus Trust for Exxon Shares
    Units
  Score Component
  Prime Component (File No. 7-8609)

This security is listed and registered on one or more other national securities exchange and is reported in the consolidated transaction reporting system.

Interested persons are invited to submit on or before October 10, 1985 written data, views and arguments concerning the above-referenced application. Persons desiring to make written comments should file three copies thereof with the Secretary of the Securities and Exchange Commission, Washington, D.C. 20549. Following this opportunity for hearing, the Commission will approve the application if it finds, based upon all the information available to it, that the extensions of unlisted trading privileges pursuant to such applications are consistent with the maintenance of fair and orderly markets and the protection of investors.

For the Commission, by the Division of Market Regulation, pursuant to delegated authority.

John Wheeler,

Secretary.

[FR Doc. 85-23151 Filed 9-26-85; 8:45 am]

BILLING CODE 8010-01-M

Self-Regulatory Organizations; Applications for Unlisted Trading Privileges and of Opportunity for Hearing; Midwest Stock Exchange, Inc.

September 19, 1985.

The above named national securities exchange has filed applications with the Securities and Exchange Commission pursuant to section 12(f)(1)(B) of the Securities Exchange Act of 1934 and Rule 12f-1 thereunder, for unlisted trading privileges in the following securities:

Eastern Air Lines, Inc.
  Purchase 1 Common at $16.00 (Warrants A) (File No. 7-8610)
Eastern Air Lines, Inc.
  Purchase 1 Common at $10.00 (Warrant) (File No. 7-8611)
Wickes Companies
  Common Stock, $0.10 Par Value (File No. 7-8612)
Occidental Petroleum Corporation
  Purchase 1 share of Common at $24.00 (Warrants) (File No. 8613)
American Royalty Trust
  Units (File No. 7-8614)
Willcox & Gibbs
  Common Stock, $1.00 Par Value (File No. 7-8615)
Knogo Corporation
  Common Stock, $.01 Par Value (File No. 7-8616)
General Development
  Common Stock, $.01 Par Value (File No. 7-8617)
Nord Resources Corporation
  Common Stock, $.01 Par Value (File

No. 7–8618)
Lomas Mortgage Corporation
Common Stock, $.01 Par Value (File No. 7–8619)
Plains Petroleum Company
Common Stock. (File No. 7–8620)
Golden Nuggets
1988 Warrants (File No. 7–8621)
R.J. Reynolds Industries, Inc.
Series C Cumulative Preferred Stock (File No. 7–8622).

These securities are listed and registered on one or more other national securities exchange and are reported in the consolidated transaction reporting system.

Interested persons are invited to submit on or before October 10, 1985, written data, views and arguments concerning the above-referenced applications. Persons desiring to make written comments should file three copies therefore with the Secretary of the Securities and Exchange Commission, Washington, D.C. 20549. Following this opportunity for hearing, the Commission will approve the applications if it finds, based upon all the information available to it, that the extensions of unlisted trading privileges pursuant to such applications are consistent with the maintenance of fair and orderly markets and the protection of investors.

For the Commission, by the Division of Market Regulation, pursuant to delegated authority.

John Wheeler,

Secretary.

[FR Doc. 85–23150 Filed 9–26–85; 8:45 am]

BILLING CODE 8010-01-M

Self-Regulatory Organizations; Applications for Unlisted Trading Privileges and of Opportunity for Hearing; Midwest Stock Exchange, Inc

September 19, 1985.

The above named national securities exchange has filed applications with the Securities and Exchange Commission pursuant to section 12(f)(1)(B) of the Securities Exchange Act of 1934 and Rule 12f–1 thereunder, for unlisted trading privileges in the following securities:

Americus Trust for Exxon Shares
10,000,000 Units of Fractional Undivided Interest (File No. 7–8606)
Americus Trust for Exxon Shares
10,000,000 Prime Components of Unites of Fractional Undivided Interest (File No. 7–8607)
Americus Trust for Exxon Shares
10,000,000 Scores Components of Units of Fractional Undivided Interest (File No. 7–8608).

These securities are listed and registered on one or more other national securities exchange and are reported in the consolidated transaction reporting system.

Interested persons are invited to submit on or before October 10, 1985, written data, views and arguments concerning the above-referenced applications. Persons desiring to make written comments should file three copies thereof with the Secretary of the Securities Exchange Commission, Washington, DC 20549. Following this opportunity for hearing, the Commission will approve the applications if it finds, based upon all the information available to it, that the extensions of unlisted trading privileges pursuant to such applications are consistent with the maintenance of fair and orderly markets and the protection of investors.

For the Commission, by the Division of Market Regulation, pursuant to delegated authority.

John Wheeler,

Secretary.

[FR Doc. 23149 Filed 9–26–85; 8:45 am]

BILLING CODE 8010-01-M

DEPARTMENT OF TRANSPORTATION

National Highway Traffic Safety Administration

[Docket No. 85–13; Notice 1]

Preliminary Evaluation Report on the Voluntary Tire Registration System, Request for Public Comment

AGENCY: National Highway Traffic Safety Administration (NHTSA), DOT.

ACTION: Request for comments.

SUMMARY: This notice announces the publication by NHTSA of a Preliminary Evaluation Report concerning the Voluntary Tire Registration System. This preliminary staff report evaluates whether the Voluntary Tire Registration System increased the proportion of tires which are registered, the extent to which dealers and distributors have encouraged first purchasers to register their tires and the extent to which the dealers and distributors followed the registration procedures (49 CFR Part 574). The agency seeks public review and comment on this preliminary evaluation prior to making the determinations required by the Naitonal Traffic and Motor Vehicle Safety Act.

DATE: Comments must be received no later than December 26, 1985.

ADDRESSES: Interested persons may obtain a copy of the report free of charge by contacting Mr. Richard Jordan, Office of Management Services, National Highway Traffic Safety Administration, Room 6115, 400 Seventh Street, SW., Washington, DC 20590 (202–426–0874). All comments should refer to the docket and notice number of this notice and be submitted to: Docket Section, Room 5109, Nassif Building, 400 Seventh Street, SW., Washington, DC 20590. [Docket hours, 8:00 a.m.–4:00 p.m., Monday through Friday.]

FOR FURTHER INFORMATION CONTACT: Mr. Frank G. Ephraim, Director, Office of Program Evaluation, Plans and Programs, National Highway Traffic Safety Administration, Room 5208, 400 Seventh Street, SW., Washington, DC 20590 (202–426–1574).

SUPPLEMENTARY INFORMATION: The Motor Vehicle Safety and Cost Savings Authorization Act of 1982 (96 STAT 1619, Pub. L. 97–331) directed that changes be made in how new tires are registered. The Act removed the requirement that independent tire dealers register new tires for the consumer (mandatory tire registration), and instead directed the Secretary of Transportation to issue a regulation requiring the dealer to hand the consumer a tire registration card which the purchaser may complete and return directly to the manufacturer of the tire. This new procedure is called voluntary tire registration (VTR).

The overriding concerns expressed by the Congress were to:

- Improve the proportion of tires sold which are registered to asure that the manufacturers could reach the first purcahser of the tire in the event of recall; and
- Reduce the burdens placed on the independent dealer. The Congress specified that the mandatory registration system was to remain in effect for stores owned or controlled by tire manufacturers.

The Act requires the Secretary to evaluate the effect of VTR after two years and to make a determination of what changes to make in the system to assure a high rate of registration.

When Congress passed the Authorization Act, the data then available showed that all original equipment tires were properly registered, but only 46.6 percent of the replacement tires were registered. While 80 to 90 percent of the tires sold by the company-owned stores were registered, only 20 percent of the tires sold by independent dealerships were. These data were submitted to the House Committee by NHTSA and were based on informal surveys of the domestic tire manufacturers taken by NHTSA in 1973, 1974 and 1979.

The preliminary evaluation examined whether voluntary registration increased the proportion of tires which are registered, the extent to which dealers and distributors have encouraged first purchasers to register their tires and the extent to which the dealers and distributors have followed registration procedures. The data reviewed included:

- The tire recall history between 1978 and 1984.
- The percentage of tires which were registered under both mandatory and voluntary registration, for both the independent dealers and distributors now covered by VTR, and, as a comparison group, for the company-owned or controlled outlets still subject to mandatory registration procedures.
- Attitudes and practices of independent tire dealers and distributors toward VTR.
- Consumer experience with registration when purchasing tires, including whether dealers mentioned VTR or gave the consumer a tire registration card.

The principal findings and tentative conclusions of the preliminary report are the following:

- Registration rates for tires sold by independent dealers dropped from 18.1 percent under mandatory procedures to 10.8 percent after the Final Rule on the voluntary registration system had been adopted. There was no comparable decline among company-owned outlets.
- The registration rates due to voluntary registration may have declined to as low as 8.4 percent because many of the registrations were due to a computer-assisted registration system and to other dealers continuing to use mandatory procedures.
- It was found that a sufficient number of registration forms are supplied to dealers and distributors by brand name owners.
- There are no records of any registrations for tires sold by over 70 percent of independent dealers (This estimate is based on information from only one manufacturer—but the largest one). Many of the other dealers have low registration rates.
- Tire purchasers want to be notified by manufacturers in the event of tire defects, but are under the misapprehension that their independent tire dealer is taking care of this for them.

NHTSA welcomes public review of the preliminary evaluation report and invites the public to submit comments.

It is requested but not required that 10 copies of comments be submitted.

Those persons desiring to be notified upon receipt of their comments in the rules docket should enclose, in the envelope with their comments, a self-addressed stamped postcard. Upon receiving the comments, the docket supervisor will return the postcard by mail.

Authority: 15 U.S.C. 1418; delegation of authority at 49 CFR 1.50 and 501.8.

Issued on: September 24, 1985.

Adele Spielberger,

Associate Administrator for Plans and Policy.

[FR Doc. 85-23060 Filed 9-26-85; 8:45 am]

BILLING CODE 4910-22-M

Sunshine Act Meetings

This section of the FEDERAL REGISTER contains notices of meetings published under the "Government in the Sunshine Act" (Pub. L. 94-409) 5 U.S.C. 552b(e)(3).

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1

CONSUMER PRODUCT SAFETY COMMISSION

TIME AND DATE: Commission Meeting, Tuesday, October 1, 1985, 2:00 p.m.

LOCATION: Third Floor Hearing Room 1111, 18th Street, NW., Washington, DC.

STATUS: Open to the public.

MATTERS TO BE CONSIDERED: Strong Sensitizers—Staff and Panel Representative.[1]

The staff will brief the Commission concerning draft proposed definitions to supplement the Federal Hazardous Substances Act definition of "strong sensitizer."

For a Recorded Message Containing the Latest Agenda Information, Call: 301–492–5709.

CONTACT PERSON FOR ADDITIONAL INFORMATION: Sheldon D. Butts, Office of the Secretary, 5401 Westbard Ave., Bethesda, Md. 20207, 301–492–6800.

Dated: September 25, 1985.

Sheldon D. Butts,

Deputy Secretary.

[FR Doc. 85–23262 Filed 9–25–85; 2:20 pm]

BILLING CODE 6355-01-M

CONSUMER PRODUCT SAFETY COMMISSION

TIME AND DATE: Commission Meeting, Wednesday, October 2, 1985, 9:30 a.m.

LOCATION: Room 456, 5401 Westbard Avenue, Bethesda, MD.

STATUS: Open to the public.

MATTERS TO BE CONSIDERED: The Commission will consider the Fiscal Year 1987 Budget.

For a recorded message containing the latest agenda information, call: 301–492–5709.

CONTACT PERSON FOR ADDITIONAL INFORMATION: Sheldon D. Butts, Office of the Secretary, 5401 Westbard Ave., Bethesda, MD. 20207, 301–492–6800

Dated: September 25, 1985.

Sheldon D. Butts,

Deputy Secretary.

[FR Doc. 85–23263 Filed 9–25–85; 2:20 pm]

BILLING CODE 6355-01-M

3

EQUAL EMPLOYMENT OPPORTUNITY COMMISSION

DATE AND TIME: 2:00 PM (Eastern Time), Tuesday, October 8, 1985

PLACE: Clarence M. Mitchell, Jr., Conference Room No. 200–C on the 2nd Floor of the Columbia Plaza Office Building, 2401 "E" Street, NW., Washington, DC 20507.

STATUS: Part will be open to the public and part will be closed to the public.

MATTERS TO BE CONSIDERED:

1. Announcement of Notation Vote(s).
2. A Report on Commission Operations.

CLOSED: Litigation Authorization; General Counsel Recommendations.

Note.—Any matter not discussed or concluded may be carried over to a later meeting. (In addition to publishing notices on EEOC Commission meetings in the Federal Register, the Commission also provides a recorded announcement a full week in advance on future Commission sessions. Please telephone (202) 634–6748 at all times for information on these meetings).

CONTACT PERSON FOR MORE INFORMATION: Cynthia C. Matthews, Executive Officer, Executive Secretariat at (202) 634–6748.

Dated: September 25, 1985.

Cynthia C. Matthews,

Executive Officer, Executive Secretariat.

This Notice Issued September 25, 1985.

[FR Doc. 85–23267 Filed 9–25–85; 3:23 pm]

BILLING CODE 6750-06

4

FEDERAL DEPOSIT INSURANCE CORPORATION

Notice of Changes in Subject Matter of Agency Meeting.

Purusant to the provisions of subsection (e)(2) of the "Government in the Sunshine Act" (5 U.S.C. 552b(e)(2)), notice is hereby given that at its open meeting held at 2:00 p.m. on Monday, September 23, 1985, the Corporation's Board of Directors determined, on motion of Chairman William M. Isaac, seconded by Mr. Michael A. Mancusi, acting in the place and stead of Director H. Joe Selby (Acting Comptroller of the Currency), that Corporation business required the withdrawal from the agenda for consideration at the meeting, on less than seven days' notice to the public, of the following matter:

Application of Metropolitan Bank St. Paul, St. Paul, Minnesota, an insured State nonmember bank, for consent to purchase the assets of and assume the liability to pay deposits made in Metro Thrift Company, Inc., St. Paul, Minnesota, a non-FDIC-insured institution.

The Board further determined, on motion of Chairman William M. Isaac, seconded by Mr. Michael A. Mancusi, acting in the place and stead of Director H. Joe Selby (Acting Comptroller of the Currency), that Corporation business required the addition to the agenda for consideration at this meeting, on less than seven days' notice to the public, of the following matter:

Application of Bank of Dodge County, Chester, Georgia, an insured State nonmember bank, for consent to merge, under its charter and title, with Colony Interim, Inc., Fitzgerald, Georgia, in organization.

By the same majority vote, the Board further determined that no earlier notice of these changes in the subject matter of the meeting was practicable.

Dated: September 24, 1985.

Federal Deposit Insurance Corporation.

Hoyle L. Robinson,

Executive Secretary.

[FR Doc. 85–23243 Filed 9–25–85; 12:51 pm]

BILLING CODE 6714-01-M

5

FEDERAL DEPOSIT INSURANCE CORPORATION

Notice of Changes in Subject Matter of Agency Meeting.

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[1] In this matter the Commission decided to waive its rules concerning outside participation in their meetings and will permit the panel representative to take part in the discussion.

Pursuant to the provisions of subsection (e)(2) of the "Government in the Sunshine Act" (5 U.S.C. 552b(e)(2)), notice is hereby given that at its closed meeting held at 2:30 p.m. on Monday, September 23, 1985, the Corporation's Board of Directors determined, on motion of Chairman William M. Isaac, seconded by Mr. Michael A. Mancusi, acting in the place and stead of Director H. Joe Selby (Acting Comptroller of the Currency), that Corporation business required the addition to the agenda for consideration at the meeting, on less than seven days' notice to the public, of the following matters:

Application of Standard Chartered Bank, London, England, for Federal deposit insurance of deposits received at and recorded for the accounts of its branch located at 900 Fourth Avenue, Suite 1515, Seattle, Washington.

Application of Bank of China, Beijing, People's Republic of China, for Federal deposit insurance of deposits received at and recorded for the accounts of its branch to be located at 42–44 East Broadway, New York, New York.

The Board further determined, by the same majority vote, that no earlier notice of these changes in the subject matter of the meeting was practicable; that the public interest did not require consideration of the matters in a meeting open to public observation; and that the matters could be considered in a closed meeting by authority of subsections (c)(6), (c)(8), and (c)(9)(A)(ii) of the "Government in the Sunshine Act" (5 U.S.C. 552b(c)(6), (c)(8), and (c)(9)(A)(ii)).

Dated: September 24, 1985.

Federal Deposit Insurance Corporation.

Hoyle L. Robinson,

Executive Secretary.

[FR Doc. 85-23244 Filed 9-25-85; 12:51 pm]

BILLING CODE 6714-01-M

6

FEDERAL RESERVE SYSTEM

"FEDERAL REGISTER" CITATION OF PREVIOUS ANNOUNCEMENT: 50 FR 38243, September 20, 1985.

PREVIOUSLY ANNOUNCED TIME AND DATE OF THE MEETING: 10:00 a.m., Wednesday, September 25, 1985.

CHANGES IN THE MEETING: One of the items announced for inclusion at this meeting was consideration of any agenda items carried forward from a previous meeting; the following such closed item(s) was added:

Federal Reserve Bank and Branch director appointments. (This item was originally announced for a closed meeting on September 16, 1985.)

CONTACT PERSON FOR MORE INFORMATION: Mr. Joseph R. Coyne, Assistant to the Board; (202) 452–3204.

Dated: September 25, 1985.

James McAfee,

Associate Secretary of the Board.

[FR Doc. 85-23269 Filed 9-25-85; 3:39 pm]

BILLING CODE 6210-01-M

7

NATIONAL CREDIT UNION ADMINISTRATION

TIME AND DATE: 9:30 a.m., Wednesday, October 2, 1985.

PLACE: 1776 G Street, NW., Washington, DC, Filene Board Room.

STATUS: Closed.

MATTERS TO BE CONSIDERED:

1. Administrative Action under Section 206 of the Federal Credit Union Act. Closed pursuant to exemptions (8) and (9)(A)(ii).

2. Reports to the Board. Closed pursuant to exemptions (8) and (9)(A)(ii).

3. Personnel Actions. Closed pursuant to exemptions (2) and (6).

FOR MORE INFORMATION CONTACT: Rosemary Brady, Secretary of the Board, Telephone (202) 357–1100.

Rosemary Brady,

Secretary of the Board.

[FR Doc. 85-23246 Filed 9-25-85; 1:20 pm]

BILLING CODE 7535-01-M

8

PACIFIC NORTHWEST ELECTRIC POWER AND CONSERVATION PLANNING COUNCIL

ACTION: Addition of item to meeting agenda.

DATE OF MEETING: September 18–19, 1985.

PLACE: Council offices, Suite 1100, 850 SW Broadway, Portland, Oregon.

SUMMARY: The Government in the Sunshine Act, 5 U.S.C. 552b, requires **Federal Register** notice whenever an agency adds an item to its meeting agenda after the meeting has been publicly announced. At its September 18, 1985 meeting in Portland, Oregon, the Northwest Power Planning Council voted unanimously to add "Consideration of Model Conservation Standards Rulemaking" to the agenda for its September 19, 1985 meeting. The Council also found that Council business required the addition and that no earlier announcement of the addition was possible.

CONTACT PERSON FOR MORE INFORMATION: Ms. Bess Atkins, (503) 222–5161, 1–800–222–3355 (toll-free in Idaho, Montana and Washington) or 1–800–452–2324 (toll-free in Oregon).

Rich Applegate,

Executive Assistant.

[FR Doc. 85-23222 Filed 9-25-85; 10:18 am]

BILLING CODE 0000-00-M

federal register

Friday
September 27, 1985

Part II

Environmental Protection Agency

40 CFR Parts 796, 797, and 798
Toxic Substances Control Act Test Guidelines; Final Rules

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Parts 796, 797, and 798

FRL 2896-1

[OPTS-46014]

Toxic Substances Control Act Test Guidelines

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: This rule introduces Parts 796, 797, and 798 which consist of Toxic Substances Control Act (TSCA) test guidelines. These guidelines were previously prepared for publication by EPA. Elsewhere in this issue of the **Federal Register** certain test guidelines, which have been published as Organization for Economic Cooperation and Development (OECD) Guidelines for the Testing of Chemicals are also codified as additional TSCA test guidelines.

EFFECTIVE DATE: Effective on September 27, 1985.

FOR FURTHER INFORMATION CONTACT: Edward A. Klein, Director, TSCA Assistance Office (TS-799), Office of Toxic Substances, Rm. E-543, 401 M St., SW., Washington, D.C. 20460. Toll free: (800-424-9065). In Washington, D.C.: (554-1404). Outside the USA: (Operator-202-554-1404).

SUPPLEMENTARY INFORMATION: This final rule codifies TSCA test guidelines which were previously prepared for publication by EPA. Elsewhere in this issue of the **Federal Register**, certain test guidelines which have been published by OECD are also codified as TSCA test guidelines.

Section 4(b)(1) of the Toxic Substances Control Act (TSCA) specifies that test rules shall include standards for the development of test data. This action codifies guidelines which may be used to establish test standards in future TSCA section 4 test rules.

These guidelines, which are now introduced into Parts 796 (chemical fate), 797 (environmental effects), and 798 (health effects) of 40 CFR, have been previously published or prepared for publication by EPA. These guidelines present generally formulated procedures for laboratory testing of an effect or characteristic deemed important for the evaluation of health and environmental hazards of a chemical. The TSCA guidelines have been rigorously peer reviewed prior to publication. Most of these guidelines have also been offered for public comment (44 FR 27334, May 9, 1979; 44 FR 44054, July 26, 1979; 45 FR 75753, November 21, 1980; 47 FR 13013, March 26, 1982). The Agency reviews and updates its TSCA test guidelines once a year according to the process described in the **Federal Register** of September 22, 1982 (47 FR 41857).

The Agency had originally planned to incorporate by reference the pertinent TSCA guidelines into the chemical specific test rules. However, under the provisions of 1 CFR Part 51, material generated by a particular government agency is not usually acceptable for incorporation by reference unless the material meets certain criteria set forth in 1 CFR 51.7 (a) and (b). Because the Director of the Federal Register has rejected the OTS request for permission to incorporate by reference the TSCA guidelines, the Agency finds it necessary to codify these guidelines into the Code of Federal Regulations with no substantive changes. This codification will make chemical-specific rules under Part 799 more usable and understandable. It is expected that modification of existing guidelines and addition of new guidelines will occur later as the state-of-the-art evolves or the need for them warrants. Elsewhere in this issue of the **Federal Register** certain OECD guidelines are also codified into the TSCA test guidelines.

Codification of these guidelines does not impose any regulatory obligation on any person who may be subject to a TSCA section 4 test rule. Specific guidelines will not become mandatory test standards until they are promulgated as such in individual section 4 rulemakings. When promulgated in such test rules, the pertinent TSCA guidelines will become test standards for only that particular section 4 rule and will not serve as generic test standards. EPA may propose modifications to the various guidelines as they are utilized for chemical-specific test rules. In each chemical-specific rule, the proposed test standards and any modifications will be subject to public comment.

List of Subjects in 40 CFR Parts 796, 797, and 798

Testing, Environmental protection, Chemical fate, Environmental effects, Health effects, Chemicals.

Dated: September 23, 1985.

John A. Moore

Assistant Administrator for Pesticides and Toxic Substances.

Therefore, 40 CFR Chapter I is amended by adding new Parts 796, 797, and 798 to read as follows:

PART 796—CHEMICAL FATE TESTING GUIDELINES

Subpart A—[Reserved]

Subpart B—Physical and Chemical Properties

Sec.

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Subpart A—[Reserved]

Subpart B—Physical and Chemical Properties

§ 796.1550 Partition Coefficient (n-Octanol/Water).

(a) *Introduction*—(1) *Background and purpose.* (i) Bioconcentration, the accumulation of a substance in living tissues or other organic matter as a result of net chemical uptake from the medium (e.g., water), is a factor in determining the movement of a chemical in the environment and the potential effects of the chemical on biota. Hydrophobic chemicals that are present in the aqueous environment at subtoxic concentrations may accumulate to toxic levels once inside organisms, presumably through diffusion into nonpolar cell components, where they accumulate because of their greater solubility. Further movement of the substance in living tissues may occur as a result of ingestion of lower trophic level organisms, i.e., food chain effects.

(ii) The tendency of an organic chemical to bioconcentrate in living cells can be inferred from the value of the octanol/water partition coefficient.

K_{ow} (Neely et al. 1974) under paragraph (d)(5) of this section. Chemicals with K_{ow} less than 10 will not significantly partition into, or tend to accumulate in, living cells. Chemicals with K_{ow} greater than 10^6 will tend to accumulate. Chemicals that exist in the environment at subtoxic levels may bioconcentrate to toxic levels once inside organisms.

(iii) This test guideline describes a detailed and commonly used procedure for determining the octanol/water partition coefficient of organic chemicals.

(2) *Definitions and units.* (i) The octanol/water partition coefficient (K_{ow}) is defined as the equilibrium ratio of the molar concentrations of a chemical in n-octanol and water, in dilute solution. K_{ow} is a constant for a given chemical at a given temperature. Since K_{ow} is the ratio of two molar concentrations, it is a dimensionless quantity. Sometimes K_{ow} is reported as log 10 K_{ow}. The mathematical statement of K_{ow} is:

Equation 1

$$K_{ow} = \frac{C_{octanol}}{C_{water}}$$

where C is the molar concentration of the solute in n-octanol and water at equilibrium at a given temperature.

(ii) The distribution law applies only to individual molecular species in solution. If a molecule dissociates or associates in octanol and water, than equation 1 under paragraph (a)(2)(i) of this section must be modified. In general, if α represents the fraction of the total solute that is dissociated or associated, assuming that either association or dissociation occurs in each solvent, then

Equation 2

$$K_{ow} = \frac{(1-\alpha_{oct.})\,C_{oct.}}{(1-\alpha_{water})\,C_{water}}$$

since $(1-\alpha)$ gives the fraction of unchanged molecules in each phase. For the special case where no association takes place in octanol, equation 2 reduces to

Equation 3

$$K_{ow} = \frac{C_{oct.}}{(1-\alpha_{water})\,C_{water}}$$

where α water represents the fraction of the total solute that has dissociated in water.

(3) *Principle of the test method.* The conventional method for determining the octanol/water partition coefficient is carried out by distributing a chemical between n-octanol and water in a vessel at constant temperature and measuring the concentration in the two liquid phases after equilibration (e.g., Fujita et al. 1964 under paragraph (d)(2) of this section; Hansch and Anderson 1967 under paragraph (d)(3) of this section; Leo et al. 1971 under paragraph (d)(4) of this section; Chiou et al. 1977 under paragraph (d)(1) of this section).

(4) *Applicability and specificity.* The test guideline is designed to determine the octanol/water partition coefficient of solid or liquid organic chemicals in the range 10 to 10^6. For chemicals whose values lie outside this range, K_{ow} should be characterized as less than 10 or greater than 10^6 with no further quantification.

(b) *Test procedures*—(1) *Test conditions*—(i) *Special laboratory equipment.* (A) A thermostatic bath, chamber, or room with a shaker and temperature control as specified in Temperature Control below.

(B) An ultracentrifuge with temperature control as specified in Temperature Control below.

(C) Stainless steel or glass centrifuge tubes with sealable caps. Special glass centrifuge tubes can be used up to approximately 12,000 G and stainless steel tubes can be used at high G values.

(D) A mechanical shaker.

(E) A pH meter capable of resolving differences of 0.1 pH unit or less.

(ii) *Temperature control.* It is recommended that the temperature of the water bath, or chamber, or room, and the ultracentrifuge be controlled to (25±1) °C.

(iii) *Solvents.* It is extremely important that n-octanol, purified as described in paragraph (b)(2)(i)(A) of this section and distilled or reagent grade water, i.e., ASTM Type II water or an equivalent grade, be used. ASTM Type II water is described in ASTM D1193–77, "Standard Specification for Reagent Water." ASTM D1193–77 is available for inspection at the Office of the Federal Register, Rm. 8401, 1100 L St., NW., Washington, DC. This incorporation by reference was approved by the Director of the Office of the Federal Register. This material is incorporated as it exists on the date of approval and a notice of any change in this material will be published in the **Federal Register**. Copies of the incorporated material may be obtained from the Document Control Officer (TS–793), Office of Toxic Substances, EPA, Rm. 107, 401 M St., SW., Washington, DC 20460, and from the American Society for Testing and Materials (ASTM), 1916 Race Street, Philadelphia, PA 19103.

(iv) *Concentration of solute.* It is extremely important that all experiments be carried out at solute concentration $C < 0.01M$ (Molar) in octanol and water and well below the solubility in either phase.

(v) *Equilibration time.* In general, 1 hour of gentle agitation is sufficient to reach equilibrium. For surfactants, at least 16 hours is required to reach equilibrium.

(vi) *Octanol/water volume ratio.* It is recommended that the ratio of the volumes of the two liquids be adjusted as appropriate for the relative solubility of the chemical in octanol and water. By adjusting the volumes, concentration errors (resulting from analytical errors) are minimized and errors resulting from dividing large numbers by small numbers are kept to a minimum.

(vii) *Chemical analysis of the octanol and water phases.* In determining the K_{ow} value for any given solute, it is important that both the octanol and water phases be analyzed for the chemical. An analytical method should be selected that is most applicable to the analysis of the specific chemical. Chromatographic methods are preferable because of their compound specificity in analyzing the parent chemical without interference from impurities. Whenever practicable, the chosen analytical method should have a precision with ±5 percent

(viii) *Emulsification and ultracentrifugation.* It is important that gentle shaking be used to minimize the formation of emulsions. Ultracentrifugation is necessary to separate troublesome emulsions and to separate the octanol and water phases. Therefore, it is very important that ultracentrifugation be carried out at 25 °C for 20 minutes in a temperature controlled ultracentrifuge. The acceleration (G) value required to break the emulsion and to achieve complete separation of the octanol and water phases can be determined by trial-and-error experimentation.

(ix) *Equilibration vessel.* (A) If feasible, equilibration should be carried out in a centrifuge tube (stainless steel or glass) with a sealable cap. It is important that the centrifuge tubes be almost completely full. In this way, partitioning with air will be minimized, especially for volatile chemicals, and the mixture will be completely mixed.

(B) Very hydrophobic chemicals, with K_{ow} in the order of 10^4 to 10^6, require relatively large volumes of the aqueous phase. Hence, for these chemicals, it is recommended that equilibration be carried out in a large ground-glass stoppered flask.

(x) *Speciation effects.* (A) The octanol/water partition coefficient, K_{ow}, has been defined in paragraph (a)(2) of this section. The mathematical statement of K_{ow} is given by equation (1) under paragraph (a)(2)(i) of this section.

(B) If the chemical does not associate or dissociate in octanol and water, then use equation 1 under paragraph (a)(2)(i) of this section and determine K_{ow} at molar concentrations $C<0.01M$ and $C_1=0.01C$.

(C) If the chemical associates in octanol or water or in both liquids, then use equation 1 under paragraph (a)(2)(i) of this section and determine K_{ow} at molar concentrations $C<0.01M$, $C_1=0.1C$, $C_2=0.01C$, $C_3=0.001C$ . . . . When K_{ow} is constant at two molar concentrations differing by a factor of 10, then the effect of association has been minimized or eliminated.

(D) If a molecule dissociates or associates in octanol and water, then it is extremely important that equation 1 under paragraph (a)(2)(i) of this section be modified to take into account such speciation changes as ionization, aggregation, and hydration. For the special case, where no association takes place in octanol and only dissociation takes place in water, equation 3 under paragraph (a)(2)(ii) of this section can be used. For chemicals that reversibly ionize or pronate (e.g., carboxylic acids, phenols, or anilines), use equation 3 under paragraph (a)(2)(ii) of this section with water buffered at pH 5.0, 7.0, and 0.0. It is recommended that buffers described in paragraph (b)(2)(i)(B) of this section be used.

(xi) *Prerinsing of all transfer vessels.* It is important that all transfer vessels be prerinsed with a portion of the equilibrium phase prior to transfer for analysis. This is especially important for very hydrophobic chemicals.

(2) *Preparations*—(i) *Reagents and Solutions*—(A) *Octanol and Water.* Very pure *n*-octanol can be obtained as follows: wash pure *n*-octanol (minimum 98 percent pure) sequentially with 0.1N H_2SO_4, with 0.1N NaOH, then with distilled water until neutral. Dry the *n*-octanol with magnesium sulfate and distill twice in a good distillation column under reduced pressure (b.p. about 80 °C at 0.27 kPa (2 torr)). It is important that the octanol produced be at least 99.9 percent pure. Alternatively, a grade equivalent to Fisher Scientific Co. No. A-402 "Certified Octanol-1" can be used. It is important that distilled or reagent grade (ASTM Type II) water be used.

(B) *Buffer solutions.* Prepare buffer solutions using reagent grade chemicals in distilled or reagent grade water as follows:

pH 5.0—To 250 mL of 0.1M potassium hydrogen phthalate add 113 mL of 0.1M sodium hydroxide; adjust final volume to 500 mL with reagent grade water.

pH 7.0—To 250 mL of 0.1 potassium dihydrogen phosphate add 145 mL of 0.1M sodium hydroxide; adjust final volume to 500 mL with reagent grade water.

pH 9.0—To 250 mL of 0.07M borax add 69 mL of 0.1M HCl; adjust final volume to 500 mL with reagent grade water.

Check the pH of each buffer solution at 25 °C with a pH meter and adjust to pH 5.0, 7.0, or 9.0, if necessary.

(C) *Presaturation of the solvents.* Before a partitioning experiment is carried out, prepare octanol saturated with water and water saturated with octanol. Add purified *n*-octanol to a large stock bottle and sufficient distilled water to saturate it. Shake the flask for 24 hours on a mechanical shaker. Then allow sufficient time for the mixture to stand so that the two phases separate. Repeat this procedure using another large stock bottle containing distilled water and sufficient octanol to saturate it. The desired quantities of the presaturated solvents can be taken from these stock bottles for each partition experiment.

(D) *Preparation of test solution.* Prepare a 10^{-2} to 10^{-3} M solution of the test material in octanol.

(3) *Performance of the test.* (i) Add a small volume of the octanol test solution (1 to 5 mL) to a centrifuge tube with a sealable cap as described in paragraph (b)(1)(ix) of this section.

(ii) Add the required volume of water to the centrifuge tube as described in paragraph (b)(1)(vi) of this section. The volume of water required is variable, depending upon the amount of chemical required for the analysis. Generally, 20–40 mL of water should be sufficient. Make sure that the centrifuge tube is almost completely full. In this way, partitioning with air will be minimized. This is important, especially when determining K_{ow} for volatile chemicals.

(iii) Equilibrate the samples at 25 °C in constant temperature bath, chamber, or room by gently shaking the centrifuge tube for 1 hour. Avoid vigorous shaking that may cause troublesome emulsions to form. For surfactants, a minimum of 16 hours of shaking is required as described in paragraph (b)(1)(v) of this section.

(iv) Centrifuge the samples at 25 °C for 20 minutes to break any emulsion and to separate the octanol and water phases. Evidence for breaking the emulsion and separation of the water and octanol phases can be obtained using a turbidimeter. The acceleration (G) value required to break the emulsion and to achieve complete separation of the octanol and water phases can be determined by trial-and-error experimentation.

(v) Sample the octanol and water phases as follows:

(A) Withdraw by pipet a known volume of the octanol phase (approximately ½ or less of the total octanol phase) and transfer to an analysis cell or diluting solvent. Before transferring the aliquot of the octanol phase, wipe the outside of the pipet with a paper tissue.

(B) Remove by pipet the remainder of the octanol phase including the interfacial layer and discard.

(C) Insert another clean pipet close to the bottom of the centrifuge tube and carefully withdraw a known volume of the aqueous phase. Wipe the bottom exterior part of the pipet with a tissue and discharge the aqueous sample directly into an analysis cell or extraction solvent. Do not allow the extraction solvent to contact the pipet stem.

(vi) Select an analytical method that is most applicable to the analysis of the specific chemical as described in paragraph (b)(1)(vii) of this section. Determine the concentration in the octanol and water phases. Express the concentration of the chemical in octanol and water in moles/liter (M).

(vii) Determine the partition coefficient in triplicate (steps i through vii) at two concentrations of the test material $C<0.01M$ and $C_1=0.1C$ as described in paragraph (b)(1)(x) of this section. If K_{ow} is not constant at C and C_1, then association effects should be considered. Therefore, follow steps 1 through 7 at lower concentrations until K_{ow} is constant at two concentrations differing by a factor of 10 as described in paragraph (b)(1)(x) of this section.

(viii) Very hydrophobic chemicals (with K_{ow} on the order of 10^4 to 10^6) required relatively large volumes of the aqueous phase as described in paragraph (b)(1)(vi) and (ix) of this section. Hence, for very hydrophobic materials, equilibrate the octanol and water phases in a large ground-glass stoppered flask as described above in step (iii). For the final phase separation, transfer the two phase mixture to centrifuge tubes that have been prerinsed with some of the aqueous phase; centrifuge as described in step (iv); withdraw aliquots from each centrifuge tube as described in step (v); and recombine for analysis.

[Note.—Prerinse all transfer tubes with the water phase.] Complete steps (vi) and (vii) to determine K_{ow}.

(ix) For materials that reversibly ionize or protanate, determine K_{ow} at pH 5.0, 7.0, and 0.0 as described in paragraph (b)(1)(x) of this section. Follow steps (i) through (vii) using the buffered aqueous solutions described in paragraph (b)(2)(i)(B) of this section. Using the acid dissociation constant and the concentration of the chemical in the aqueous phase [C_{water}], the term can be calculated. The concentration of undissociated chemical can be determined from α and C_{water}.

(c) *Data and reporting*— (1) *Test report.* For each individual determination, report the octanol/water partition coefficient at each concentration of the test substance, including the molar concentration of chemical in each phase [C_{oct} and C_{water}]. In addition, report the mean value of K_{ow}, and the standard deviation.

(2) *Specific analytical and recovery procedures.* (i) Provide a detailed description or reference for the analytical procedure used, including the calibration data and precision; and (ii) if extraction methods were used to separate the solute from the octanol and aqueous phases, provide a description of the extraction data.

(3) *Other test conditions.* Report the experimental (G) value required to break the emulsion and to achieve separation of the octanol and water phases.

(d) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Chiou, C.T., Freed, V.H., Schmedding, D.W., Kohnert, R.L. "Partition Coefficient and Bioaccumulation of Selected Organic Chemicals," *Environmental Science and Technology*, 11:475 (1977).

(2) Fujita, T., Iwasa, J., Hansch, C. "A New Substituent Constant Derived from Partition Coefficients," *American Chemical Society Journal*, 86:5175 (1964).

(3) Hansch, C., Anderson, S.M., "The Effect of Intermolecular Hydrophobic Bonding on Partition Coefficients," *Journal of Organic Chemistry*, 23:2583 (1977).

(4) Leo, A., Hansch, C., Elkins, D. "Partition Coefficients and Their Uses," *Chemical Reviews*, 71:525 (1971).

(5) Neely, W.B., Branson, D.R., Blau, G.E. "Partition Coefficient to Measure Bioconcentration Potential of Organic Chemicals in Fish," *Environmental Science and Technology*, 8:113 (1974).

§ 796.1570 Partition Coefficient (n-Octanol/Water)—Estimation by Liquid Chromatography.

(a) *Introduction*—(1) *Background and purpose.* (i) Since the pioneering work of Hansch and Fujita (1964) under paragraph (d)(1) of this section in the measurement and estimation of the octanol/water partition coefficient (K_{ow}), this property has become the cornerstone of a myriad of structure-activity relationships (SAR). Hansch and Leo (1979) under paragraph (d)(2) of this section have used the coefficient extensively for correlating structural changes in drugs with changes observed in biological, biochemical or toxic effects. These correlations are then used to predict the effect of a new drug for which a K_{ow} could be measured.

(ii) In the study of the environmental fate of organic chemicals, the coefficient has become a key parameter. It has been shown to be correlated to water solubility, soil/sediment adsorption coefficient, and bioconcentration. The importance of this property to SAR is indicated by its discussion in the first chapter of Lyman, Reehl and Rosenblatt's (1982) under paragraph (d)(3) of this section comprehensive compendium of methods for estimating the behavior of organic compounds in the environment. These authors consider the measurement or estimation of the coefficient to be the necessary first step in assessing the fate of new chemicals.

(iii) Of the three properties that can be estimated from K_{ow}, water solubility is the most important because it affects both the fate and transport of chemicals. For example, highly soluble chemicals become quickly distributed by the hydrologic cycle, have low adsorption coefficients for soils and sediments, and tend to be more easily degraded by microorganisms. In addition, chemical transformation processes such as hydrolysis and oxidation tend to occur more readily if a compound is soluble.

(iv) Direct correlations between K_{ow} and both the soil/sediment adsorption coefficient and the bioconcentration factor are to be expected. In these cases compounds that are more soluble in octanol (more hydrophobic) would be expected to partition out of the water and onto the organic portion of soils/sediments and into lipophilic tissue. The relationship between K_{ow} and the bioconcentration factor, as developed by Neely et al. (1974) under paragraph (d)(4) of this section, and other similar relationships, are the principal means of estimating bioconcentration factors. These factors are then used to predict the potential for a chemical to accumulate in living tissue. As a rough estimate, chemicals with K_{ow} less than 10 will not accumulate in tissue while those with K_{ow} greater than 10^6 will. Thus, although a chemical may be present in the aqueous environment at subtoxic concentrations, if its K_{ow} is greater than 10^6 it would accumulate to levels that may be toxic not only to the organism but also to the consumers of that organism.

(v) This test guideline describes a rapid, inexpensive method based on reverse phase-high pressure liquid chromatography (RP-HPLC) for estimating the octanol/water partition coefficient as developed by Veith et al. (1979) under paragraph (d)(5) of this section. It is not intended, however, to replace the standard shake-flask method described in § 796.1550, and should be used keeping in mind the limitations described herein. The RP-HPLC method is intended to give quick estimates of K_{ow}, particularly for very hydrophobic substances and mixtures that cannot be analyzed using the shake-flask method.

(2) *Definitions.* (i) The octanol/water partition coefficient (K_{ow}), as estimated by this test method, is the ratio of the equilibrium molar concentrations of a chemical in n-octanol and water, in dilute solution; as such it is a dimensionless quantity. K_{ow} is a constant for a given chemical at a given temperature. Because K_{ow} can assume such a wide range of values, from less than one to greater than a million, depending on the structure of the compound, K_{ow} is often reported as log K_{ow}.

(ii) The retention time, t_R, is the time in minutes elapsed between sample injection into the chromatograph and the peak maximum (concentration) as recorded on a chromatogram. The retention time is characteristic of the substance, the liquid phase flow rate, and the stationary phase, at a given temperature. With proper flow and temperature control, it can be reproduced to within one percent and used to identify multiple peaks. Although several substances can have nearly identical retention times, each substance has only one retention time. This retention time is not influenced by the presence of other components. Retention times for this method vary between several minutes for substances with a lower K_{ow} to thirty minutes or greater for substances with higher K_{ow}'s.

(3) *Principle of the test method.* This test method is based on a reverse-phase high pressure chromatographic (HPLC) separation procedure developed by Veith et al. (1979) under paragraph (d)(5)

of this section. The test substance (solute) is injected onto an HPLC column containing a support onto which a long-chain hydrocarbon has been permanently bonded. A methanol/water solvent system is used to elute the solute which is subsequently analyzed using an ultraviolet absorption detector, gas chromatograph, liquid scintillator or other suitable detector. During elution, the solute moves alone the column by partitioning between the mobile phase and the stationary hydrocarbon phase. The retention time on the column is a function of the hydrophobicity of the solute: A water soluble solute has a short retention time while a hydrophobic solute has a long retention time. Once the retention time is measured on the chromatogram, the K_{ow} of the substance is estimated from a previously established linear regression equation between log t_R and log K_{ow}. The relationship between these two variables is determined through a calibration step that involves injecting into the chromatograph a mixture of six reference chemicals having a range of retention times and known octanol/water partition coefficients. The retention time for each chemical is measured and a plot of log t_R *vs.* log K_{ow} is made. The data are also correlated using a linear regression and the resulting equation is used to calculate log K_{ow} from the log t_R of test substances; the correlation coefficient of the linear regression gives a measure of the "goodness of fit" of the calibration data to a straight line.

(4) *Applicability and specificity.* The test method described in this guideline is designed to calculate an estimated value of the octanol/water partition coefficient using an empirically derived equation that relates the K_{ow} of a substance to its experimentally determined retention time on a HPLC column. It must be emphasized that the shake-flask method in § 796.1550 remains the conventional method for determining K_{ow}. The HPLC method described herein is a rapid procedure for estimating log K_{ow} for a single substance or a mixture of substances. Estimates of log K_{ow} should be limited to within two log units of the minimum and maximum values of the calibration substances, i.e., the method is applicable to substances with log K_{ow} between zero and eight. In the range of two to six log units, estimates are within 22.5±20.1 percent of the values reported in the literature obtained using other methods (Veith et al. 1979) under paragraph (d)(5) of this section.

(b) *Test procedures*—(1) *Test conditions*—(i) *Special laboratory equipment.* (A) A liquid chromatograph equipped with a 6000 psi pump, a high-pressure stopflow injector, and appropriate recorder.

(B) A preparative scale reverse phase column (250 mm × 8 mm under paragraph (a)(4) of this section), e.g., Varian Preparative Micropak C–H, consisting of a stainless steel tube filled with 10 micron LiChrosprb to which octadecylsilane is permanently bonded.

(C) For chemicals that absorb in the ultraviolet (i.e., aromatics), either 254–nm fixed wavelength detector or 190 to 600 nm variable wavelength detector, can be used. For chemicals that cannot be detected in the ultraviolet, a fraction collector can be used to collect fractions at suitable intervals (0.50 to 1.0-minutes near the retention time) for analysis by gas chromatography, liquid scintillation, or other suitable, sensitive, analytical detector.

(ii) *Purity of solvents and reagents.* All solvents (water, methanol, acetone, and cyclohexane) and reagents used in this test procedure should be reagent or HPLC grade and contain no impurities that could interfere with the determination of the retention time of the test compound. Water meeting ASTM Type II standards or an equivalent grade is recommended to minimize the effects of dissolved salts and other impurities. ASTM Type II water is described in ASTM D 1193–77, "Standard Specification for Reagent Water". ASTM D 1193–77 is available for inspection at the Office of the Federal Register, Rm. 8401, 1100 L St., NW., Washington, DC. This incorporation by reference was approved by the Director of the Office of the Federal Register. This material is incorporated as it exists on the date of approval and a notice of any change in this material will be published in the **Federal Register.** Copies of the incorporated material may be obtained from the Document Control Officer (TS–793), Office of Toxic Substances, EPA, Rm. 107, 401 M St., SW., Washington, DC 20460, and from the American Society for Testing and Materials (ASTM), 1916 Race Street, Philadelphia, PA 19103.

(2) *Preparation of reagents and solutions*—(i) *Solvents.* For column elution and preparation of buffers, mix chromatographic or reagent grade methanol and water in an 85:15 v/v ratio.

(ii) *Calibration mixture.* Prepare a standard calibration solution containing 200 mg/L of each of the substances listed in the following Table 1 dissolved in acetone and cyclohexane (3:1 v/v) other suitable solvent. Twenty microliters of this solution injected into the chromatograph should give an adequate recorder response (25 percent of scale) for calibration purposes. However, both the concentration and amount injected may be increased or decreased without affecting the retention times, since t_R is independent of concentration in dilute solutions.

TABLE 1—MEASURED OCTANOL/WATER PARTITION COEFFICIENTS AND TYPICAL HPLC RETENTION TIMES FOR THE CHEMICALS USED IN THE CALIBRATION MIXTURE

Chemical	Literature log K_{ow}	Measured typical retention time	
		Minutes	Log minutes
Benzene	2.13	4.12	0.61
Bromobenzene	2.99	7.09	0.85
Biphenyl	3.76	8.85	0.95
Bibenzyl	4.81	15.87	1.20
p,p'-DDE	5.69	21.98	1.34
2,4,5,2',5'-PCB	6.11	31.58	1.50

(iii) *Test solution.* Solutions of the test substance(s) are prepared similar to the calibration mixture: by dissolving the substance(s) to be tested in a 3:1 mixture of acetone and cyclohexane. The concentration of the substance(s), as determined by trial and error, should be sufficient to produce a chromatographic peak of at least 25 percent of the recorder scale.

(3) *Performance of the test.* (i) After conditioning the column with 85:15 methanol-water or buffered methanol-water, chromatograph the calibration mixture by injecting 20 microliters of the mixture into the column. Elute the column using a solvent flow rate of about 2.0 ml/min at a pressure of approximately 1200 psi. Determine the retention time for each substance in the mixture. The calibration mixture must be chromatographed daily because the retention time is sensitive to variations in the flow rate, temperature, solvent ratio, and the retention properties of the column.

(ii) Chromatograph 20 microliters of the test solution(s) directly following column calibration, using an identical flow rate and pump pressure. Determine the retention time(s) for the test substance(s).

(c) *Data and reporting*—(1) *Test report.* (i) Using the measured retention times of the substances in the calibration mixture and the log K_{ow} for each substance in Table 1 under paragraph (b)(2)(ii) of this section make a plot of log t_R *vs.* log K_{ow}. From the data used in making this plot compute a linear regression equation of the form:

$$\log K_{ow} = m \log t_R + b$$

i.e., $y = mx + b$. For each set of test conditions (flow rate, pressure) report this equation, its correlation coefficient and the data used in its calculation.

(ii) Calculate an estimated log K_{ow} for each test substance from its retention time and corresponding regression equation. Report the retention time, its logarithm and log K_{ow} along with the above data.

(2) *Specific analytical procedures.* (i) Provide a detailed description of, or reference for, the liquid chromatograph, separation column, and detector.

(ii) Report the temperature at which the test(s) were conducted.

(iii) Give a description of any problems (and their rectification) or changes in the test procedures.

(d) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Hansch, C., Fujita, T. "A Method for the Correlation of Biological Activity and Chemical Structure," *American Chemical Society Journal*, 86:1616 (1964).

(2) Hansch, C., Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*. (J. Wiley & Sons, New York, 1979).

(3) Lyman, W.J., Reehl, W.F., Rosenblatt, D.H. *Handbook of Chemical Property Estimation Methods: Environmental Behavior of Organic Compounds*. (McGraw-Hill Book Co., New York, 1982).

(4) Neely, W.B., Branson, D.R., Blau, G.E. "Partition Coefficient to Measure Bioconcentration Potential of Organic Chemicals in Fish," *Environmental Science Technology*, 8:113 (1974).

(5) Veith, G.D., Austin, N.M., Morris, R.T. "A Rapid Method for Estimating Log P for Organic Chemicals," *Water Research*, 13:43 (1979).

§ 796.1720 Octanol/Water Partition Coefficient, Generator Column Method.

(a) *Introduction*—(1) *Background and purpose.* (i) Since the pioneering work of Fujita and Hansch (1964) under paragraph (d)(2) of this section in the measurement and estimation of the octanol/water partition coefficient (K_{ow}), this property has become the cornerstone of a myriad of structure-activity relationships (SAR). Hansch and Leo (1979) under paragraph (d)(3) of this section have used the coefficient extensively for correlating structural changes in drugs with changes observed in biological, biochemical or toxic effects. These correlations are then used to predict the effect of a new drug for which a K_{ow} could be measured.

(ii) In the study of the environmental fate of organic chemicals, the coefficient has become a key parameter. It has been shown to be correlated to water solubility, soil/sediment sorption coefficient, and bioconcentration. The importance of this property to SAR is indicated by its discussion in the first chapter of Lyman, Reehl and Rosenblatt's (1982) under paragraph (d)(4) of this section comprehensive compendium of methods for estimating the behavior of organic compounds in the environment. These authors consider the measurement or estimation of the coefficient to be the necessary first step in assessing the fate of new chemicals.

(iii) Of the three properties that can be estimated from K_{ow}, water solubility is the most important because it affects both the fate and transport of chemicals. For example, highly soluble chemicals become quickly distributed by the hydrologic cycle, have low sorption coefficients for soils and sediments, and tend to be more easily degraded by microorganisms. In addition, chemical transformation processes such as hydrolysis, direct photolysis, indirect photolysis (oxidation) and tend to occur more readily if a compound is soluble.

(iv) Direct correlations between K_{ow} and both the soil/sediment sorption coefficient and the bioconcentration factor are to be expected. In these cases compounds that are more soluble in octanol (more hydrophobic) would be expected to partition out of the water and into the organic portion of soils/sediments and into lipophilic tissue. The relationship between K_{ow} and the bioconcentration factor, as developed by Neely et al. (1974) under paragraph (d)(7) of this section, and other similar relationships, are the principal means of estimating bioconcentration factors. These factors are then used to predict the potential for a chemical to accumulate in living tissue.

(v) This test method describes a method for determining the octanol/water partition coefficient based on the dynamic coupled column liquid chromatographic technique, a technique commonly referred to as the generator column method. This method was the basis for a previous test method for water solubility, § 796.1860 and closely follows that section. The method described herein can be used in place of the standard shake-flask method described in § 796.1550 for compounds with a log K_{ow} greater than 1.0.

(2) *Definitions and units.* (i) The octanol/water partition coefficient (K_{ow}) is defined as the ratio of the molar concentrations of a chemical in n-octanol and water, in dilute solution. The coefficient K_{ow} is a constant for a given chemical at a given temperature. Since K_{ow} is the ratio of two molar concentrations, it is a dimensionless quantity. Sometimes K_{ow} is reported as log K_{ow}. The mathematical statement of K_{ow} is:

Equation 1

$$K_{ow} = \frac{C_{octanol}}{C_{water}},$$

where $C_{octanol}$ and C_{water} are the molar concentration of the solute in n-octanol and water, respectively, at a given temperature. This test procedure determines K_{ow} at 25±0.05 °C.

(ii) A "generator column" is used to partition the test substance between the octanol and water phases. The column in Figure 1 under paragraph (b)(1)(i)(A)(2) of this section is packed with a solid support and is coated with the test substance at a fixed concentration in n-octanol. The test substance is eluted from the column with water and the aqueous solution leaving the column represents the equilibrium concentration of the test substance that has partitioned from the octanol phase into the water phase. Preparation of the generator column is described under paragraph (b)(1)(i) of this section.

(iii) An "extractor column" is used to extract the solute from the aqueous solution produced by the generator column. After extraction onto a bonded chromatographic support, the solute is eluted with a solvent/water mixture and subsequently analyzed by high pressure liquid chromatography (HPLC). A detailed description of the preparation of the extractor column is given in paragraph (b)(1)(i) of this section.

(iv) The "sample loop" is a 1/16 in. O.D. (1.6mm) stainless steel tube with an internal volume between 20 and 50 μL. The loop is attached to the sample injection valve of the HPLC and is used to inject standard solutions into the mobile phase of the HPLC when determining the response factor for the recording integrator. The exact volume of the loop must be determined as described in paragraph (b)(3)(ii)(C)(*1*) of this section when the HPLC method is used.

(v) The "response factor" (RF) is the solute concentration required to give a one unit area chromatographic peak or one unit output from the HPLC recording integrator at a particular recorder and detector attenuation. The factor is required to convert from units of area to units of concentration. The determination of the response factor is given in paragraph (b)(3)(ii)(C)(*2*) of this section.

(3) *Principle of the test method.* (i) This test method is based on the dynamic coupled column liquid chromatographic (DCCLC) technique for determining the aqueous solubility of organic compounds that was initially developed by May et al. (1978) under paragraph (d) (5) and (6) of this section, modified by DeVoe et al. (1981) under paragraph (d)(1) of this section, and finalized by Wasik et al. (1981) under paragraph (d)(8) of this section. The DCCLC technique utilizes a generator column, extractor column and HPLC coupled or interconnected to provide a continuous closed flow system. Aqueous solutions of the test compound are produced by pumping water through the generator column that is packed with a solid support coated with an approximately 1.0 percent (w/w) solution of the compound in octanol. The aqueous solution leaving the column represents the equilibrium concentration of the test chemical which has partitioned from the octanol phase into the water phase. The compound is extracted from the aqueous solution onto an extractor column, then eluted from the extractor column with a solvent/water mixture and subsequently analyzed by HPLC using an ultraviolet absorption detector operating at a suitable wavelength. Chromatogram peaks are recorded and integrated using a recording integrator. The concentration of the compound in the effluent from the generator column is determined from the mass of the compound (solute) extracted from a measured volume of water (solvent). The octanol/water partition coefficient is calculated from the ratio of the molar concentration of the solute in the 1.0 percent (w/w) octanol and molar concentration of the solute in water as determined using the generator column technique.

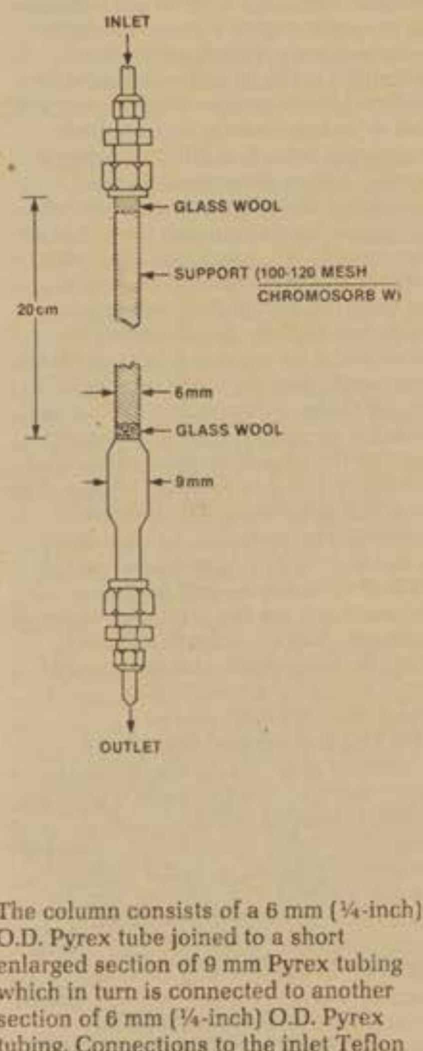
(ii) Since the HPLC method is only applicable to compounds that absorb in the ultraviolet, an alternate gas chromatographic (GC) method is used for those compounds that do not absorb in the ultraviolet. In the GC method the saturated solutions produced in the generator column are extracted using an appropriate organic solvent that is subsequently injected into the GC for analysis of the test compound.

(4) *Applicability and specificity.* The test guideline is designed to determine the octanol/water partition coefficient of solid or liquid organic chemicals in the range log K_{ow} 1.0 to >6.0 (10 to $>10^6$).

(b) *Test Procedure*—(1) *Test conditions*—(i) *Special laboratory equipment.* (A) (*1*) Generator column—Either of two different methods for connecting to the generator column are used depending on whether the eluted aqueous phase is analyzed by HPLC (Procedure A) or by solvent extraction followed by GC analysis of solvent extract (Procedure B).

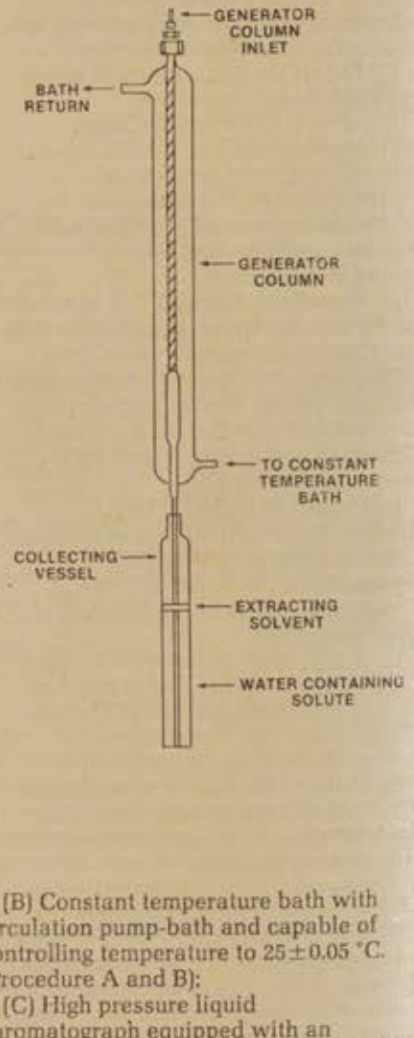
(*2*) The design of the generator column is shown in the following Figure 1:

Figure 1—Generator column



The column consists of a 6 mm (¼-inch) O.D. Pyrex tube joined to a short enlarged section of 9 mm Pyrex tubing which in turn is connected to another section of 6 mm (¼-inch) O.D. Pyrex tubing. Connections to the inlet Teflon tubing (⅛-inch O.D.) and to the outlet stainless steel tubing (¹⁄₁₆-inch O.D.) are made by means of stainless steel fittings with Teflon ferrules. The column is enclosed in a water jacket for temperature control as shown in the following Figure 2:

Figure 2—Setup showing generator column enclosed in a water jacket and overall arrangement of the apparatus used in GC method



(B) Constant temperature bath with circulation pump-bath and capable of controlling temperature to 25±0.05 °C. (Procedure A and B):

(C) High pressure liquid chromatograph equipped with an ultraviolet absorption detector operating at a suitable wavelength and a recording integrator (Procedure A);

(D) Extractor column—6.6 x 0.6 cm stainless steel tube with end fittings containing 5 micron frits filled with a superficially porous phase packing (Bondapack C_{18} Corasil: Water Associates) (Procedure A);

(E) Two 6-port high pressure rotary switching valves (Procedure A);

(F) Collection vessel under paragraph (b)(1)(i)(A)(2) Figure 2 of this section 8 x ¾ inch section of Pyrex tubing with a flat bottom connected to a short section of ¾-inch O.D. borosilicate glass tubing.

The collecting vessel is sealed with a ⅜-inch Teflon cap fitting (Procedure B); and

(C) Gas chromatograph equipped with a detector sensitive to the solute of interest (Procedure B).

(ii) *Purity of Octanol and Water.* Purified n-octanol, described in paragraph (b)(2) of this section, and water meeting ASTM Type II standards, or an equivalent grade, are recommended to minimize the effects of dissolved salts and other impurities. ASTM Type II water is described in ASTM D 1193–77, "Standard Specification for Reagent Water". ASTM D 1193–77 is available for inspection at the Office of the Federal Register, Rm. 8401, 1100 L St., NW., Washington, DC. This incorporation by reference was approved by the Director of the Office of the Federal Register. This material is incorporated as it exists on the date of approval and a notice of any change in this material will be published in the **Federal Register**. Copies of the incorporated material may be obtained from the Document Control Officer (TS–793), Office of Toxic Substances, EPA, Rm. 107, 401 M St., SW., Washington, DC 20460, and from the American Society for Testing and Materials (ASTM), 1916 Race Street, Philadelphia, PA 19103.

(iii) *Purity of Solvents.* It is important that all solvents used in this method be reagent or HPLC grade and contain no impurities which could interfere with the determination of the test compound.

(iv) *Reference Compounds.* In order to ensure that the HPLC system is working properly, at least two of the following five reference compounds should be run. The values obtained should be within ±0.1 log unit of the reference value

	Log K_{ow}
n-Propylacetate	1.24
2-Heptanone	1.08
Naphthalene	3.34
n-Propylbenzene	3.69
n-Octane	5.18

Reference compounds should be reagent or HPLC grade to avoid interferences by impurities.

(2) *Preparation of reagents and solutions*—(i) *Octanol and Water.* Very pure *n*-octanol can be obtained as follows: wash pure *n*-octanol (minimum 98 percent pure) sequentially with 0.1N H_2SO_4, with 0.1N NaOH, then with distilled water until neutral. Dry the *n*-octanol with magnesium sulfate and distill twice in a good distillation column under reduced pressure [b.p. about 80 °C at 0.27 kPa (2 torr)]. The octanol produced should be at least 99.9 percent pure. Alternatively, a grade equivalent to Fisher Scientific Co. No. A–402 "Certified Octanol-1" can be used. Distilled or reagent grade water should be used throughout the test procedure; ASTM Type II water as described in ASTM D1193–77, "Standard Specification for Water", is recommended. ASTM D1193–77 is available for inspection at the Office of the Federal Register, Rm. 8401, 1100 L St., NW., Washington, DC. This incorporation by reference was approved by the Director of the Office of the Federal Register. This material is incorporated as it exists on the date of approval and a notice of any change in this material will be published in the **Federal Register**. Copies of the incorporated material may be obtained from the Document Control Officer (TS–793), Office of Toxic Substances, EPA, Rm. 107, 401 M St., SW., Washington, DC 20460, and from the American Society for Testing and Materials (ASTM), 1916 Race Street, Philadelphia, PA 19103.

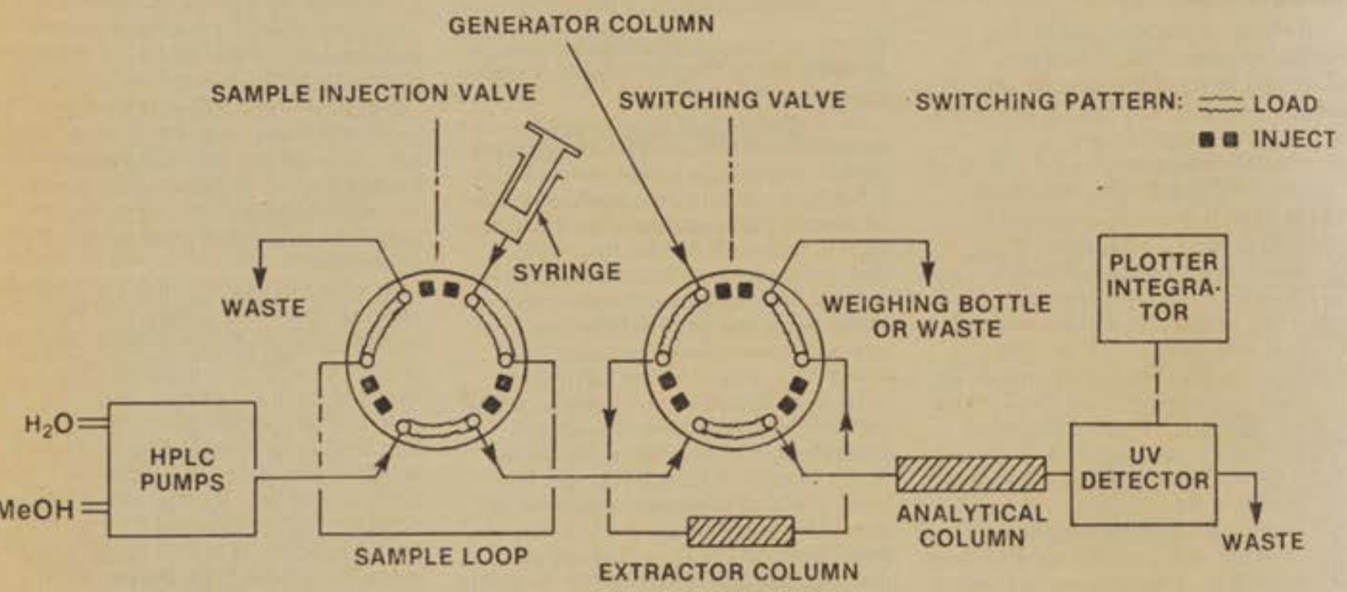
(ii) [Reserved]

(3) *Performance of the Test.* Initially, an approximately 1.0 percent (w/w) solution of the test substance in *n*-octanol is prepared. Precise measurement of the solute concentration in this solution is required for the K_{ow} calculation. Subsequently, the 1.0 percent (w/w) solution is coated on the generator column and using either Procedure A or B as described below, the molar concentration of the test substance in reagent grade water is determined.

(i) *Test solution.* The test solution consists of an approximately 1.0 percent (w/w) solution of the test substance in octanol. A sufficient quantity (about 10–20 ml) of the test solution should be prepared to coat the generator column. The solution is prepared by accurately weighing out, using a tared bottle, quantities of both the test substance and octanol required to make a 1.0 percent (w/w) solution. When the weights are measured precisely (to the nearest 0.1 mg, knowing the density of octanol (0.827 g/ml at 25 °C), then the molar concentration of the test substance in the octanol is sufficiently accurate for the purposes of the test procedure. If desired, however, a separate analytical determination (e.g., by GC) may be used to check the concentration in the test solution. If storage is required, the test solution should be kept stoppered to prevent volatilization of the test chemical.

(ii) *Procedure A—HPLC Method.* (A) Procedure A covers the determination of the aqueous solubility of compounds which absorb in the ultraviolet. The HPLC analytical system is shown schematically in the following Figure 3:

Figure 3—Schematic of HPLC—generator column flow system



Two reciprocating piston pumps deliver the mobile phase (water or solvent/water mixture) through two 6-port high pressure rotary valves and a 30 x 0.6 cm C_{18} analytical column to an ultraviolet absorption detector operating at a suitable wavelength. Chromatogram peaks are recorded and integrated with a recording integrator. One of the 6-port valves is the sample injection valve used for injecting samples of standard solutions of the solute in an appropriate concentration for determining response factors or standard solutions of basic chromate for determining the sample loop volume. The other 6-port valve in the system serves as a switching valve for the extractor column which is used to remove solute from the aqueous solutions.

(B) The general procedure for analyzing the aqueous phase after equilibration is as follows; a detailed procedure is given in part d. of this section:

(*1*) Direct the aqueous solution from the generator column to "Waste" in Figure 3 under paragraph (b)(3)(ii)(A) of this section with the switching valve in the inject position in order to equilibrate internal surfaces with the solution, thus insuring that the analyzed sample would not be depleted by solute adsorption on surfaces upstream from the valve.

(*2*) At the same time, water is pumped from the HPLC pumps in order to displace the solvent from the extractor column.

(*3*) The switching valve is next changed to the load position to divert a sample of the solution from the generator column through the extractor column, and the liquid leaving the extractor column is collected in a tared weighing bottle. During this extraction step, the HPLC mobile phase is changed to a solvent/water mixture to condition the analytical column.

(*4*) After the desired volume of sample is extracted, the switching valve is returned to the inject position for elution from the extractor column and analysis. Provided that all of the solute was adsorbed by the extractor column during the extraction step, the chromatographic peak represents all of the solute in the extracted sample.

(*5*) The solute concentration in the aqueous phase is calculated from the peak area, the weight of the extracted liquid collected in the weighing bottle, and the response factor.

(C)(*1*) *Determination of the Sample Loop Volume.* Accurate measurement of the sample loop may be accomplished by using the spectrophotometric method of Devoe et al. (1981) under paragraph (d)(1) of this section. For this method measure absorbance, A_{loop}, at 373 nm for at least three solutions, each of which is prepared by collecting from the sample valve an appropriate number, n, of loopfuls of an aqueous stock solution of K_2CrO_4 (1.3 percent by weight) and diluting to 50 mL with 0.2 percent KOH. (For a 20 μL loop, use n=5; for a 50 μL loop, use n=2.) Also measure the absorbance, A_{stock}, of the same stock solution after diluting 1:500 with 0.2 percent KOH. Calculate the loop volume to the nearest 0.1 μL using the relation:

$$V_{loop}=(A_{loop}/A_{stock})(10^{-4}/n)$$

(*2*) *Determination of the Response Factor (RF).* (*i*) For all determinations adjust the mobile phase solvent/water ratio and flow rate to obtain a reasonable retention time on the HPLC column. For example, typical concentrations of organic solvent in the mobile phase range from 50 to 100 percent while flow rates range from 1 to 3 mL/min; these conditions often give a 3 to 5 minute retention time.

(*ii*) Prepare standard solutions of known concentrations of the solute in a suitable solvent. Concentrations must give a recorder response within the maximum response of the detector. Inject samples of each standard solution into the HPLC system using the calibrated sample loop. Obtain an average peak area from at least three injections of each standard sample at a set detector absorbance unit full scale (AUFS), i.e., at the same absorbance scale attenuation setting.

(*iii*) Calculate the response factor from the following equation:

$$\text{Response Factor (RF)} = \frac{\text{Concentration (M)}}{\text{(Average Area) (AUFS)}}.$$

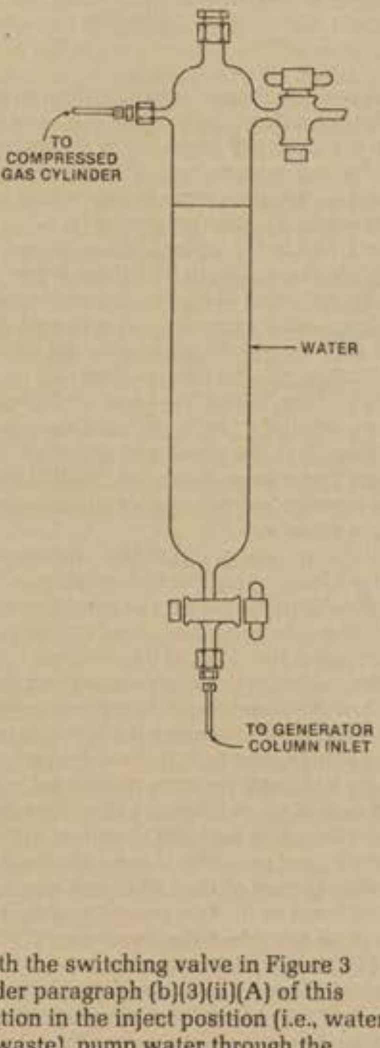
(*3*) *Loading of the Generator Column.* (*i*) The design of the generator column was described in paragraph (b)(1)(i) of this section and is shown in Figure 1 under paragraph (b)(1)(i)(A)(*2*) of this section. To pack the column, a plug of silanized glass wool is inserted into one end of the 6 mm Pyrex tubing. Silanized diatomaceous silica support (about 0.5g of 100–120 mesh Chromosorb W chromatographic support material) is poured into the tube with tapping and retained with a second plug of silanized glass wool.

(*ii*) The column is loaded by pulling the test solution through the dry support with gentle suction and then allowing the excess solution to drain out. After loading the column, draw water up through the column to remove any entrapped air.

(*4*) *Analysis of the solute.* Use the following procedure to collect and analyze the solute:

(*i*) Pump water to the generator column by means of a minipump or pressurized water reservoir as shown in the following Figure 4:

Figure 4—Water reservoir for GC method



With the switching valve in Figure 3 under paragraph (b)(3)(ii)(A) of this section in the inject position (i.e., water to waste), pump water through the generator column at a flow rate of approximately 1 mL/min for approximately 15 minutes to bring the system into equilibrium.

(*ii*) Flush out the organic solvent that remains in the system from previous runs by changing the mobile phase to 100 percent H_2O and allowing the water to reach the HPLC detector, as indicated by a negative reading. As soon as this occurs, place a 25 mL weighing bottle (weighed to the nearest mg) at the waste position and immediately turn the switching valve to the load position.

(*iii*) Collect an amount of water from the generator column (as determined by trial and error) in the weighing bottle, corresponding to the amount of solute adsorbed by the extractor column that gives a reasonable detector response. During this extraction step, switch back to the original HPLC mobile phase composition, i.e., solvent/water mixture, to condition the HPLC analytical column.

(*iv*) After the desired volume of sample has been extracted, turn the switching valve back to the inject position in Figure 3 under paragraph (b)(3)(ii)(A) of this section. As soon as switching valve is turned to the inject position, remove the weighing bottle, cap it and replace it with the waste container; at the same time turn on the recording integrator. The solvent/water mobile phase will elute the solute from the extractor column and transfer the solute to the HPLC analytical column.

(*v*) Determine the weight of water collected to the nearest mg and record the corresponding peak area. Using the same AUFS setting repeat the analysis of the solute at least two more times and determine the average ratio of peak area to grams of water collected. Calculate the solute solubility in water using the following equation:

$S = (997\ g/L)(RF)(V_{loop})(AUFS)(R)$

where

S = solubility (M)
RF = response factor
V_{loop} = sample loop volume (L)
R = ratio of area to grams of water.

(iii) *Procedure B—GC Method.* In the GC method, aqueous solutions from the generator column enter a collecting vessel in Figure 2 under paragraph (b)(1)(i)(A)(*2*) of this section containing a known weight of extracting solvent which is immiscible in water. The outlet of the generator column is positioned such that the aqueous phase always enters below the extracting solvent. After the aqueous phase is collected, the collecting vessel is stoppered and the quantity of aqueous phase is determined by weighing. The solvent and the aqueous phase are equilibrated by slowly rotating the collecting vessel. A small amount of the extracting solvent is then removed and injected into a gas chromatograph equipped with an appropriate detector. The solute concentration in the aqueous phase is determined from a calibration curve constructed using known concentrations of the solute.

(A) *Determination of calibration curve.* (*1*) Prepare solute standard solutions of concentrations covering the expected range of the solute solubility. Select a column and optimum GC operating conditions for resolution between the solute and solvent and the solute and extracting solvent. Inject a known volume of each standard solution into the injection port of the GC. For each standard solution determine the average of the ratio R of peak area to volume (in μL) for the chromatographic peak of interest from at least three separate injections.

(*2*) After running all the standard solutions, determine the coefficients, a and b, using linear regression analysis on the equation of concentration (C) vs. R in the form

$C = aR + b.$

(B) *Loading of the Generator Column.* The generator column is packed and loaded with solute in the same manner as for the HPLC method in paragraph (b)(3)(ii) of this section. As shown in Figure 2 under paragraph (b)(1)(i)(A)(*2*) of this section, attach approximately 20 cm of straight stainless steel tubing to the bottom of the generator column. Connect the top of the generator column to a water reservoir in Figure 4 under paragraph (b)(3)(ii)(C)(*4*)(*i*) of this section using Teflon tubing. Use air or nitrogen pressure (5 PSI) from an air or nitrogen cylinder to force water from the reservoir through the column. Collect water in an Erlenmeyer flask for approximately 15 minutes while the solute concentration in water equilibrates; longer time may be required for less soluble compounds.

(C) *Collection and extraction of the solute.* During the equilibration time, add a known weight of extracting solvent to a collection vessel which can be capped. The extracting solvent should cover the bottom of the collection vessel to a depth sufficient to submerge the collecting tube but still maintain 100:1 water/solvent ratio. Record the weight (to the nearest mg) of a collection vessel with cap and extracting solvent. Place the collection vessel under the generator column so that water from the collecting tube enters below the level of the extracting solvent in Figure 2 under paragraph (b)(1)(i)(A)(*2*) of this section. When the collection vessel is filled, remove it from under the generator column, replace cap, and weigh the filled vessel. Determine the weight of water collected. Before analyzing for the solute, gently rotate the collection vessel contents for approximately 30 min., controlling the rate of rotation so as not to form an emulsion; rotating the flask end over end five times per minute is sufficient.

(D) Analysis of the solute. (*1*) After rotating, allow the collection vessel to stand for approximately 30 minutes; then remove a known volume of the extracting solvent from the vessel using a microliter syringe and inject in into the G.C. Record the ratio of peak area to volume injected and, from the regression equation of the calibration line, determine the concentration of solute in the extracting solvent. The molar concentration of solute in water C(M) is determined from the following equation

$$C(M) = (C_{es}) \frac{d_{H_2O} \; g_{es}}{d_{es} \; g_{H_2O}}$$

where C_{es} is the molar concentration of solute in extracting solvent, d_{H_2O} and d_{es} are the densities in gm/ml of water and extracting solvent, respectively, and g_{es} and g_{H_2O} are the grams of extracting solvent and water, respectively, contained in the collection vessels.

(*2*) Make replicate injections from each collecting vessel to determine the average solute concentration in water for each vessel. To make sure the generator column has reached equilibrium, run at least two additional (for a total of three) collection vessels and analyze the extracted solute as described above. Calculate (CM) from the average solute concentration in the three vessels.

(iv) *Analysis of reference compounds.* Prior to analyzing the test solution, make duplicate runs on at least two of the five reference compounds given in paragraph (b)(1)(iv) of this section. When using the reference compounds, follow the same procedure previously described for preparing the test solution and running the test. If the average value obtained for each compound is 0.1 log unit of the reference value, then the test procedure and HPLC system are functioning properly; if not a thorough checking over of the HPLC and careful adherence to the test procedures should be done to correct the discrepancy.

(v) *Modification of procedures for potential problems—Decomposition of the test compound.* If the test compound decomposes in one or more of the aqueous solvents required during the period of the test at a rate such that an accurate value for water solubility cannot be obtained, then it will be necessary to carry out detailed transformation studies; e.g., hydrolysis under § 796.3500. If decomposition is due to aqueous photolysis, then it will be necessary to carry out water solubility studies in the dark, under red or yellow lights, or by any other suitable method to eliminate this transformation process.

(c) *Data and reporting*—(1) *Test report.* (i) For the test solution, report the weights to the nearest 0.1 mg of the test substance and octanol. Also report the weight percent and molar concentration of the test substance in the octanol, the density of *n*-octanol at 25 °C is 0.827 gm/ml.

(ii) For each run provide the molar concentration of the test substance in water for each of three determinations, the mean value, and the standard deviation.

(iii) For each of the three determinations calculate the octanol/water partition coefficient as the ratio of the molar concentration of the test substance in octanol to the molar concentration in water. Also calculate and report the mean K_{ow} and its standard deviation. Values of K_{ow} may be reported as their logarithms if desired.

(iv) Report the temperature (± 0.05 °C) at which the generator column was controlled during the test.

(v) For each reference compound report the individual values of log K_{ow} and the average of the two runs.

(vi) For compounds that decompose at a rate such that a precise value for the solubility cannot be obtained, provide a statement to that effect.

(2) *Specific analytical, calibration and recovery procedures* (i)—(A) For the HPLC method describe and/or report:

(*1*) The method used to determine the sample loop volume and the average and standard deviation of that volume.

(*2*) The average and standard deviation of the response factor.

(*3*) Any changes made or problems encountered in the test procedures.

(B) For the GC method report:

(*1*) The column and GC operating conditions of temperature and flow rate.

(*2*) The average and standard deviation of the average area/μL obtained for each of the standard solutions.

(*3*) The form of the regression equation obtained in the calibration procedure.

(*4*) The extracting solvent used.

(*5*) The average and standard deviation of solute concentration in each collection vessel.

(*6*) Any changes made or problems encountered in the test procedure.

(C) If the concentration of the test substance in *n*-octanol is determined by an independent analytical method such as gas chromatography, provide a complete description of the method.

(ii) [Reserved]

(d) *References.* For additional background information on this test guideline the following reference should be consulted:

(1) DeVoe, H., Miller, M.M., Wasik, S.P. "Generator Columns and High Pressure Liquid Chromatography for Determining Aqueous Solubilities and Octanol-Water Partition Coefficients of Hydrophobic Substances," *Journal of Research of the National Bureau of Standards,* 86:361–366 (1981).

(2) Fujita, T., Iwasa, J., Hansch, C. "A New Substituent Constant, Derived from Partition Coefficients." *American Chemical Society Journal,* 86:5175 (1964).

(3) Hansch, C., Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology.* (New York: J. Wiley & Sons, 1979).

(4) Lyman, W.J., Reehl, W.F., Rosenblatt, D.H. *Handbook of Chemical Property Estimation Methods, Environmental Behavior of Organic Compounds.* (McGraw Hill Book Co., New York, 1982).

(5) May, W.E., Wasik, S.P., Freeman, D.H. "Determination of the Aqueous Solubility of Polynuclear Aromatic Hydrocarbons by a Coupled Column Liquid Chromatographic Technique," *Analytical Chemistry,* 50:175–179 (1978).

(6) May, W.E., Wasik, S.P., Freeman, D.H. "Determination of the Solubility Behavior of Some Polycyclic Aromatic Hydrocarbons in Water," *Analytical Chemistry,* 50:997–1000 (1978).

(7) Neely, W.B., Bronson, D.R., Blau, G.E. "Partition Coefficient to Measure Bioconcentration Potential of Organic Chemicals in Fish," *Environmental Science and Technology,* 8:113 (1974).

(8) Wasik, S.P., Tewari, Y.B., Miller, M.M., Martire, D.E. "Octanol/Water Partition Coefficient and Aqueous Solubilities of Organic Compounds," Report NBSIR 81-2406, National Bureau of Standards, U.S. Department of Commerce, Washington, D.C. (1981).

§ 796.1840 Water Solubility.

(a) *Introduction*—(1) *Background and purpose.* (i) The water solubility of a compound can be defined as the equilibrium concentration of the compound in a saturated aqueous solution at a given temperature and pressure. The water solubility of a chemical is an important factor in determining the environmental movement and distribution of any substance. Chemicals that are relatively water soluble are more likely to be widely distributed by the hydrologic cycle than those which are relatively insoluble.

(ii) Water provides the medium in which many organisms live, and water is a major component of the internal environment of all living organisms (except for dormant stages of certain life forms). Even organisms which are adapted to life in a gaseous environment require water for normal functioning. Water is thus the medium through which most other chemicals are transported to and into living cells. As a result, the extent to which chemicals dissolve in water will be a major determinant for movement through the environment and entry into living systems.

(iii) The water solubility of a chemical has an effect on its adsorption on and desorption from soils and sediments and on volatilization from aqueous media. The more soluble a chemical substance is, the more likely it is to desorb from soils and sediments and the less likely it is to volatilize from water. The extent of chemical transformations via hydrolysis, photolysis, oxidation, reduction, and biodegradation in water depends on the chemical being soluble in water (i.e., homogeneous kinetics). Finally, the design of most chemical tests and many ecological and health tests requires precise knowledge of the water solubility of the chemical to be tested.

(iv) Procedures in this test guideline have been described to enable sponsors to determine the water solubility for solid and liquid organic compounds.

(2) *Definitions and units.* (i) "Colloidal dispersion" is a mixture resembling a true solution but containing one or more substances that are finely divided but large enough to prevent passage through a semipermeable membrane. It consists of particles which are larger than molecules, which settle out very slowly with time, which scatter a beam of light, and which are too small for resolution with an ordinary light microscope.

(ii) A "concentration vs. time study" results in a graph which plots the measured concentration of a given compound in a solution as a function of elapsed time. Usually, it provides a more reliable determination of equilibrium water solubility of hydrophobic compounds than can be obtained by single measurements of separate samples.

(iii) "Concentration" of a solution is the amount of solute in a given amount of solvent and can be expressed as a weight/weight or weight/volume relationship. The conversion from a weight relationship to one of volume incorporates density as a factor. For dilute aqueous solutions, the density of the solvent is approximately equal to the density of the solutions; thus, concentrations in mg/dm^3 are approximately equal to $10^{-3}g/10^3g$ or parts per million (ppm); ones in $\mu g/dm^3$ are approximately equal to $10^{-6}g/10^3g$ or parts per billion (ppb). In addition, concentration can be expressed in terms of molarity, normality, molality, and mole fraction. For example, to convert from weight/volume to molarity one incorporates molecular mass as a factor.

(iv) "Density" is the mass of a unit volume of a material. It is a function of temperature, hence the temperature at which it is measured should be specified. For a solid, it is the density of the impermeable portion rather than the bulk density. For solids and liquids, suitable units of measurement are g/cm^3. The density of a solution is the mass of a unit volume of the solution and suitable units of measurement are g/cm^3.

(v) An "oversaturated (supersaturated) solution" is a solution that contains a greater concentration of a solute than is possible at equilibrium under fixed conditions of temperature and pressure.

(vi) A "saturated solution" is a solution in which the dissolved solute is in equilibrium with an excess of undissolved solute; or a solution in equilibrium such that at a fixed temperature and pressure, the concentration of the solution is at its maximum value and will not change even in the presence of an excess of solute.

(vii) A "solution" is a homogeneous mixture of two or more substances constituting a single phase.

(3) *Principle of the test method.* The test method is based on the conventional method of preparing saturated aqueous solutions. The method involves the coating of the compound to the walls of a vessel, adding water (i.e., very pure water, buffer solution, or artificial seawater), and determining the concentration of the compound in the water as a function of time at a fixed temperature. When the concentration reaches a plateau, equilibrium has been achieved, and the water is saturated with the compound. Specific procedures have been incorporated in this test guideline to measure the water solubility of very hydrophobic compounds and to alleviate the problems of colloids and emulsions usually formed.

(4) *Applicability and specificity.* (i) Procedures have been described in this test guideline to determine the saturated water solubility for liquid or solid compounds. The water solubility can be determined in very pure water, buffer solution for compounds that reversibly

ionize or protonate, or in artificial seawater as a function of temperature (i.e., in the range of temperatures of environmental concern). Water solubility is usually not useful for gases because their solubility in water is measured when the gas above the water is at a partial pressure of one atmosphere which is several orders of magnitude greater than those existing under environmental conditions. A more important parameter for gases is Henry's law constant which is the ratio of the vapor pressure of the compound to solution concentration at low partial pressures.

(ii) This test guideline is designed to determine the saturated water solubility of a solid or liquid test chemical in the range infinity to 10 parts per billion (ppb). For chemicals whose solubility is below 10 ppb, the water solubility should be characterized as "less than 10 ppb" with no further quantification.

(b) *Description of the test procedure*—(1) *Test conditions*—(i) *Special laboratory equipment.* (A) A thermostatic bath with temperature control (±1 °C) in the approximate range of 5–30 °C.

(B) An ultracentrifuge with temperature control (±1 °C) in the approximate range of 5–30 °C and capable of obtaining acceleration (G) values to 39,000 or higher.

(C) A pH meter capable of resolving differences of 0.1 pH units or less.

(D) Centrifuge tubes with sealable caps: special glass tubes can be used up to approximately 12,000 G; tubes to be used at G value >12,000 should be made of stainless steel.

(ii) *Purity of water.* Reagent grade water, e.g., water meeting ASTM Type IIA standards or an equivalent grade, is highly recommended to minimize biodegradation and to minimize the effects of dissolved salts on water solubility. ASTM Type IIA water is described in ASTM D 1193–77, "Standard Specification for Reagent Water". ASTM D 1193–77 is available for inspection at the Office of the Federal Register, Rm. 8401, 1100 L. St., NW., Washington, DC. This incorporation by reference was approved by the Director of the Office of the Federal Register. This material is incorporated as it exists on the date of approval and a notice of any change in this material will be published in the Federal Register. Copies of the incorporated material may be obtained from the Document Control Officer (TS–793), Office of Toxic Substances, EPA, RM. 107, 401 M St., SW., Washington, DC 20460, and from the American Society for Testing and Materials (ASTM), 1916 Race Street, Philadelphia, PA 19103.

(iii) *Purity of solvents.* It is important that all solvents used for coating test compounds on the walls of vessels and in separation and analytical technique be reagent grade and contain no impurities which will interfere with the determination of the test compound.

(iv) *Seawater.* It is recommended that artificial seawater be used to determine the saturated water solubility in seawater. The preparation of artificial seawater is described in paragraph (b)(2)(ii) of this section.

(v) *Agitation and equilibration time.* It is important that contact time of test compounds with water be sufficient to obtain a saturated solution. The length of time necessary will depend upon such variables as the size of the vessel, the extent and degree of agitation, the properties of the compound and particle size. To increase the rate of solution of hydrophobic compounds, a mild agitation is recommended. For hydrophobic compounds a minimum time of one day is required.

(vi) *Effects of colloids and emulsions: Centrifugation.* (A) It is important that gentle shaking be used to minimize the formation of colloids. The presence of colloids and emulsions will lead to solubility values that are higher than those in a true saturated solution. This is a common problem with hydrophobic solids and liquids but can usually be overcome by centrifugation. It is recommended that centrifugation be conducted in tightly sealed tubes that are almost filled to capacity to avoid partitioning with air.

(B) It is extremely important that centrifugation be carried out at two or three different G values (minimum of 12,000 G) for at least 30 minutes at 25 °C until concentration changes are small. For hydrophobic compounds (solubility < 10 ppm), it is extremely important that the acceleration G values differ by 10,000 G and include a determination of 39,000 or higher.

(vii) *Effect of pH on solubility.* It is recommended that all experiments be carried out at pH's 5.0, 7.0, and 9.0 for any chemical which reversibly ionizes or protonates (e.g., carboxylic acids, phenols, amines). Buffers described in paragraph (b)(2)(i) of this section can be used.

(viii) *Analysis of saturated solutions.* Any suitable analytical method may be used; where practicable, precision should be within ±5 percent. Preferred analytical methods are those that are specific for the compound to be tested, to the exclusion of other compounds. Chromatographic methods which incorporate separation, and therefore, specification, are recommended.

(ix) *Adsorption to glass or other surfaces.* Hydrophobic compounds have a tendency to adsorb to glass or other surfaces, e.g., stainless steel. Thus, when transferring the solution to any glass vessel or container, it is essential to pre-rinse the surfaces of the vessel or container with the solution. Failure to do so will lead to solubility values that are lower than those of true equilibrium water solubility because the compound will adsorb to the unrinsed surface. However, when hydrophobic compounds are extracted with organic solvent, the extraction vessels should not be pre-rinsed since this would lead to solubility values that are greater than those of true equilibrium water solubility.

(2) *Preparation of reagents and solutions*—(i) *Buffer solutions.* Prepare buffer solutions using reagent grade water as follows:

pH 5.0—To 250 mL of 0.1M potassium hydrogen phthalate add 113 mL of 0.1M sodium hydroxide; adjust the final volume to 500 mL with reagent water.

pH 7.0—To 250 mL of 0.1M potassium dihydrogen phosphate add 145 mL of 0.1M sodium hydroxide; adjust the final volume to 500 mL with reagent gradewater.

pH 9.0—To 250 mL of 0.075M borax add 69 ml of 0.1M HCL; adjust the final volume to 500 mL with reagent gradewater.

Check the pH of each buffer solution with a pH meter at 25 °C and adjust to pH 5.0, 7.0, or 9.0, if necessary. If the pH of the solution has changed by ±0.2 pH units or more after the addition of the test compound, then a more concentrated buffer is required for that pH determination. The sponsor should then choose a more suitable buffer.

(ii) *Artificial seawater.* Add the reagent-grade chemicals listed in the following Table 1 in the specified amounts and order to 890 mL of reagent-grade water. It is important that each chemical be dissolved before another one is added.

TABLE 1—CONSTITUENTS OF ARTIFICIAL SEAWATER[1]

Chemical	Amount
NaF	3 mg
$SrCl_2 \cdot 6H_2O$	20 mg
H_3BO_3	30 mg
KBr	1,000 mg
KCl	700 mg
$CaCl_2 \cdot 2H_2O$	1.47 g
Na_2SO_4	4.00 g
$MgCl_2 \cdot 6H_2O$	10.78 g
$NaCl$	23.50 g
$Na_2SiO_3 \cdot 9H_2O$	20 mg
$NaHCO_3$	200 mg

[1] If the resulting solution is diluted to 1 cubic decimeter (1 liter), the salinity should be 34 ±0.5 g/kg and the pH 8.0 ±0.2. The desired test salinity is attained by dilution at time of use.

(3) *Performance of the test.* Determine the saturated water solubility of the test compound at 25 °C in reagent grade water or buffer solution, if appropriate. Under certain circumstances, it may be necessary to determine the water solubility of a test compound at 25 °C in artificial seawater. The water solubility can also be determined at other temperatures of environmental concern by adjusting the temperature of the water bath to the appropriate temperature.

(i) *Procedure for the determination of solids and liquids in water at 25 °C.* (A) Dissolve a sufficient amount of the solid compound in a suitable volatile organic solvent and coat on the walls of a vessel. Viscous liquids may be coated on vessels in a similar fashion; non-viscous liquids do not require solvents. Remove the solvent under reduced pressure or with a pure nitrogen gas stream. When all the solvent is removed, add reagent grade water or, for compounds which reversibly ionize or protonate, the appropriate buffer solution and slowly stir or agitate the mixture under temperature control. Mixing may be accomplished by use of a Teflon coated stirring bar and should be continued for a minimum of 24 hours before aliquots are withdrawn. Prior to taking aliquots, the mixture should be left to stand at constant temperature for at least one hour to permit separation of any small particles. To determine the concentration of the compound in the aqueous phase, aliquots should be centrifuged at two or three different G values (minimum of 12,000 G) for at least 30 minutes at 25 °C until concentration changes are small. The concentration value so obtained is plotted against the time of mixing. At a latter time, aliquots are again taken and analyzed in the same fashion to produce another data point on a concentration vs. time plot. When the concentration reaches a plateau, equilibrium is assumed. For hydrophobic compounds (solubility < 10 ppm) it is extremely important that the acceleration (G) values differ by 10,000 G and include a determination at 39,000 G or higher.

(B) For more soluble compounds (solubility > 500 ppm) coating the walls of the vessel is not necessary and filtration may be substituted for centrifugation. Use filters which are adequate to remove suspended particles. If the concentration of the solute exceeds 10 g/dm^3, then determine the density of the solution. This can be done by weighing known volumes of the solution at the same temperature as the constant temperature bath. Sufficient solution should be used so that each determination is made on a fresh aliquot. Carry out solubility and density experiments in triplicate.

(ii) *Modification of Procedures for Potential Problems*—(A) *Interference of Soluble Impurities.* Interference by soluble impurities in the test sample can be avoided by the use of an analytical technique that is specific to the compound being tested. If this is not practical, interferences can sometimes be minimized by repeatedly preparing saturated solutions from the same sample chemical until the concentration of the impurity has been depleted.

(B) *Decomposition of the test compound.* If the test compound decomposes in one or more of the aqueous solvents required during the period of the test at a rate such that an accurate value for water solubility cannot be obtained, then it will be necessary to carry out detailed transformation studies e.g., hydrolysis. If decomposition is due to aqueous photolysis, then it will be necessary to carry out the water solubility studies in the dark, under red or yellow lights, or by any other suitable method to eliminate this transformation process.

(c) *Data and reporting*—(1) *Test report.* (i) For each set of conditions, (e.g., temperature, pure water, buffer solution, artificial seawater) required for the study, provide the water solubility value for each of three determinations, the mean value, and the standard deviation.

(ii) For compounds that decompose at a rate such that a precise value for the water solubility cannot be obtained, provide a statement to that effect.

(iii) For compounds with water solubility below 10 ppb, report the value as "less than 10 ppb."

(iv) For compounds with water solubility greater than 10 g/dm^3, report the density of the solution at each required temperature.

(2) *Specific analytical and recovery procedures.* (i) Provide a detailed description or references for the analytical procedure used, including the calibration data and precision; and

(ii) If extraction methods were used to separate the solute from the aqueous solution provide a description of the extraction method as well as the recovery data.

§ 796.1860 Water Solubility (Generator Column Method).

(a) *Introduction*—(1) *Background and purpose.* (i) The water solubility of a chemical is defined as the equilibrium concentration of the chemical in a saturated aqueous solution at a given temperature and pressure. The aqueous phase solubility is an important factor in governing the movement, distribution, and rate of degradation of chemicals in the environment. Substances that are relatively water soluble are more likely to be widely distributed by the hydrologic cycle than those which are relatively insoluble. Furthermore, substances with higher water solubility are more likely to undergo microbial or chemical degradation in the environment because dissolution makes them "available" to interact and, therefore, react with other chemicals and microorganisms. Both the extent and rate of degradation via hydrolysis, photolysis, oxidation, reduction, and biodegradation depend on a chemical being soluble in water (i.e., homogeneous kinetics).

(ii) Water provides the medium in which many organisms live, and water is a major component of the internal environment of all living organisms (except for dormant stages of certain life forms). Even organisms which are adapted to life in a gaseous environment require water for normal functioning. Water is thus the medium through which most other chemicals are transported to and into living cells. As a result, the extent to which chemicals dissolve in water will be a major determinant for movement through the environment and entry into living systems.

(iii) The water solubility of a chemical also has an effect on its adsorption on and desorption from soils and sediments, and on volatilization from aqueous media. The more soluble a chemical substance is, the less likely it is to adsorb to soils and sediments and the less likely it is to volatilize from water. Finally, the design of most chemical tests and many ecological and health tests requires precise knowledge of the water solubility of the chemical to be tested.

(2) *Definitions and units.* (i) "Concentration" of a solution is the amount of solute in a given amount of solvent or solution and can be expressed as a weight/weight or weight/volume relationship. The conversion from a weight relationship to one of volume incorporates density as a factor. For dilute aqueous solutions, the density of the solvent is approximately equal to the density of the solution; thus, concentrations in mg/L are approximately equal to 10^{-3} g/10^3 g or parts per million (ppm); ones in µg/L are approximately equal to 10^{-6} g/10^3 g or parts per billion (ppb). In addition, concentration can be expressed in terms of molarity, normality, molality, and

mole fraction. For example, to convert from weight/volume to molarity one incorporates molecular mass as a factor.

(ii) "Density" is the mass of a unit volume of a material. It is a function of temperature, hence the temperature at which it is measured should be specified. For a solid, it is the density of the impermeable portion rather than the bulk density. For solids and liquids, suitable units of measurement are g/cm^3. The density of a solution is the mass of a unit volume of the solution and suitable units of measurement are g/cm^3.

(iii) A "saturated solution" is a solution in which the dissolved solute is in equilibrium with an excess of undissolved solute; or a solution in equilibrium such that at a fixed temperature and pressure, the concentration of the solute in the solution is at its maximum value and will not change even in the presence of an excess of solute.

(iv) A "solution" is a homogeneous mixture of two or more substances constituting a single phase.

(v) A "generator column" is used to produce or generate saturated solutions of a solute in a solvent. The column (see Figure 1 under paragraph (b)(1)(i)(A) of this section) is packed with a solid support coated with the solute, i.e., the organic compound whose solubility is to be determined. When water (the solvent) is pumped through the column, saturated solutions of the solute are generated. Preparation of the generator column is described under paragraph (b)(1)(i) of this section.

(vi) An "extractor column" is used to extract the solute from the saturated solutions produced by the generator column. After extraction onto a chromatographic support, the solute is eluted with a solvent/water mixture and subsequently analyzed by high pressure liquid chromatography (HPLC). A detailed description of the preparation of the extractor column is given in paragraph (b)(1)(i) of this section.

(vii) The "sample loop" is a 1/16 in. O.D. (1.6 mm) stainless steel tube with an internal volume between 20 and 50 µL. The loop is attached to the sample injection valve of the HPLC and is used to inject standard solutions into the mobile phase of the HPLC when determining the response factor for the recording integrator. The exact volume of the loop must be determined as described in paragraph (b)(3)(i)(B)(*1*) of this section when the HPLC method is used.

(viii) The "response factor" (RF) is the solute concentration required to give a one unit area chromatographic peak or one unit output from the HPLC recording integrator at a particular recorder attenuation. The factor is required to convert from units of area to units of concentration. The determination of the response factor is given in paragraph (b)(3)(i)(B)(*2*) of this section.

(3) *Principle of the test method.* (i) This test method is based on the dynamic coupled column liquid chromatographic (DCCLC) technique for determining the aqueous solubility of organic compounds that was initially developed by May et al. (1978) under paragraph (d)(2) and (3) of this section, modified by DeVoe et al. (1981) under paragraph (d)(1) of this section, and finalized by Wasik et al. (1981) under paragraph (d)(4) of this section. The DCCLC technique utilizes a generator column, extractor column and HPLC coupled or interconnected to provide a continuous closed flow system. Saturated aqueous solutions of the test compound are produced by pumping water through the generator column that is packed with a solid support coated with the compound. The compound is extracted from the saturated solution onto an extractor column, then eluted from the extractor column with a solvent/water mixture and subsequently analyzed by HPLC using an ultraviolet absorption detector operating at a suitable wavelength. Chromatogram peaks are recorded and integrated using a recording integrator. The concentration of the compound in the effluent from the generator column, i.e., the water solubility of the compound, is determined from the mass of the compound (solute) extracted from a measured volume of water (solvent).

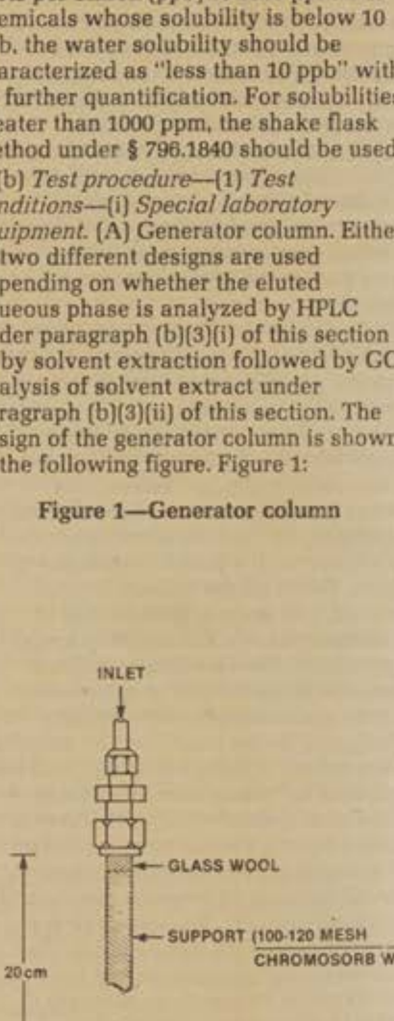
(ii) Since the HPLC method is only applicable to compounds that absorb in the ultraviolet, an alternate gas chromatographic (GC) method is used for those compounds that do not absorb in the ultraviolet. In the GC method the saturated solutions produced in the generator column are extracted using an appropriate organic solvent that is subsequently injected into the GC for analysis of the test compound.

(4) *Applicability and specificity.* (i) Procedures are described in this test guideline to determine the water solubility for liquid or solid compounds. The water solubility can be determined in very pure water, buffer solution for compounds that reversibly ionize or protonate, or in artificial seawater as a function of temperature (i.e., in the range of temperatures of environmental concern). This guideline is not applicable to the water solubility of gases.

(ii) This test guideline is designed to determine the water solubility of a solid or liquid test chemical in the range of 10 parts per billion (ppb) to 1000 ppm. For chemicals whose solubility is below 10 ppb, the water solubility should be characterized as "less than 10 ppb" with no further quantification. For solubilities greater than 1000 ppm, the shake flask method under § 796.1840 should be used.

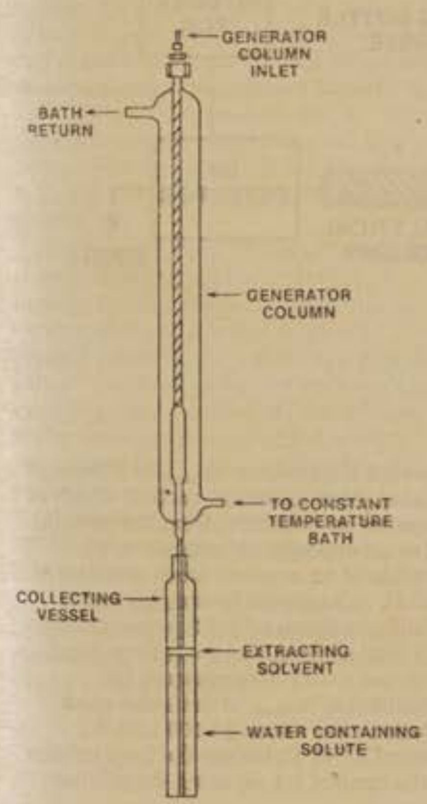
(b) *Test procedure*—(1) *Test conditions*—(i) *Special laboratory equipment.* (A) Generator column. Either of two different designs are used depending on whether the eluted aqueous phase is analyzed by HPLC under paragraph (b)(3)(i) of this section or by solvent extraction followed by GC analysis of solvent extract under paragraph (b)(3)(ii) of this section. The design of the generator column is shown in the following figure. Figure 1:

Figure 1—Generator column



The column consists of a 6 mm (¼-inch) O.D. Pyrex tube joined to a short enlarged section of 9 mm Pyrex tubing which in turn is connected to another section of 6 mm (¼-inch) O.D. Pyrex tubing. Connections to the inlet Teflon tubing (⅛-inch O.D.) and to the outlet stainless steel tubing (1/16-inch O.D.) are made by means of stainless steel fittings with Teflon ferrules. The column is enclosed in a water jacket for temperature control as shown in the following Figure 2:

Figure 2—Setup showing generator column enclosed in a water jacket and overall arrangement of the apparatus used in the GC method



(B) Constant temperature bath with circulation pump-bath and capable of controlling temperature to ± 0.05°C. (See paragraph (b)(3) (i) and (ii) of this section.)

(C) High pressure liquid chromatograph equipped with an ultraviolet absorption detector operating at a suitable wavelength and a recording integrator under paragraph (b)(3)(i) of this section.

(D) Extractor column. 6.6 x 0.6 cm stainless steel tube with end fittings containing 5 μm frits filled with a superficially porous phase packing (Bondapack C_{18}/Corasil: Waters Associates) under paragraph (b)(3)(i) of this section.

(E) Two 6-port high pressure rotary switching valves under paragraph (b)(3)(i) of this section.

(F) Collection vessel. 8 x ¾ inch section of Pyrex tubing with a flat bottom connected to a short section of ⅜-inch O.D. borosilicate glass tubing in Figure 2 under paragraph (b)(1)(i)(A) of this section. The collecting vessel is sealed with a ⅜-inch Teflon cap fitting under paragraph (b)(3)(ii) of this section.

(G) Gas chromatograph equipped with a detector sensitive to the solute of interest under paragraph (b)(3)(ii) of this section.

(ii) *Purity of water.* Water meeting ASTM Type II standards or an equivalent grade, is recommended to minimize the effects of dissolved salts and other impurities on water solubility. ASTM Type II water is described in ASTM D 1193–77, "Standard Specification for Reagent Water". ASTM D 1193–77 is available for inspection at the Office of the Federal Register, Rm. 8401, 1100 L St., NW., Washington, DC. This incorporation by reference was approved by the Director of the Office of the Federal Register. This material is incorporated as it exists on the date of approval and a notice of any change in this material will be published in the **Federal Register**. Copies of the incorporated material may be obtained from the Document Control Officer (TS-793), Office of Toxic Substances, EPA, Rm. 107, 401 M St., SW., Washington, DC 20460, and from the American Society for Testing and Materials (ASTM), 1916 Race Street, Philadelphia, PA 19103.

(iii) *Purity of solvents.* It is important that all solvents used in this method be reagent or HPLC grade and contain no impurities which could interfere with the determination of the test compound.

(iv) *Seawater.* When the water solubility in seawater is desired, the artificial seawater described in paragraph (b)(2)(ii) of this section should be used.

(v) *Effect of pH on solubility.* For chemicals that reversibly ionize or protonate (e.g., carboxylic acids, phenols, amines), experiments should be performed at pH 5.0, 7.0, and 9.0 using buffers described in paragraph (b)(2)(i) of this section.

(2) *Preparation of reagents and solutions*—(i) *Buffer solutions.* Prepare buffer solutions as follows:

(A) pH 5.0—To 250 mL of 0.1M potassium hydrogen phthalate add 113 mL of 0.1M sodium hydroxide; adjust the final volume to 500 mL with reagent grade water.

(B) pH 7.0—To 250 mL of 0.1M potassium dihydrogen phosphate add 145 mL of 0.1M sodium hydroxide; adjust the final volume to 500 mL with reagent grade water.

(C) pH 9.0—To 250 mL of 0.075M borax add 69 mL of 0.1M HCl; adjust the final volume to 500 mL with reagent grade water.

Check the pH of each buffer solution with a pH meter at 25 °C and adjust to pH 5.0, 7.0, or 9.0, if necessary. If the pH of the solution has changed by ±0.2 pH units or more after the addition of the test compound, then a more concentrated buffer is required for that pH determination. The sponsor should then choose a more suitable buffer.

(ii) *Artificial seawater.* Add the reagent-grade chemicals listed in the following Table 1 in the specified amounts and order to 890 mL of reagent-grade water. It is important that each chemical be dissolved before another one is added.

TABLE 1.—CONSTITUENTS OF ARTIFICIAL SEAWATER[1]

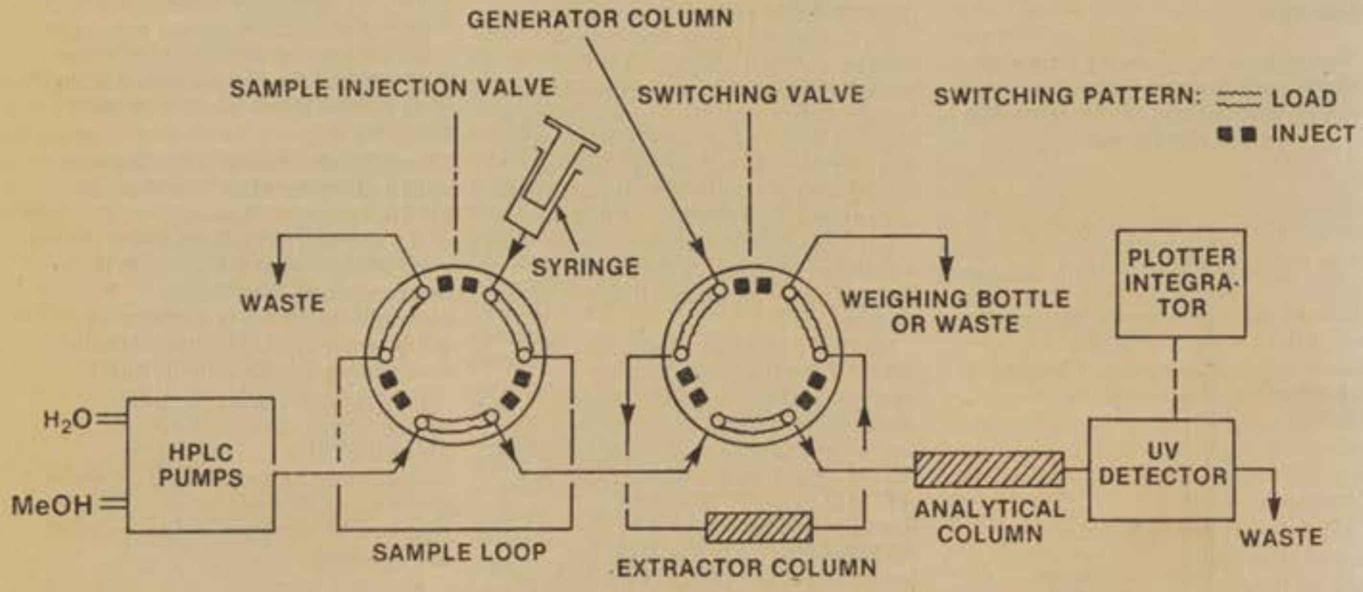
Chemical	Amount
NaF	3 mg
$SrCl_2 \cdot 6H_2O$	20 mg
H_3BO_3	30 mg
KBr	100 mg
KCl	700 mg
$CaCl_2 \cdot 2H_2O$	1.47 g
Na_2SO_4	4.00 g
$MgCl_2 \cdot 6H_2O$	10.78 g
NaCl	23.50 g
$Na_2SiO_3 \cdot 9H_2O$	20 mg
$NaHCO_3$	200 mg

[1] If the resulting solution is diluted to 1 cubic decimeter (1 liter), the salinity should be 34 ±0.5 g/kg and the pH 8.0 ±0.2. The desired test salinity is attained by dilution at time of use.

(3) *Performance of the test.* Using either the procedures under paragraph (b)(3) (i) or (ii) of this section, determine the water solubility of the test compound at 25 °C in reagent grade water or buffer solution, as appropriate. Under certain circumstances, it may be necessary to determine the water solubility of a test compound at 25 °C in artificial seawater. The water solubility can also be determined at other temperatures of environmental concern by adjusting the temperature of the water bath to the appropriate temperature.

(i) *Procedure A—HPLC Method.* (A) *Scope.* (*1*) Procedure A covers the determination of the aqueous solubility of compounds which absorb in the ultraviolet. The HPLC analytical system is shown schematically in the following Figure 3:

Figure 3 —Schematic of HPLC—generator column flow system



Two reciprocating piston pumps deliver the mobile phase (water or solvent/water mixture) through two 6-port high pressure rotary valves and a 30 x 0.6 cm C_{18}/Corosil analytical column to an ultraviolet absorption detector operating at a suitable wavelength; chromatogram peaks are recorded and integrated with a recording integrator. One of the 6-port valves is the sample injection valve used for injecting samples of standard solutions of the solute in an appropriate concentration for determining response factors or standard solutions of basic chromate for determining the sample loop volume. The other 6-port valve in the system serves as a switching valve for the extractor column which is used to remove solute from the aqueous solutions.

(*2*) The general procedure for analyzing the aqueous phase is as follows; a detailed procedure is given in paragraph (b)(3)(i)(B)(*4*) of this section:

(*i*) Direct the aqueous solution to "Waste" (see Figure 3 under paragraph (b)(3)(i)(A)(*1*) of this section) with the switching valve in the inject position in order to equilibrate internal surfaces with the solution, thus insuring that the analyzed sample would not be depleted by solute adsorption on surfaces upstream from the valve.

(*ii*) At the same time, water is pumped from the HPLC pumps in order to displace the solvent from the extractor column.

(*iii*) The switching valve is next changed to the load position to divert a sample of the solution through the extractor column, and the liquid leaving this column is collected in a weighing bottle. During this extraction step, the mobile phase is changed to a solvent/water mixture to condition the analytical column.

(*iv*) After the desired volume of sample is extracted, the switching valve is returned to the inject position for elution and analysis. Provided that there is no breakthrough of solute from the extractor column during the extraction step, the chromatographic peak represents all of the solute in the sample.

(*v*) The solute concentration in the aqueous phase in calculated from the peak area and the weight of the extracted liquid collected in the weighing bottle.

(B) *Determinations.*—(*1*) *Sample Loop Volume.* Accurate measurement of the sample loop may be accomplished by using the spectrophotometric method of Devoe et al. (1981) under paragraph (d)(1) of this section. For this method measure absorbance, A_{loop}, at 373 nm of at least three solutions, each of which is prepared by collecting from the sample valve an appropriate number, n, of loopfuls of an aqueous stock solution of K_2CrO_4 (1.3 percent by weight) and diluting to 50 mL with 0.2 percent KOH. (For a 20 μL loop, use n=5; for a 50 μL loop, use n=2.) Also measure the absorbance, A_{stock}, of the same stock solution after diluting 1:500 with 0.2 percent KOH. Calculate the loop volume to the nearest 0.1 μL using the relation:

$$V_{loop}=(A_{loop}/A_{stock})(10^{-4}/n)$$

(*2*) *Response Factor (RF).* (*i*) For all determinations adjust the mobile phase solvent/water ratio and flow rate to obtain a reasonable retention time on the HPLC column. For example, typical concentrations of solvent in the mobile phase range from 50 to 100 percent while flow rates range from 1 to 3 mL/min; these conditions give a 3 to 5 minute retention time.

(*ii*) Prepare standard solutions of known concentrations of the solute in a suitable solvent. Concentrations must give a recorder response within the maximum response of the detector. Inject samples of each standard solution into the HPLC system using the calibrated sample loop. Obtain an average peak area from at least three injections of each standard sample at a set absorbance unit full scale (AUFS), i.e., at the same absorbance scale attenuation setting.

(*iii*) Calculate the response factor from the following equation:

$$\text{Response Factor (RF)} = \frac{\text{Concentration (M)}}{\text{(Average Area) (AUFS)}}$$

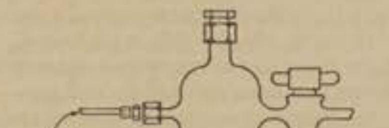
(*3*) *Loading of the Generator Column.* (*i*) The design of the generator column was described in paragraph (b)(1)(i) of this section and is shown in Figure 1 under paragraph (b)(1)(i)(A) of this section. To pack the column, a plug of silanized glass wool is inserted into one end of the 6 mm Pyrex tubing. Silanized diatomaceous silica support (about 0.5g 100–120 mesh Chromosorb W chromatographic support material) is poured into the tube with tapping and retained with a second plug of silanized glass wool.

(*ii*) If the solute is a liquid, the column is loaded by pulling the liquid solute through the dry support with gentle suction. If the solute is a solid, a 1 percent solution of the solid in a volatile solvent is added to the dry packing. The solvent is then distilled off the column under reduced pressure. After loading the column draw water up through the column to remove entrapped air.

(*4*) *Analysis of the solute.* Use the following procedure to collect and analyze the solute.

(*i*) Pump water to the generator column by means of a minipump or pressurized water reservoir as shown in the following Figure 4:

Figure 4—Water reservoir for GC method



With the switching valve (Figure 3 under paragraph (b)(3)(i)(A)(*1*) of this section) in the inject position (i.e., water to waste), pump water through the generator column at a flow rate of approximately 1 mL/min for approximately 5 minutes to bring the system into equilibrium.

(*ii*) Flush out the solvent that remains in the system from previous runs by changing the mobile phase to 100 percent H_2O and allowing the water to reach the HPLC detector, as indicated by a negative reading. As soon as this occurs, place a 25 mL weighing bottle (weighed to the nearest mg) at the waste position and immediately turn the switching valve to the load position.

(*iii*) Collect an amount of water (as determined by trial and error) in the weighing bottle, corresponding to the amount of solute adsorbed by the extractor column that gives a large on-scale detector response. During this extraction step, switch back to the original HPLC mobile phase composition, i.e., solvent/water mixture, to condition the HPLC analytical column.

(*iv*) After the desired volume of sample has been extracted, turn the switching valve back to the inject position (Figure 3 under paragraph (b)(3)(i)(A)(1) of this section); at the same time turn on the recording integrator. The solvent/water mobile phase will elute the solute from the extractor column and transfer the solute to the HPLC analytical column.

(*v*) Remove the weighing bottle, cap it and replace it with the waste container. Determine the weight of water collected to the nearest mg and record the corresponding peak area. Using the same AUFS setting repeat the analysis of the solute at least two more times and determine the average ratio of peak area to grams of water collected. Calculate the solute solubility in water using the following equation:

$s = (997\ \text{g/L})(RF)(V_{loop})(AUFS)(R)$

where

s = solubility (M)
RF = response factor
V_{loop} = sample loop volume (L)
R = ratio of area to grams of water.

(*ii*) *Procedure B–GC Method.* (*A*) *Scope.* In the GC method, aqueous solutions from the generator column enter a collecting vessel (Figure 2 under paragraph (b)(1)(i)(A) of this section) containing a known weight of extracting solvent which is immiscible in water. The outlet of the generator column is positioned such that the aqueous phase always enters below the extracting solvent. After the aqueous phase is collected, the collecting vessel is stoppered and the quantity of aqueous phase is determined by weighing. The solvent and the aqueous phase are equilibrated by slowly rotating the collecting vessel. A small amount of the extracting solvent is then removed and injected into a gas chromatograph equipped with an appropriate detector. The solute concentration in the aqueous phase is determined from a calibration curve constructed using known concentrations of the solute.

(B) *Determinations.—(1) Calibration Curve.* (i) Prepare solute standard solutions of concentrations covering the range of the solute solubility. Select a column and optimum GC operating conditions for resolution between the solute and solvent and the solute and extracting solvent. Inject a known volume of each standard solution into the injection port of the GC. For each standard solution determine the average of the ratio R of peak area to volume (in μL) for three chromatographic peaks from three injections.

(ii) After running all the standard solutions, determine the coefficients, a and b, using a linear regression equation of concentration (c) vs. R in the following form

$c = aR + b$.

(*2*) *Loading of the Generator Column.* The generator column is packed and loaded with solute in the same manner as for the HPLC method (see paragraph (b)(3)(i)(B)(*3*) of this section). As shown in Figure 2 under paragraph (b)(1)(i)(A) of this section, attach approximately 20 cm of straight stainless steel tubing to the bottom of the generator column. Connect the top of the generator column to a water reservoir (Figure 4 under paragraph (b)(3)(i)(B)(4)(i) of this section) using Teflon tubing. Use air or nitrogen pressure (5 PSI) from an air or nitrogen cylinder to force water from the reservoir through the column. Collect water in an Erlenmeyer flask for approximately 15 minutes while the solute concentration in water equilibrates; longer time may be required for less soluble compounds.

(*3*) *Collection and extraction of the solute.* During the equilibration time, add a known weight of extracting solvent to a collection vessel which can be capped. The extracting solvent should cover the bottom of the collection vessel to a depth sufficient to submerge the collecting tube but still maintain 100:1 water/solvent ratio. Record the weight (to the nearest mg) of a collection vessel with cap and extracting solvent. Place the collection vessel under the generator column so that water from the collecting tube enters below the level of the extracting solvent (Figure 2 under paragraph (b)(1)(i)(A) of this section). When the collection vessel is filled, remove it from under the generator column, replace cap, and weigh the filled vessel. Determine the weight of water collected. Before analyzing for the solute, gently shake the collection vessel contents for approximately 30 min., controlling the rate of shaking so as not to form an emulsion; rotating the flask end over end five times per minute is sufficient.

(*4*) *Analysis of the solute.* (*i*) After shaking, allow the collection vessel to stand for approximately 30 minutes; then remove a known volume of the extracting solvent from the vessel using a microliter syringe and inject it into the G.C. Record the ratio of peak area to volume injected and, from the regression equation of the calibration line, determine the concentration of solute in the extracting solvent. The concentration of solute in water C(M) is determined from the following equation:

$$C(M) = (C_{es}) \frac{d_{H_2O}}{d_{es}} \frac{g_{es}}{g_{H_2O}}$$

where C_{es} is the concentration of solute in extracting solvent (M), d_{H_2O} and d_{es} are the densities of water and extracting solvent, respectively, and g_{es} and g_{H_2O} are the grams of extracting solvent and water, respectively, contained in the collection vessel.

(*ii*) Make replicate injections from each collecting vessel to determine the average solute concentration in water for each vessel. To make sure the generator column has reached equilibrium, run at least two additional (for a total of three) collection vessels and analyze the extracted solute as described above. Calculate the water solubility of the solute from the average solute concentration in the three vessels.

(*iii*) *Modification of procedures for potential problems*—(*A*) *Decomposition of the test compound.* If the test compound decomposes in one or more of the aqueous solvents required during the period of the test at a rate such that an accurate value for water solubility cannot be obtained, then it will be necessary to carry out detailed transformation studies; e.g., hydrolysis under §796.3500. If decomposition is due to aqueous photolysis, then it will be necessary to carry out water solubility studies in the dark, under red or yellow lights, or by any other suitable method to eliminate this transformation process.

(B) [Reserved].

(*c*) *Data and reporting*— (1) *Test report.* (i) For each set of conditions, (e.g., temperature, pure water, buffer solution, artificial seawater) required for the study, provide the water solubility value for each of three determinations, the mean value, and the standard deviation.

(ii) For compounds that decompose at a rate such that a precise value for the water solubility cannot be obtained, provide a statement to that effect.

(iii) For compounds with water solubility below 10 ppb, report the value as "less than 10 ppb".

(2) *Specific analytical, calibration and recovery procedures.* (i) For the HPLC method describe and/or report:

(A) The method used to determine the sample loop volume and the average and standard deviation of that volume.

(B) The average and standard deviation of the response factor.

(C) Any changes made or problems encountered in the test procedure.

(ii) For the GC method report:

(A) The column and GC operating conditions of temperature and flow rate.

(B) The average and standard deviation of the average area/μL obtained for each of the standard solutions.

(C) The form of the regression equation obtained in the calibration procedure.

(D) The extracting solvent used.

(E) The average and standard deviation of solute concentration in each collection vessel.

(F) Any changes made or problems encountered in the test procedure.

(*d*) *References.* For additional background information on this test guideline the following references should be consulted:

(1) DeVoe, H., Miller, M.M., Wasik, S.P. "Generator columns and high pressure liquid chromatography for determining aqueous solubilities and octanol-water partition coefficients of hydrophobic substances," *Journal of Research, National Bureau of Standards,* 86:361–366 (1981).

(2) May, W.E., Wasik, S.P., Freeman, D.H. Determination of the Aqueous Solubility of Polynuclear Aromatic Hydrocarbons by a Coupled Column Liquid Chromatographic Technique, *Analytical Chemistry,* 50:175–179.

(3) May, W.E., Wasik, S.P., Freeman, D.H. "Determination of the solubility behavior of some polycyclic aromatic hydrocarbons in the water," *Analytical Chemistry,* 50:997–1000 (1978).

(4) Wasik, S.P., Tewari, Y.B., Miller, M.M., Martire, D.E. "Octanol/Water Partition Coefficient and Aqueous Solubilities of Organic Compounds," NBS Report NBSIR 81-2406. Washington, DC: National Bureau of Standards, U.S. Department of Commerce, 1981.

§ 796.1950 Vapor pressure.

(a) *Introduction*—(1) *Background and purpose.* (i) Volatilization, the evaporative loss of a chemical, depends upon the vapor pressure of chemical and on environmental conditions which influence diffusion from a surface.

Volatilization is an important source of material for airborne transport and may lead to the distribution of a chemical over wide areas and into bodies of water far from the site of release. Vapor pressure values provide indications of the tendency of pure substances to vaporize in an unperturbed situation, and thus provide a method for ranking the relative volatilities of chemicals. Vapor pressure data combined with water solubility data permit the calculation of Henry's law constant, a parameter essential to the calculation of volatility from water.

(ii) Chemicals with relatively low vapor pressures, high adsorptivity onto solids, or high solubility in water are less likely to vaporize and become airborne than chemicals with high vapor pressures or with low water solubility or low adsorptivity to solids and sediments. In addition, chemicals that are likely to be gases at ambient temperatures and which have low water solubility and low adsorptive tendencies are less likely to transport and persist in soils and water. Such chemicals are less likely to biodegrade or hydrolyze and are prime candidates for atmospheric oxidation and photolysis (e.g., smog formation or stratospheric alterations). On the other hand, nonvolatile chemicals are less frequently involved in atmosphere transport, so that concerns regarding them should focus on soils and water.

(iii) Vapor pressure data are an important consideration in the design of other chemical fate and effects tests; for example, in preventing or accounting for the loss of violatile chemicals during the course of the test.

(2) *Definitions and units.*

(i) "Desorption efficiency" of a particular compound applied to a sorbent and subsequently extracted with a solvent is the weight of the compound which can be recovered from the sorbent divided by the weight of the compound originally sorbed.

(ii) "Pascal" (Pa) is the standard international unit of vapor pressure and is defined as newtons per square meter (N/m^2). A newton is the force necessary to give acceleration of one meter per second squared to one kilogram of mass.

(iii) The "torr" is a unit of pressure which equals 133.3 pascals or 1 mm Hg at 0 °C.

(iv) "Vapor pressure" is the pressure at which a liquid or solid is in equilibrium with its vapor at a given temperature.

(v) "Volatilization" is the loss of a substance to the air from a surface or from solution by evaporation.

(3) *Principle of the test methods.* (i) The isoteniscope procedure uses a standardized technique [ASTM 1978] that was developed to measure the vapor pressure of certain liquid hydrocarbons. The sample is purified within the equipment by removing dissolved and entrained gases until the measured vapor pressure is constant, a process called "degassing." Impurities more volatile than the sample will tend to increase the observed vapor pressure and thus must be minimized or removed. Results are subject to only slight error for samples containing nonvolatile impurities.

(ii) Gas saturation (or transpiration) procedures use a current of inert gas passed through or over the test material slowly enough to ensure saturation and subsequent analysis of either the loss of material or the amount (and sometimes kind) of vapor generated. Gas saturation procedures have been described by Spencer and Cliath (1969) under paragraph (d)(2) of this section. Results are easy to obtain and can be quite precise. The same procedures also can be used to study volatilization from laboratory scale environmental simulations. Vapor pressure is computed on the assumption that the total pressure of a mixture of gases is equal to the sum of the pressures of the separate or component gases and that the ideal gas law is obeyed. The partial pressure of the vapor under study can be calculated from the total gas volume and the weight of the material vaporized. If v is the volume which contains w grams of the vaporized material having a molecular weight M, and if p is the pressure of the vapor in equilibrium at temperature T (K), then the vapor pressure, p, of the sample is calculated by

$$p=(w/M)(RT/v),$$

where R is the gas constant (8.31 Pa $m^3 mol^{-1} K^{-1}$) when the pressure is in pascals (Pa) and the volume is in cubic meters. As noted by Spencer and Cliath (1970) under paragraph (d)(3) of this section, direct vapor pressure measurements by gas saturation techniques are more directly related to the volatilization of chemicals than are other techniques.

(iii) In an effort to improve upon the procedure described by Spencer and Cliath (1969) under paragraph (d)(2) of this section, and to determine the applicability of the gas saturation method to a wide variety of chemical types and structures, EPA has sponsored research and development work at SRI International (EPA 1982) under paragraph (d)(1) of this section. The procedures described in this Test Guideline are those developed under that contract and have been evaluated with a wide variety of chemicals of differing structure and vapor pressures.

(4) *Applicability and specificity.* (i) A procedure for measuring the vapor pressure of materials released to the environment ideally would cover a wide range of vapor pressure values, at ambient temperatures. No single procedure can cover this range, so two different procedures are described in this section, each suited for a different part of the range. The isoteniscope procedure is for pure liquids with vapor pressures from 0.1 to 100 kPa. For vapor pressures of 10^{-5} to 10^3 Pa, a gas saturation procedure is to be used.

(ii) With respect to the isoteniscope method, if compounds that boil close to or form azeotropes with the test material are present, it is necessary to remove the interfering compounds and use pure test material. Impurities more volatile than the sample will tend to increase the observed vapor pressure above its true value but the purification steps will tend to remove these impurities. Soluble, nonvolatile impurities will decrease the apparent vapor pressure. However, because the isoteniscope procedure is a static, fixed-volume method in which an insignificant fraction of the liquid sample is vaporized, it is subject to only slight error for samples containing nonvolatile impurities. That is, the nonvolatile impurities will not be concentrated due to vaporization of the sample.

(iii) The gas saturation method is applicable to solid or liquid chemicals. Since the vapor pressure measurements are made at ambient temperatures, the need to extrapolate data from high temperatures is not necessary and high temperature extrapolation, which can often cause serious errors, is avoided. The method is most reliable for vapor pressures below 10^3 Pa. Above this limit, the vapor pressures are generally overestimated, probably due to aerosol formation. Finally, the gas saturation method is applicable to the determination of the vapor pressure of impure materials.

(b) *Test Procedures*—(1) *Test conditions.* (i) The apparatus in the isoteniscope method is described in paragraph (b)(2)(i) of this section.

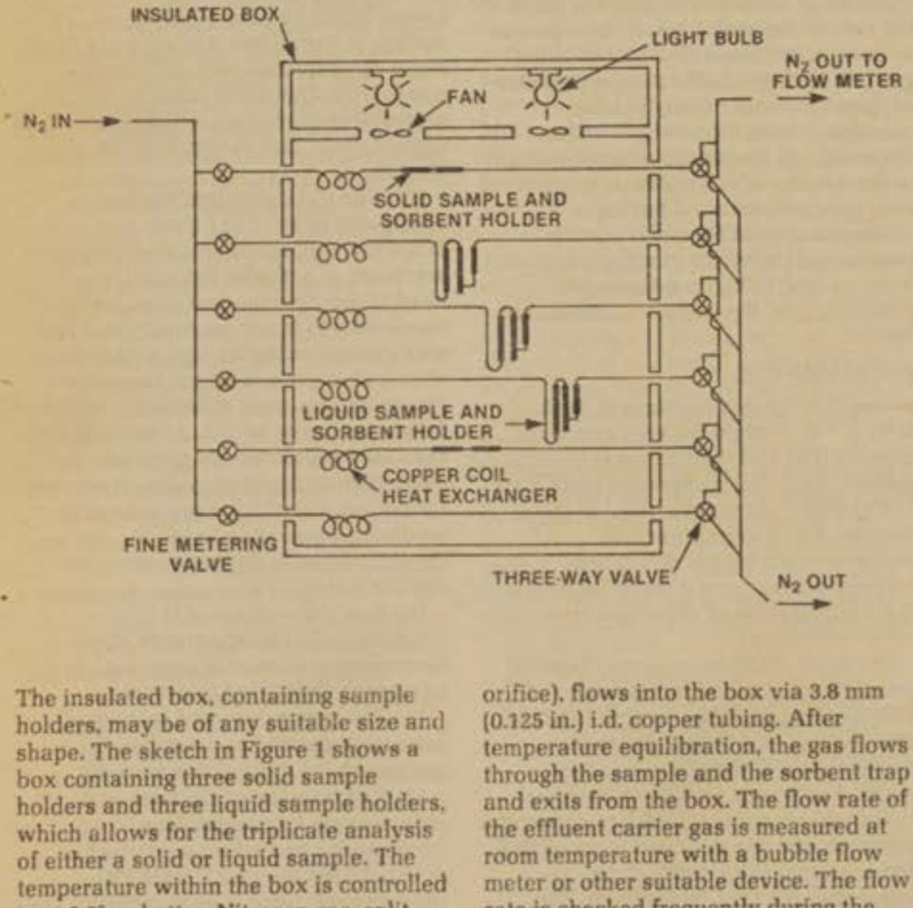
(ii) The apparatus used in the gas saturation method is described in paragraph (b)(2)(ii) of this section.

(2) *Performance of the tests*—(i) *Isoteniscope Procedure.* The isoteniscope procedure described as ANSI/ASTM Method D 2879–75 (Reapproved 1980) is applicable for the measurement of vapor pressures of liquids with vapor pressures of 0.1 to 100 kilopascals (kPa) (0.75 to 750 torr).

ASTM D 2879–75 (Reapproved 1980) is available for inspection at the Office of the Federal Register, Rm. 8401, 1100 L St., NW., Washington, DC. This incorporation by reference was approved by the Director of the Office of the Federal Register. This material is incorporated as it exists on the date of approval and a notice of any change in this material will be published in the **Federal Register**. Copies of the incorporated material may be obtained from the Document Control Officer (TS–793), Office of Toxic Substances, EPA, Rm. 107, 401 M St., SW., Washington, DC 20460, and from the American Society for Testing and Materials (ASTM), 1916 Race Street, Philadelphia, PA 19103. The isoteniscope method involves placing liquid sample in a thermostated bulb (the isoteniscope) connected to a manometer and a vacuum pump. Dissolved and entrained gases are removed from the sample in the isoteniscope by degassing the sample at reduced presssure. The vapor pressure of the sample at selected temperatures is determined by balancing the pressure due to the vapor of the sample against a known pressure of an inert gas. The vapor pressure of the test compound is determined in triplicate at 25±0.5 °C and at any other suitable temperatures (±0.5°). It is important that additional vapor pressure measurements be made at other temperatures, as necessary, to assure that there is no need for further degassing, as described in the ASTM method.

(ii) *Gas Saturation Procedure.* (A) The test procedures require the use of a constant-temperature box as depicted in the following Figure 1.

Figure 1—Schematic diagram of vapor saturation apparatus



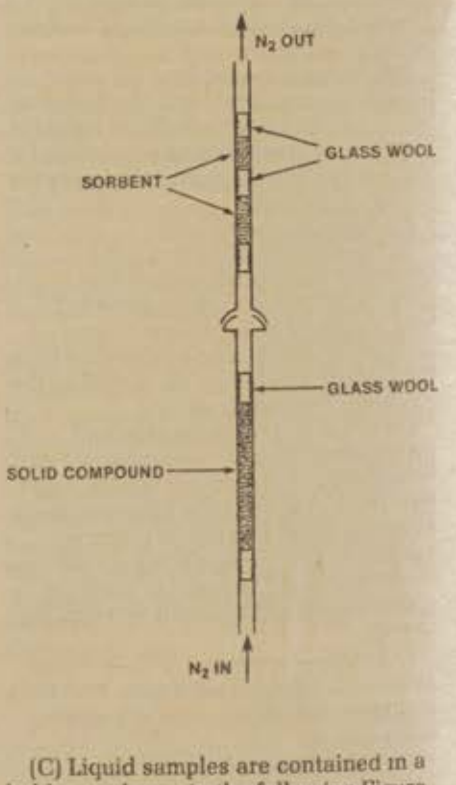
The insulated box, containing sample holders, may be of any suitable size and shape. The sketch in Figure 1 shows a box containing three solid sample holders and three liquid sample holders, which allows for the triplicate analysis of either a solid or liquid sample. The temperature within the box is controlled to ±0.5° or better. Nitrogen gas, split into six streams and controlled by fine needle valves (approximately 0.79 mm orifice), flows into the box via 3.8 mm (0.125 in.) i.d. copper tubing. After temperature equilibration, the gas flows through the sample and the sorbent trap and exits from the box. The flow rate of the effluent carrier gas is measured at room temperature with a bubble flow meter or other suitable device. The flow rate is checked frequently during the experiment to assure that there is an accurate value for the total volume of carrier gas. The flow rate is used to calculate the total volume (at room temperature) of gas that has passed through the sample and sorbent [(vol/time) × time = volume]. The vapor pressure of the test substance can be calculated from the total gas volume and the mass of sample vaporized. If v is the volume of gas that transported mass w of the vaporized test material having a molecular weight M, and if p is the equilibrium vapor pressure of the sample at temperature T, then p is calculated by the equation

$$p=(w/M)(RT/v).$$

In this equation, R is the gas constant (8.31 Pa m^3mol^{-1} K^{-1}). The pressure is expressed in pascals (Pa), the volume in cubic meters (m^3), mass in grams and T in kelvins (K). T=273.15+t, if t is measured in degrees Celsius (°C).

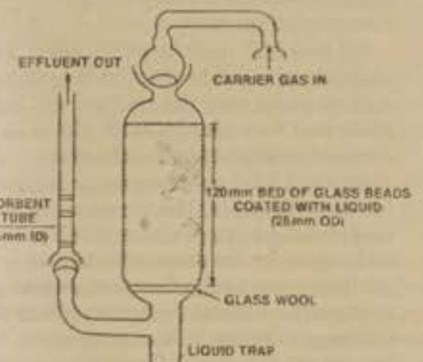
(B) Solid samples are loaded into 5 mm i.d. glass tubing between glass wool plugs. The following Figure 2 depicts a drawing of a sample holder and absorber system.

Figure 2—Solid compound sampling system



(C) Liquid samples are contained in a holder as shown in the following Figure 3.

Figure 3—Liquid compound sampling system



The most reproducible method for measuring the vapor pressure of liquids is to coat the liquid on glass beads and to pack the holder in the designated place with these beads.

(D) At very low vapor pressures and sorbent loadings, adsorption of the chemical on the glass wool separating the sample and the sorbent and on the glass surfaces may be a serious problem. Therefore, very low loadings should be avoided whenever possible. Incoming nitrogen gas (containing no interfering impurities) passes through a coarse frit and bubbles through a 38 cm column of liquid sample. The stream passes through a glass wool column to trap aerosols and then through a sorbent tube, as described above. The pressure drop across the glass wool column and the sorbent tube are negligible.

(E) With both solid and liquid samples, at the end of the sampling time, the front and backup sorbent sections are analyzed separately. The compound on each section is desorbed by adding the sorbent from that section to 1.0 ml of desorption solvent in a small vial and allowing the mixture to stand at a suitable temperature until no more test compound desorbs. It is extremely important that the desorption solvent contain no impurities which would interfere with the analytical method of choice. The resulting solutions are analyzed quantitatively by a suitable analytical method to determine the weight of sample desorbed from each section. The choice of the analytical method, sorbent, and desorption solvent is dictated by the nature of the test material. Commonly used sorbents include charcoal, Tenax GC, and XAD-2. Describe in detail the sorbent, desorption solvent, and analytical methods employed.

(F) Measure the desorption efficiency for every combination of sample, sorbent, and solvent used. The desorption efficiency is determined by injecting a known mass of sample onto a sorbent and later desorbing it and analyzing for the mass recovered. For each combination of sample, sorbent, and solvent used, carry out the determination in triplicate at each of three concentrations. Desorption efficiency may vary with the concentration of the actual sample and it is important to measure the efficiency at or near the concentration of sample under gas saturation test procedure conditions.

(G) To assure that the gas is indeed saturated with test compound vapor, sample each compound at three differing gas flow rates. Appropriate flow rates will depend on the test compound and test temperature. If the calculated vapor pressure shows no dependence on flow rate, then the gas is assumed to be saturated.

(c) *Data and reporting.* (1) Report the triplicate calculated vapor pressures for the test material at each temperature, the average calculated vapor pressure at each temperature, and the standard deviation.

(2) Provide a description of analytical methods used to analyze for the test material and all analytical results.

(3) For the isoteniscope procedure, include the plot of p vs. the reciprocal of the temperature in K, developed during the degasing step and showing linearity in the region of 298.15 K (25 °C) and any other required test temperatures.

(4) For the gas saturation procedure, include the data on the calculation of vapor pressure at three or more gas flow rates at each test temperature, showing no dependence on flow rate. Include a description of sorbents and solvents employed and the desorption efficiency calculations.

(5) Provide a description of any difficulties experienced or any other pertinent information.

(d) *References.* For additional background information on this test guideline the following references should be consulted:

(1) U.S. Environmental Protection Agency. *Evaluation of Gas Saturation Methods to Measure Vapor Pressures: Final Report,* EPA Contract No. 68-01-5117 with SRI International, Menlo Park, California (1982).

(2) Spencer, W.F. and Cliath, M.M. "Vapor Density of Dieldrin," *Journal of Agricultural and Food Chemistry,* 3:664-670 (1969).

(3) Spencer, W.F. and Cliath, M.M. "Vapor Density and Apparent Vapor Pressure of Lindane," *Journal of Agricultural and Food Chemistry,* 18:529-530 (1970).

Subpart C—Transport Processes

§ 796.2700 Soil thin-layer chromatography.

(a) *Introduction—(1) Background and purpose.* (i) Leaching of chemicals through soil is an important process which affects a chemical's distribution in the environment. If a chemical is tightly adsorbed to soil particles, it will not leach through the soil profile but will remain on the soil surface. If a chemical is weakly adsorbed, it will leach through the soil profile and may reach ground waters and then surface waters. Knowledge of the leaching potential is essential under certain circumstances for the assessment of the fate of chemicals in the environment.

(ii) Chemical leaching also affects the assessment of ecological and human health effects of chemicals. If a chemical reaches ground water, deleterious human health effects may arise due to the consumption of drinking water. If a chemical remains at the soil surface, deleterious environmental and human health effects may arise due to an increased concentration of the chemical in the zone of plant growth, possibly resulting in contamination of human food supplies.

(iii) Soil thin-layer chromatography (TLC) is a qualitative screening tool suitable for obtaining an estimate of a chemical's leaching potential. This test is one of several tests which can be used in obtaining a rough estimation of a chemical's leaching potential.

(2) *Definitions and units.* (i) "Cation exchange capacity" (CEC) is the sum total of exchangeable cations that a soil can adsorb. The CEC is expressed in milliequivalents of negative charge per 100 grams (meq/100g) or milliequivalents of negative charge per gram (meq/g) of soil.

(ii) "Particle size analysis" is the determination of the various amounts of the different particle sizes in a soil sample (i.e., sand, silt, clay) usually by sedimentation, sieving, micrometry or combinations of these methods. The names and size limits of these particles as widely used in the United States are:

Name	Diameter range
Very coarse sand	2.0 to 1.0 mm
Coarse sand	1.0 to 0.5 mm
Medium sand	0.5 to 0.25 mm
Fine sand	0.25 to 0.125 mm

Name	Diameter range
Very fine sand	0.125 to 0.062 mm
Silt	0.062 to 0.002 mm
Clay	$<$0.002 mm

(iii) "R_f" is the furthest distance traveled by a test material on a thin-layer chromatography plate divided by the distance traveled by a solvent front (arbitrarily set at 10.0 cm in soil TLC studies).

(iv) "Soil" is the unconsolidated mineral material on the immediate surface of the earth that serves as a natural medium for the growth of land plants; its formation and properties are determined by various factors such as parent material, climate, macro- and microorganisms, topography, and time.

(v) "Soil aggregate" is the combination or arrangement of soil separates (sand, silt, clay) into secondary units. These units may be arranged in the profile in a distinctive characteristic pattern that can be classified on the basis of size, shape, and degree of distinctness into classes, type, and grades.

(vi) "Soil classification" is the systematic arrangement of soils into groups or categories. Broad groupings are made on the basis of general characteristics, subdivisions, on the basis of more detailed differences in specific properties. The soil classification system used today in the United States is the 7th Approximation Comprehensive System. The ranking of subdivisions under the system is: order, suborder, great group, family and series.

(vii) "Soil horizon" is a layer of soil approximately parallel to the land surface. Adjacent layers differ in physical, chemical, and biological properties or characteristics such as color, structure, texture, consistency, kinds, and numbers of organisms present, and degree of acidity or alkalinity.

(viii) "Soil order" is the broadest category of soil classification and is based on general similarities of physical/chemical properties. The formation by similar genetic processes causes these similarities. The soil orders found in the United States are: Alfisol, Aridisol, Entisol, Histosol, Inceptisol, Mollisol, Oxisol, Spodosol, Ultisol, and Vertisol.

(ix) "Soil organic matter" is the organic fraction of the soil; it includes plant and animal residues at various stages of decomposition, cells and tissues of soil organisms, and substances synthesized by the microbial population.

(x) "Soil pH" is the negative logarithm to the base 10 of the hydrogen ion activity of a soil as determined by means of a suitable sensing electrode coupled with a suitable reference electrode at a 1:1 soil:water ratio.

(xi) "Soil series" is the basic unit of soil classification and is a subdivision of a family. A series consists of soils that were developed under comparable climatic and vegetational conditions. The soils comprising a series are essentially alike in all major profile characteristics except for the texture of the "A" horizon (i.e., the surface layer of soil).

(xii) "Soil texture" refers to the classification of soils based on the relative proportions of the various soil separates present. The soil textural classes are: clay, sandy clay, silty clay, clay loam, silty clay loam, sandy clay loam, loam, silt loam, silt, sandy loam, loamy sand, and sand.

(3) *Principle of the test method.* (i) Before 1968, methods of investigating the mobility of nonvolatile organic chemicals within soils were based on the use of field analysis, soil adsorption isotherms, and soil columns. In 1968, Helling and Turner introduced soil thin-layer chromatography (soil TLC) as an alternative procedure; it is analogous to conventional TLC, with the use of soil instead of silica gels, oxides, etc., as the adsorbent phase.

(ii) The papers by Helling (1968, 1971) under paragraph (d) (5), (6), and (7) of this section and Helling and Turner (1968) under paragraph (d)(3) of this section were the basis of this test guideline. The soil and colloid chemistry literature and the analytical chemistry literature substantiate the experimental conditions specified in the guideline.

(iii) The soil TLC offers many desirable features. First, mobility results are reproducible. Mass transfer and diffusion components are distinguishable. The method has relatively modest requirements for chemicals, soils, laboratory space, and equipment. It yields data that are amenable to statistical analyses. A chemical extraction-mass balance procedure to elicit information on degradation and chemical transformations occurring at colloid interfaces can be incorporated into this test. The ease with which the R_f and mass balance are performed will depend upon the physical/chemical properties of the test chemical and the availability of suitable analytical techniques for measuring the chemical.

(4) *Applicability and specificity.* (i) Soil TLC can be used to determine the soil mobility of sparingly water soluble to infinitely soluble chemicals. In general, a chemical having a water solubility of less than 0.5 ppm need not be tested since the literature indicates that these chemicals are, in general, immobile, see Goring and Hamaker (1972) under paragraph (d)(1) of this section. However, this does not preclude future soil adsorption/transformation testing of these chemicals if more refined data are needed for the assessment process.

(ii) Soil TLC may be used to test the mobility of volatile chemicals by placing a clean plate over the spotted soil TLC plate and then placing both plates in a closed chromatographic chamber.

(iii) Soil TLC was originally designed for use with soils. The literature shows no published use of this method with sediments as the absorbent phase, probably due to the fact that sediment surface properties change significantly during air drying. It is extremely important that the TLC plate with the adsorbent be air dried before leaching studies can be undertaken.

(b) *Test procedures*—(1) *Test conditions.* (i) Equipment required: Distilled-deionized water adjusted to pH 7 by boiling to remove CO_2; clean glass plates (TLC); glass rods or a variable thickness plate spreader; masking tape; closed chromatographic chambers; analytical instrumentation necessary and appropriate for the detection and quantitative analysis of the test chemical.

(ii) The test procedure may be run at 23±5 °C.

(iii) It is recommended that three replicate plates for each soil be used.

(2) *Test procedures.* (i) To reduce aggregate size before or during sieving, crush and grind the air-dried soil very, very gently.

(ii) Sieve air-dried soils with a 250 micrometer sieve.

(iii) Add water to the sieved soil until a smooth, moderately fluid slurry is attained (approximately ¾ ml H_2O added for each gram of soil).

(iv) Spread the slurry evenly and quickly across the clean glass plate using a variable thickness plate spreader, a glass rod, or other available method. If a glass rod is used, control the layer thickness by affixing multiple layers of masking tape along the plate edges. Soil layer thickness should be 0.50–0.75 mm.

(v) Air dry the plates at 25 °C for a minimum of 24 hours after uniform slurry application is achieved.

(vi) Scribe a horizontal line 11.5 cm above the base through the soil layer down to the glass so as to stop solvent movement.

(vii) Spot the test chemical, in solution, 1.5 cm above the base. For radiolabeled materials, 0.5–5 µg containing 0.01–0.03 μCi of ^{14}C labeled compound may be used.

(viii) If the compound is volatile, it is extremely important that a clean plate be placed over the soil TLC plate to impede volatilization.

(ix) Immerse the plate with the base down at some angle from the vertical in a closed chromatographic chamber containing H_2O at a height of 0.5 cm.

(x) Allow the solvent front to migrate to the 11.5 cm line before removing the plates from the chamber.

(xi) Determine the R_f values. Zonal extraction, plate scanning, or any other method or combination of methods suitable for detection of the parent test chemical may be used.

(xii) Determine the amount of the parent test chemical on the entire soil TLC plate after test chemical migration. Any method or combination of methods suitable for the extraction and quantitative detection of the parent test chemical may be used.

(c) *Data and reporting.* Report the following information. (1) Temperature at which the test was conducted.

(2) Amount of the test chemical applied and amount recovered from the plates.

(3) Detailed description of the analytical technique used in the R_f determination, the chemical extraction, and the quantitative recovery and analysis of the parent chemical.

(4) The mean frontal R_f value with the standard deviation for each soil tested.

(5) A photograph or diagram of the TLC plate which shows the entire leaching pattern (from 1.5 to 11.5 cm).

(6) Soil information: soil order, series, texture, sampling location, horizon, general clay fraction mineralogy.

(7) Soil physical/chemical properties: percent sand, percent silt and percent clay (particle size analysis); percent organic matter; pH (soil-to-water ratio, 1:1); and cation exchange capacity.

(d) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Goring, C.A.I., Hamaker, J.W. *Organic Chemicals in the Soil Environment.* Vol. I & II (New York: Marcel Dekker, Inc., 1972).

(2) Helling, C.S. "Pesticide mobility investigations using soil thin-layer chromatography," *American Society for Agronomy Abstracts* (1968).

(3) Helling, C.S., Turner, B.C. "Pesticide mobility: Determination by soil thin layer chromatography," *Science,* 162:562 (1968).

(4) Helling, C.S., "Movement of s-triazine herbicides in soils," *Residue Review,* 32:175–210 (1970).

(5) Helling, C.S. "Pesticide mobility in soils I. Parameters of soil thin layer chromatography," *Soil Science Society of America Proceedings,* 35:732–737 (1971).

(6) Helling, C.S. "Pesticide mobility in soils II. Applications of soil thin layer chromatography," *Soil Science Society of America Proceedings,* 35:737–743 (1971).

(7) Helling, C.S. "Pesticide mobility in soils III. Influence of soil properties," *Soil Science of America Proceedings,* 35:743–748 (1971).

§ 796.2750 Sediment and soil adsorption isotherm.

(a) *Introduction*—(1) *Background and purpose.* The adsorption of chemicals to sediments and soils is an important process that affects a chemical's distribution in the environment. If a chemical is adsorbed to soil particles, it will remain on the soil surface and will not reach ground water. If a chemical is not adsorbed, it will leach through the soil profile and may reach ground waters and then surface waters. Similarly, if a chemical adsorbed to sediment, it will accumulate in the bed and suspended load of aquatic systems. If a chemical is not adsorbed to sediment, it will accumulate in the water column of aquatic systems. Information on the adsorption potential is needed under certain circumstances to assess the transport of chemicals in the environment. This section describes procedures that will enable sponsors to determine the adsorption isotherm of a chemical on sediments and soils.

(2) *Definitions and units.* (i) The "cation exchange capacity" (CEC) is the sum total of exchangeable cations that a sediment or soil can adsorb. The CEC is expressed in milliequivalents of negative charge per 100 grams (meq/100g) or milliequivalents of negative charge per gram (meq/g) of soil or sediment.

(ii) "Clay mineral analysis" is the estimation or determination of the kinds of clay-size minerals and the amount present in a sediment or soil.

(iii) "Organic matter" is the organic fraction of the sediment or soil; it includes plant and animal residues at various stages of decomposition, cells and tissues of soil organisms, and substances synthesized by the microbial population.

(iv) "Particle size analysis" is the determination of the various amounts of the different particle sizes in a sample (i.e., sand, silt, clay), usually by sedimentation, sieving, micrometry, or combinations of these methods. The names and diameter range commonly used in the United States are:

Name	Diameter range
Very coarse sand	2.0 to 1.0 mm
Coarse sand	1.0 to 0.5 mm
Medium sand	0.5 to 0.25 mm
Fine sand	0.25 to 0.125 mm
Very fine sand	0.125 to 0.062 mm
Silt	0.062 to 0.002 mm
Clay	<0.002 mm

(v) The "pH" of a sediment or soil is the negative logarithm to the base ten of the hydrogen ion activity of the sediment or soil suspension. It is usually measured by a suitable sensing electrode coupled with a suitable reference electrode at a 1/1 solid/solution ratio by weight.

(vi) The adsorption ratio, "K_d," is the amount of test chemical adsorbed by a sediment or soil (i.e., the solid phase) divided by the amount of test chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio.

(vii) "Sediment" is the unconsolidated inorganic and organic material that is suspended in and being transported by surface water, or has settled out and has deposited into beds.

(viii) "Soil" is the unconsolidated mineral material on the immediate surface of the earth that serves as a natural medium for the growth of land plants. Its formation and properties are determined by various factors such as parent material, climate, macro- and microorganisms, topography, and time.

(ix) "Soil aggregate" is the combination or arrangement of soil separates (sand, silt, clay) into secondary units. These units may be arranged in the soil profile in a distinctive characteristic pattern that can be classified according to size, shape, and degree of distinctness into classes, types, and grades.

(x) "Soil classification" is the systematic arrangement of soils into groups or categories. Broad groupings are based on general soil characteristics while subdivisions are based on more detailed differences in specific properties. The soil classification system used in this standard and the one used today in the United States is the 7th Approximation-Comprehensive System. The ranking of subdivisions under this system is: Order, Suborder, Great group, family, and series.

(xi) A "soil horizon" is a layer of soil approximately parallel to the land surface. Adjacent layers differ in physical, chemical, and biological properties such as color, structure, texture, consistency, kinds and numbers of organisms present, and degree of acidity or alkalinity.

(xii) "Soil Order" is the broadest category of soil classification and is based on the general similarities of soil physical/chemical properties. The formation of soil by similar general genetic processes causes these similarities. The Soil Orders found in the United States are: Alfisol, Aridisol, Entisol, Histosol, Inceptisol, Mollisol, Oxisol, Spodosol, Ultisol, and Vertisol.

(xiii) "Soil series" is the basic unit of soil classification and is a subdivision of a family. A series consists of soils that were developed under comparable climatic and vegetational conditions. The soils comprising a series are essentially alike in all major profile characteristics except for the texture of the "A" horizon (i.e., the surface layer of soil).

(xiv) "Soil texture" is a classification of soils that is based on the relative proportions of the various soil separates present. The soil textural classes are: clay, sandy clay, silty clay, clay loam, silty clay loam, sandy clay loam, loam, silt loam, silt, sandy loam, loamy sand, and sand.

(3) *Principle of the test method.* (i) The extent of adsorption of a chemical onto sediment or soil is measured, using this test guideline, by equilibrating aqueous solutions containing different, but environmentally realistic, concentrations of the test chemical with a known quantity of sediment or soil. After equilibrium is reached, the distribution of the chemical between the water phase and the solid phase is quantitatively measured by a suitable analytical method. Then, sorption constants are calculated by using the Freundlich equation:

Equation 1

$x/m = C_s = KC_e^{1/n}$.

where:

C_e = Equilibrium concentration of the chemical in the solution phase

C_s = Equilibrium concentration of the chemical in the solid phase

K = Freundlich adsorption coefficient

m = The mass of the solid in grams

$^{1/n}$ = Exponent where n is a constant

x = The mass in micrograms of the chemical adsorbed by m grams of solid.

Logarithmetic transformation of the Freundlich equation yields the following linear relationship:

Equation 2

$\log C_s = \log K + (1/n) \log C_e$

(ii) In order to estimate the environmental movement of the test chemical, the values K and 1/n are compared with the values of other chemicals whose behavior in soil and sediment systems is well-documented in scientific literature.

(iii) The adsorption isotherm (AI) test has many desirable features. First, adsorption results are highly reproducible. The test provides excellent quantitative data readily amenable to statistical analyses. Also, it has relatively modest requirements for chemicals, soils, laboratory space, and equipment. It allows solution phase organic chemical determinations that are relatively uncomplicated. A chemical extraction-mass balance procedure to elicit information on chemical transformations occurring at colloid interfaces can be incorporated into this test. The ease of performing the isotherm test and mass balance will depend upon the physical/chemical properties of the test chemical and the availability of suitable analytical techniques to measure the chemical.

(iv) The papers by Aharonson and Kafkafi (1975) under paragraph (d)(1) of this section, Harvey (1974) under paragraph (d)(3) of this section, Murray (1975) under paragraph (d)(4) of this section, Saltzman (1972) under paragraph (d)(5) of this section, Weber (1971) under paragraph (d)(6) of this section, and Wu (1975) under paragraph (d)(7) of this section served as the basis for this section. The soil and colloid chemistry literature and the analytical chemistry literature substantiate the experimental conditions and procedures specified in this guideline as accepted, standard procedures.

(4) *Applicability and specificity.* The AI Test Guideline can be used to determine the soil and sediment adsorption potential of sparingly water soluble to infinitely soluble chemicals. In general, a chemical having a water solubility of less than 0.5 ppm need not be tested with soil as the solid phase, since the literature indicates that these chemicals are, in general, immobile in soils, see Goring and Hamaker (1972) under paragraph (d)(2) of this section. However, this does not preclude future soil adsorption/transformation testing of these chemicals if more refined data are needed for the assessment process.

(b) *Test procedures*—(1) *Test conditions*—(i) *Special laboratory equipment.* (A) Equilibrating solutions that contain, besides the test chemical, 0.01M calcium nitrate dissolved in sterilized, distilled-deionized H_2O adjusted to neutral pH 7 by boiling to remove CO_2.

(B) Containers that are composed of material that (*1*) adsorb negligible amounts of test chemical, and (*2*) withstand high speed centrifugation. The volume of the container is not a major consideration; however, it is extremely important that the amount of soil or sediment and the solid/solution ratio used in the study result in minimal container headspace. It is also extremely important that the containers be sterilized before use.

(C) A 150 micron (100 mesh) stainless-steel or brass sieve.

(D) Drying oven, with circulating air, that can attain 100 °C.

(E) Vortex mixer or a comparable device.

(F) Rotary shaker or a comparable device.

(G) High speed temperature-controlled centrifuge capable of sedimenting particles greater than 0.5 micron from aqueous solution.

(ii) *Temperature.* It is recommended that the test procedure be performed at 23±5 °C.

(iii) *Replications.* It is recommended that three replications of the experimental treatments be used.

(iv) *Soil pretreatment.* It is extremely important that these soil pretreatment steps be performed under the following conditions:

(A) Decrease the water content, air or oven-dry soils at or below 50 °C.

(B) Reduce aggregate size before and during sieving, crush and grind dried soil very gently.

(C) Eliminate microbial growth during the test period using a chemical or physical treatment that does not alter or minimally alters the soil surface properties.

(D) Sieve soils with a 100 mesh stainless-steel or brass sieve.

(E) Store all solutions and soils at temperatures between 0 and 5 °C.

(v) *Sediment pretreatment.* It is extremely important that these sediment pretreatment steps be performed under the following conditions:

(A) Decrease the H_2O content by air or oven-drying sediments at or below 50 °C. Sediments should not be dried completely and should remain moist at all times prior to testing and analysis.

(B) Eliminate microbial growth during the test period by using a chemical and/or physical treatment that does not alter or minimally alters the colloid surface's properties.

(C) Store at temperatures between 0 and 5 °C.

(vi) *Solid/solution ratio.* It is recommended that the solid/solution ratio be equal to or greater than 1/10. If possible, the ratios should be equal to or greater than 1/5. The sediment or soil dry weight after drying for a 24-hour minimum at 90 °C is recommended for use as the weight of the solid for ratio and data calculations.

(vii) *Equilibration time.* The equilibration time will depend upon the length of time needed for the parent chemical to attain an equilibrium distribution between the solid phase and the aqueous solution phase. It is recommended that the equilibration time be determined by the following procedure:

(A) Equilibrate one solution containing a known concentration of the test chemical with the sediment or soil in a solid/solution ratio not exceeding 1/10 and preferably equal to or greater than 1/5. It is important that the concentration of the test chemical in the equilibrating solution (*1*) does not exceed one-half of its solubility and (*2*) should be 10 ppm or less at the end of the equilibration period.

(B) Measure the concentration of the chemical in the solution phase at frequent intervals during the equilibration period.

(C) Determine the equilibration time by plotting the measured concentration versus time of sampling; the equilibration time is the minimum period of time needed to establish a rate of change of solution concentration of 5 percent or less per 24 hours.

(viii) *Centrifugation time.* Calculate the centrifugation time, t_c, necessary to remove particles from solution greater than approximately 0.5 micron (5×10^{-5} micron) equivalent diameter (which represents all particles except the fine clay fraction) using the following equation:

Equation 3

$$t_c(\text{min}) = 1.41 \times 10^9 [\log(R_2/R_1)]/N^2$$

where:

t_c = centrifuge time in minutes

R_2 = distance from centrifuge spindle to deposition surface of centrifuge

R_1 = distance from spindle to surface of the sample

N = number of revolutions of the centrifuge per minute.

(ix) *Storage of solutions.* If the chemical analysis is delayed during the course of the experiment, store all solutions between 0 and 5 °C.

(x) *Solvents for extraction.* It is extremely important that (A) the purity of the solvent used to extract the chemical that is adsorbed on the sediment or soil is analytical grade or better and (B) the minimum solubility of the test chemical in the solvent is 10 g/l.

(2) *Test procedure* (i) *Equilibration.* Add six solutions containing different concentrations of the test chemical to at least one gram of each solid. The initial concentration of the test chemical in these solutions will depend on the affinity the chemical has for the sediment or soil. Therefore, after equilibrium is attained, it is extremely important that the highest concentration of the test chemical in the equilibrating solution does not exceed 10 ppm, is at least one order of magnitude greater than the lowest concentration reported, and does not exceed one half of its solubility.

(A) Immediately after the solutions are added to the solids, tightly cap the containers and vigorously agitate them for several minutes with a vortex mixture or similar device.

(B) Shake the containers throughout the equilibration period at a rate that suspends all solids in the solution phase.

(ii) *Centrifugation.* When the equilibration time has expired, centrifuge the containers for t_c minutes.

(iii) *Chemical extraction.* (A) After centrifugation, remove the supernatant aqueous phase from the solid-solution mixture.

(B) Extract the chemical adsorbed on the sediment or soil colloid surfaces with solvent.

(iv) *Chemical analysis.* Determine the amount of parent test chemical in the aqueous equilibrating solution and organic solvent extractions. Use any method or combination of methods suitable for the identification and quantitative detection of the parent test chemical.

(c) *Reporting.* Report the following information:

(1) Temperature at which the test was conducted.

(2) Detailed description of the analytical technique(s) used in the chemical extraction, recovery, and quantitative analysis of the parent chemical.

(3) Amount of parent test chemical applied, the amount recovered, and the percent recovered.

(4) Extent of adsorption by containers and the approach used to correct the data for adsorption by containers.

(5) The individual observations, the mean values, and graphical plots of x/m as a function of C_e for each sediment or soil for (i) the equilibration time determination and (ii) the isotherm determination.

(6) The quantities K, n, and l/n.

(7) Soil information: Soil Order, series, texture, sampling location, horizon, general clay fraction mineralogy.

(8) Sediment information: sampling location, general clay fraction mineralogy.

(9) Sediment and soil physical-chemical properties: percent sand, silt, and clay (particle size analysis); percent organic matter; pH (1/1 solids/H_2O); and cation exchange capacity.

(10) The procedures used to determine the physical/chemical properties listed under paragraphs (c) (7) through (9) of this section.

(d) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Aharonson, N., Kafkafi, U. "Adsorption, mobility and persistence of thiabendazole and methyl 2-benzimidasole carbamate in soils," *Journal of Agricultural and Food Chemistry,* 23:720–724 (1975).

(2) Goring, C.A.I., Hamaker, J.W., (eds). *Organic Chemicals in the Soil Environment.* Vol. I & II (New York: Marcel Dekker, Inc., 1972).

(3) Harvey, R.G. et al. "Soil adsorption and volatility of dinitroaniline herbicides," *Weed Science,* 22:120–124 (1974).

(4) Murray, D.S. et al. "Comparative adsorption, desorption, and mobility of dipropetryn and prometryn in soil," *Journal of Agricultural and Food Chemistry,* 23:578–581 (1973).

(5) Saltzman, S.L. et al. "Adsorption, desorption of parathion as affected by soil organic matter," *Journal of Agricultural and Food Chemistry,* 20:1224–1226 (1972).

(6) Weber, J.B. "Model soil system, herbicide leaching, and sorption," *Weed Science,* 19:145–160 (1971).

(7) Wu, C.H., et al. "Napropamide adsorption, desorption, and movement in soils," *Weed Science,* 23:454–457 (1975).

Subpart D—Transformation Processes

§ 796.3100 Aerobic aquatic biodegradation.

(a) *Introduction*—(1) *Purpose.* (i) This Guideline is designed to develop data on the rate and extent of aerobic biodegradation that might occur when chemical substances are released to aquatic environments. A high biodegradability result in this test provides evidence that the test substance will be biodegradable in natural aerobic freshwater environments.

(ii) On the contrary, a low biodegradation result may have other causes than poor biodegradability of the test substance. Inhibition of the microbial inoculum by the test substance at the test concentration may be observed. In such cases, further work is needed to assess the aerobic aquatic biodegradability and to determine the concentrations at which toxic effects are evident. An estimate of the expected environmental concentration will help to put toxic effects into perspective.

(2) *Definitions.* (i) "Adaptation" is the process by which a substance induces the synthesis of any degradative

enzymes necessary to catalyze the transformation of that substance.

(ii) "Ultimate Biodegradability" is the breakdown of an organic compound to CO_2, water, the oxides or mineral salts of other elements and/or to products associated with normal metabolic processes of microorganisms.

(iii) "Ready Biodegradability" is an expression used to describe those substances which, in certain biodegradation test procedures, produce positive results that are unequivocal and which lead to the reasonable assumption that the substance will undergo rapid and ultimate biodegradation in aerobic aquatic environments.

(3) *Principle of the test method.* This Guideline method is based on the method described by William Gledhill (1975) under paragraph (d)(1) of this section. The method consists of a 2-week inoculum buildup period during which soil and sewage microorganisms are provided the opportunity to adapt to the test compound. This inoculum is added to a specially equipped Erlenmeyer flask containing a defined medium with test substance. A reservoir holding barium hydroxide solution is suspended in the test flask. After inoculation, the test flasks are sparged with CO_2-free air, sealed, and incubated, with shaking in the dark. Periodically, samples of the test mixture containing water-soluble test substances are analyzed for dissolved organic carbon (DOC) and the $Ba(OH)_2$ from the reservoirs is titrated to measure the amount of CO_2 evolved. Differences in the extent of DOC disappearance and CO_2 evolution between control flasks containing no test substance, and flasks containing test substance are used to estimate the degree of ultimate biodegradation.

(4) *Prerequisites.* The total organic carbon (TOC) content of the test substance should be calculated or, if this is not possible, analyzed, to enable the percent of theoretical yield of carbon dioxide and percent of DOC loss to be calculated.

(5) *Guideline information.* (i) Information on the relative proportions of the major components of the test substance will be useful in interpreting the results obtained, particularly in those cases where the result lies close to a "pass level."

(ii) Information on the toxicity of the chemical may be useful in the interpretation of low results and in the selection of appropriate test concentrations.

(6) *Reference substances.* Where investigating a chemical substance, reference compounds may be useful and an inventory of suitable reference compounds needs to be identified. In order to check the activity of the inoculum the use of a reference compound is desirable. Aniline, sodium citrate, dextrose, phthalic acid and trimellitic acid will exhibit ultimate biodegradation under the conditions of this Test Guideline method. These reference substances must yield 60 percent of theoretical maximum CO_2 and show a removal of 70 percent DOC within 28 days. Otherwise the test is regarded as invalid and should be repeated using an inoculum from a different source.

(7) *Reproducibility.* The reproducibility of the method has not yet been determined; however it is believed to be appropriate for a screening test which has solely an acceptance but no rejective function.

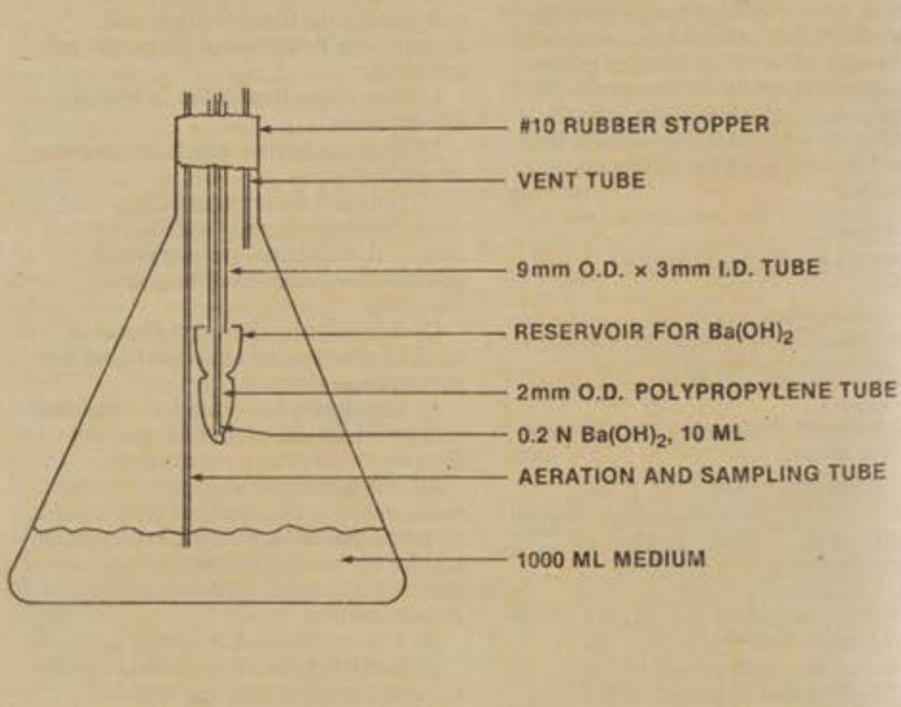
(8) *Sensitivity.* The sensitivity of the method is determined by the ability to measure the endogenous CO_2 production of the inoculum in the blank flask and by the sensitivity limit of the dissolved organic carbon analysis. If the test is adapted to handle ^{14}C-labeled test substances, test substance concentrations can be much lower.

(9) *Possibility of standardization.* This possibility exists. The major difficulty is to standardize the inoculum in such a way that interlaboratory reproducibility is ensured.

(10) *Possibility of automation.* None at present, although parts of the analyses may be automated.

(b) *Test procedures*—(1) *Preparations*—(i) *Apparatus.* The shake flask apparatus under the following Figure 1 contains 10 mL of 0.2N $Ba(OH)_2$ in an open container suspended over 1 liter of culture medium in a 2-liter Erlenmeyer flask.

Figure 1—Shake-flask system for carbon dioxide evolution



The $Ba(OH)_2$ container is made by placing a constriction just above the 10 mL mark of a 50 mL heavy-duty centrifuge tube and attaching the centrifuge tube to a 2 mm I.D. × 9 mm O.D. glass tube by means of 3 glass support rods. The centrifuge tube opening is large enough to permit CO_2 to diffuse into the $Ba(OH)_2$, while the constriction permits transferal of the flask to and from the shaker without $Ba(OH)_2$ spillage into the medium. For periodic removal and addition of base from the center well, a polypropylene capillary tube, attached at one end to a 10 ml disposable syringe, is inserted through the 9 mm O.D. glass tube into the $Ba(OH)_2$ reservoir. The reservoir access port is easily sealed during incubation with a serum bottle stopper. Two glass tubes are added for sparging, venting, and medium sampling. The tops of these tubes are connected with a short section of flexible tubing during incubation.

(ii) *Reagents and stock solutions*

(A) Stock solutions, I, II, and III under the following Table 1.

(B) Yeast extract.

(C) Vitamin-free casamino acids.

(D) 70 percent O_2 in nitrogen or CO_2-free air.

(E) 0.2N $Ba(OH)_2$.

(F) 0.1 N HCl.

(G) 20 percent H_2SO_4.

(H) Phenolphthalein.

(I) Dilution water—distilled, deionized water (DIW).

(iii) *Soil Inoculum.* A fresh sample of an organically rich soil is used as the inoculum in the ultimate biodegradation test. Soil is collected, prepared, and stored according to the recommendations of Pramer and Bartha (1972) under paragraph (d)(2) of this section. The soil surface is cleared of litter and a soil sample is obtained 10 to 20 cm below the surface. The sample is screened through a sieve with 2 to 5 mm openings and stored in a polyethylene bag at 2 to 4 °C for not more than 30 days prior to use. The soil is never allowed to air-dry, and should not be frozen during storage.

TABLE 1—MEDIUM EMPLOYED FOR ASSAY OF CO_2 EVOLUTION

Solution [1]	Compound	Stock Solution Conc (g/L)
I	NH_4Cl	35
	KNO_3	15
	$K_2HPO_4 \cdot 3H_2O$	75
	$NaH_2PO_4 \cdot H_2O$	25
II [2]	KCl	10
	$MgSO_4$	20
	$FeSO_4 \cdot 7H_2O$	1
III	$CaCl_2$	5
	$ZnCl_2$	0.05
	$MnCl_2 \cdot 4H_2O$	0.5
	$CuCl_2$	0.05
	$CoCl_2$	0.001
	H_3BO_3	0.001
	MoO_3	0.0004

[1] = Each liter of test medium contains 1 mL of each solution.

[2] = Final pH is adjusted to 3.0 with 0.10 N HCl.

(iv) *Acclimation Medium.* Acclimation medium is prepared by adding, for each liter of distilled, deionized water (DIW): 1 mL each of solutions I, II, and III in Table 1 in paragraph (b)(1)(ii)(A) of this section, 1.0 gm of soil inoculum (prepared according to paragraph (b)(1)(iii) of this section), 2.0 mL of aerated mixed liquor (obtained from an activated sludge treatment plant not more than 2 days prior to commencing the acclimation phase, and stored in the interim at 4 °C) and 50 mL raw domestic influent sewage. This medium is mixed for 15 minutes and filtered through a glass wool plug in a glass funnel. The filtrate is permitted to stand for 1 hour, refiltered through glass wool, and supplemented with 25 mg/L each of Difco vitamin-free casamino acids and yeast extract. Appropriate volumes are added to 2-liter Erlenmeyer flasks. Test compounds are added incrementally during the acclimation period at concentrations equivalent to 4, 8, and 8 mg/L carbon on days 0, 7, and 11, respectively. On day 14, the medium is refiltered through glass wool prior to use in the test. For evaluating the biodegradability of a series of functionally or structurally related chemicals, media from all inoculum flasks may be combined before final filtration.

(2) *Procedures.* (i) Inoculum (100 mL of acclimation medium) is added to 900 mL DIW containing 1 mL each of solutions I, II, and III in Table 1 under paragraph (b)(1)(ii)(A) of this section in a 2-liter Erlenmeyer flask. Test compound equivalent to 10 mg/liter carbon is added to each of the replicate flasks containing the test medium. Ten mL of 0.2 N Ba $(OH)_2$ are added to the suspended reservoir in each flask and duplicate 10 mL samples of $Ba(OH)_2$ are also saved as titration blanks for analysis with test samples. Flasks are sparged with CO_2-free air (for volatile test materials, sparging is done prior to addition of the chemical), sealed, and placed on a gyrotary shaker (approximately 125 rpm) at 20 to 25 °C in the dark. For each set of experiments, each test, reference, inhibited, and control system should be analyzed at time zero and at a minimum of four other times from time zero through day 28. Sampling must be made with sufficient frequency to allow for a smooth plot of biodegradation with time. Sampling times should be varied by the investigator as deemed appropriate to match the rate of degradation of the test substance. Tests may be terminated when biodegradation reaches a plateau and is consistent (±10 percent) over 3 consecutive days or on day 28, whichever occurs first. For chemicals which are water soluble at the test concentration, an adequate volume (5 to 10 mL) of medium is removed for DOC analysis. Each sample for DOC analysis should be filtered through a membrane filter of 0.45 micrometer pore diameter before DOC analysis. For all test and reference compounds, $Ba(OH)_2$ from the center well is removed for analysis. The center well is rinsed with 10 mL CO_2-free DIW and is refilled with fresh base. Rinse water is combined with the $Ba(OH)_2$ sample to be analyzed. Flasks are resealed and placed on the shaker. On the day prior to terminating the test, 3 mL of 20 percent H_2SO_4 are added to the medium to release carbonate bound CO_2.

(ii) For each set of experiments, each test substance should be tested in triplicate.

(iii) For each set of experiments, one or two reference compounds are included to assess the microbial activity of the test medium. Duplicate reference flasks are prepared by adding reference compound equivalent to 10 mg/liter carbon to each of two flasks containing the test medium. Reference compounds which are positive for ultimate biodegradability include: sodium citrate, dextrose, phthalic acid, trimellitic acid, and aniline.

(iv) For each test set, triplicate controls receiving inoculated medium and no test compound, plus all test and reference flasks, are analyzed for CO_2 evolution and DOC removal. Results from analysis of the control flasks (DOC, CO_2 evolution, etc.) are subtracted from corresponding experimental flasks containing test compound in order to arrive at the net effect due to the test compound.

(v) A test system containing a growth inhibitor should be established as a control for each substance tested for biodegradation by this method. That inhibited system must contain the same amount of water, mineral nutrients, inoculum, and test substance used in the uninhibited test systems, plus 50 mg/L mercuric chloride ($HgCl_2$) to inhibit microbial activity.

(vi) Flasks should be incubated in the dark to minimize both photochemical reactions and algal growth. Appropriate sterile controls or controls containing a metabolic inhibitor, such as 50 mg/L $HgCl_2$, are needed to correct for interferences due to non-biological degradation. With volatile organic materials, sparging with CO_2-free air is performed only once, just prior to addition of the test chemical. Analyses for CO_2 evolution and DOC removal are conducted within 2 to 3 hours of sampling to minimize interferences which may occur in storage. All glassware should be free of organic carbon contaminants.

(3) *Analytical measurements.* The quantity of CO_2 evolved is measured by titration of the entire $Ba(OH)_2$ sample (10 mL $Ba(OH)_2$ + 10 mL rinse water) with 0.1 N HCl to the phenolphthalein end point. $Ba(OH)_2$ blanks are also supplemented with 10 mL CO_2-free DIW

and titrated in a similar manner. Samples (5 mL) for DOC are centrifuged and/or filtered and supernatant or filtrate analyzed by a suitable total organic carbon method.

(c) *Data and reporting*—(1) *Treatment of results.* (i) Test compound (10 mg carbon) is theoretically converted to 0.833 mmol CO_2. Absorbed CO_2 precipitates as $BaCO_3$ from $Ba(OH)_2$, causing a reduction in alkalinity by the equivalent of 16.67 mL of 0.1 N HCl for complete conversion of the test compound carbon to CO_2. Therefore, the percent theoretical CO_2 evolved from the test compound is calculated at any sampling time from the formula:

Percent CO_2 evolution = [(TF–CF)/16.67] 100 (for 10 mg/L test compound carbon)

where:

TF = mL 0.1 N HCl required to titrate $Ba(OH)_2$ samples from the test flask
CF = mL 0.1 N HCl required to titrate $Ba(OH)_2$ samples from the control flask.

(ii) The cumulative percent CO_2 evolution at any sample time is calculated as the summation of the percent CO_2 evolved at all sample points of the test.

(iii) The percent DOC disappearance from the test compound is calculated from the following equation:

Percent DOC Removal = $[1-(DTF_x-DCF_x)/(DTF_o-DCF_o)]$ 100

where:

DTF = Dissolved organic carbon from test flask
DCF = Dissolved organic carbon from control flask
o = Day zero measurements
x = Day of measurements during test.

(iv) The difference between the amount of 0.1 N HCl used for the $Ba(OH)_2$ titration blank samples and the $Ba(OH)_2$ samples from the control units (no test compound) is an indication of the activity of the microorganisms in the test system. In general, this difference is approximately 1 to 3 mL of 0.1 N HCl at each sampling time. A finding of no difference in the titration volumes between these two samples indicates a poor inoculum. In this case, the validity of the test results is questionable and the test set should be rerun beginning with the acclimation phase.

(v) CO_2 evolution in the reference flasks is also indicative of the activity of the microbial test system. The suggested reference compounds should all yield final CO_2 evolution values in the range 80 to 100 percent of theoretical CO_2. If, for any test set, the percent theoretical CO_2 evolution value for the reference flasks is outside this range, the test results are considered invalid and the test is rerun.

(vi) Inhibition by the test compound is indicated by lower CO_2 evolution in the test flasks than in the control flasks. If inhibition is noted, the study for this compound is rerun beginning with the acclimation phase. During the test phase for inhibitory compounds, the test chemical is added incrementally according to the schedule: Day 0 – 0.5 mg/liter as organic carbon, Day 2—1 mg/liter C, Day 4—1.5 mg/liter C, Day 7—2 mg/liter C, Day 10—5 mg/liter C. For this case, the $Ba(OH)_2$ is sampled on Day 10, and weekly thereafter. The total test duration remains 28 days.

(vii) The use of ^{14}C-labeled chemicals is not required. If appropriately labeled test substance is readily available and if the investigator chooses to use this procedure with labeled test substance, this is an acceptable alternative. If this option is chosen, the investigator may use lower test substance concentrations if those concentrations are more representative of environmental levels.

(2) *Test report.* (i) For each test and reference compound, the following data should be reported.

(ii) Information on the inoculum, including source, collection date, handling, storage and adaptation possibilities (i.e., that the inoculum might have been exposed to the test substance either before or after collection and prior to use in the test).

(iii) Results from each test, reference, inhibited (with $HgCl_2$) and control system at each sampling time, including an average result for the triplicate test substance systems and the standard deviation for that average.

(iv) Average cumulative percent theoretical CO_2 evolution over the test duration.

(v) Dissolved organic carbon due to test compound at each sampling time (DTF–DCF).

(vi) Average percent DOC removal at each sampling time.

(vii) Twenty-eight day standard deviation for percent CO_2 evolution and DOC removal.

(d) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Gledhill, W.E. "Screening Test for Assessment of Ultimate Biodegradability: Linear Alkyl Benzene Sulfonate," *Applied Microbiology*, 30:922–929 (1975).

(2) Pramer, D., Bartha, R. "Preparation and Processing of Soil Samples for Biodegradation Testing," *Environmental Letters*, 2:217–224 (1972).

§ 796.3140 Anaerobic biodegradability of organic chemicals.

(a) *Introduction*—(1) *Purpose.* (i) This section has been developed for screening for anaerobic biodegradability of organic chemicals. A high biodegradability result in this test provides evidence that the test substance will be biodegradable in sewage-treatment plant anaerobic digestors and in many natural anaerobic environments such as swamps, flooded soils and surface water sediments.

(ii) On the contrary, a low biodegradation result may have other causes than poor biodegradability of the test substance. Inhibition of the microbial inoculum by the substance at the test concentration may be observed. In such cases further work is needed to assess the anaerobic biodegradability and to determine the concentrations at which toxic effects are evident. An estimate of the expected environmental concentration will help to put toxic effects into perspective.

(2) *Principle of the test method.* (i) This section is based on anaerobic biodegradability methods referenced in paragraph (d) of this section.

(ii) A chemically defined anaerobic medium, containing resazurin as an oxidation/reduction indicator and 10 percent (v/v) primary anaerobic digestor sludge from a waste treatment plant, is dispensed in 100 mL portions into 160 mL capacity serum bottles. Selected bottles are supplemented with test substance at a concentration equivalent to 50 mg/L as organic carbon. Gas production is measured with a pressure transducer. The extent of biodegradation is determined by comparing gas production from blank control bottles and bottles containing the test substance.

(iii) The average cumulative gas production (CH_4 + CO_2), in mL, is reported for blank controls, solvent controls, test substances and any reference compounds. Also reported is the percent of theoretical anaerobic biodegradation at test completion or 56 days (whichever comes first) and the standard deviation between replicate bottles.

(3) *Prerequisites.* The total organic carbon content of the test material should be calculated or, if this is not possible, analyzed, to enable the theoretical yield of carbon dioxide and methane to be calculated.

(4) *Guidance information.* (i) Information on the relative proportions of the major components of the test material will be useful in interpreting the results obtained, particularly in

those cases where the result lies close to a "pass level."

(ii) Information on the toxicity of the chemical may be useful in the interpretation of low results and in the selection of appropriate test concentrations.

(5) *Reference substances.* In some cases, when investigating a substance, reference substances may be useful and an inventory of suitable reference substances needs to be identified. In order to check the activity of the inoculum the use of a reference substance is desirable. Ethanol may be used for this purpose. The ethanol must exhibit anaerobic biodegradation (as gas production) greater than 50 percent of the theoretical maximum within 56 days. Otherwise the test is regarded as invalid and should be repeated using an inoculum from a different source.

(6) *Applicability.* The method is only applicable to those organic test substances which, at the concentration used in the test, are not inhibitory to bacteria.

(7) *Reproducibility.* The reproducibility of the method has not yet been determined; however, it is believed to be appropriate for a screening test which has solely an acceptance but no rejective function.

(8) *Sensitivity.* The sensitivity of the method is largely determined by the necessity to compare gas production in test-substance bottles with gas production in blank control bottles. The method suggests the use of test substance at a concentration of 50 mg/L as organic carbon. This concentration will produce a maximum of 10.5 mL of CH_4 and CO_2 gases at 35 °C. Actual measured gas production will be less due to (i) incomplete conversion of all of the organic carbon into CH_4 and CO_2 and (ii) the extent to which the CO_2 and CH_4 remain solubilized in the aqueous phase. The use of test substance at 50 mg/L as organic carbon represents a compromise between the desire to maximize gas production and thus the sensitivity of the test, and the desire to minimize the possibility of toxicity to the microbial population.

(9) *Possibility of standardization.* This possibility exists. The major difficulty is to standardize the inoculum in such a way that interlaboratory reproducibility is ensured.

(10) *Possibility of automation.* None at present.

(b) *Test procedures*—(1) *Preparations*—(i) *Apparatus.* (A) If gas production is measured with a pressure transducer, apparatus such as a 20-gauge syringe needle attached by means of an inert capillary tube to a three-way valve (Hamilton Mininert valve 3-FLM-IX or equivalent) fitted to a pressure transducer (Unimeasure 100-500 ohm/2mA pressure transducer or equivalent) and an appropriate ohmmeter (e.g. Digitec Model 2120).

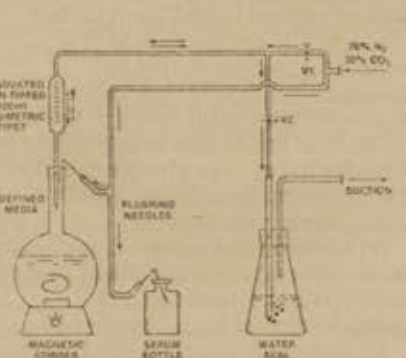
(B) If gas production is measured with a syringe, apparatus such as a 20 mL capacity gas-tight glass syringe fitted with a 20-gauge syringe needle.

(C) If methane (CH_4) and carbon dioxide (CO_2) are quantified using an analytical procedure specific for CH_4 and CO_2, apparatus necessary to carry out that analysis, such as a gas chromatograph equipped with a suitable detector.

(D) An incubator sufficient to store the test bottles at 35±1 °C for the duration of the test.

(E) Apparatus suitable for the maintenance of anaerobic conditions during medium preparation and inoculation, such as that shown in the following Figure 1:

Figure 1—Schematic diagram of apparatus suitable for maintenance of anaerobic conditions during medium preparation and inoculation



(F) A supply of 160 mL capacity serum bottles with butyl rubber stoppers.

(ii) *Nutrient medium*—(A) *Stock solutions.* (*1*) S–1 Prepare a solution in distilled water containing resazurin at 0.5 g/L.

(*2*) S–2 Dissolve 20 g ammonium monohydrogen phosphate, $(NH_4)_2HPO_4$, and 100 g ammonium chloride, NH_4Cl, in distilled water and dilute to 1 L.

(*3*) S–3 Dissolve 18 g calcium chloride, $CaCl_2$; 180 g magnesium chloride, $MgCl_2.6H_2O$; 130 g potassium chloride, KCl; 2 g manganous chloride, $MnCl_2.4H_2O$; 3 g cobalt chloride,$CoCl_2.4H_2O$; 0.6 g boric acid, H_3BO_3; 0.23 g cupric chloride, $CuCl_2$; 1.0 g sodium molybdate, $Na_2MoO_4.2H_2O$ and 0.2 g zinc chloride, $ZnCl_2$, in distilled water and dilute to 1 L.

(*4*) S–4 Dissolve 368 g ferrous chloride, $FeCl_2.4H_2O$, in distilled water and dilute to 1 L.

(*5*) S–5 Dissolve 50 g sodium sulfide, $Na_2S.9H_2O$, in distilled water and dilute 1L.

(B) *Reagents.* Sodium bicarbonate, $NaHCO_3$.

(iii) *Inoculum.* (A) The inoculum should consist of sludge from an anaerobic sludge digestor. It is recommended that well-mixed primary sludge from a digestor with a sludge retention time of 15 to 25 days be used. At the time of collection, the sludge should be sieved through a 2 mm mesh screen.

(B) Most sludges can be stored for up to 2 weeks at 4 °C, if necessary, but it is recommended that fresh sludge be used.

(C) Care should be taken to minimize exposure of the sludge to oxygen during collection, handling and storage.

(2) *Procedure*—(i) *Inoculated medium.* (A) Prereduced medium is prepared by adding 8 mL of stock solution S–1, 8 mL of S–2 and 40 mL of S–3 to approximately 3500 mL of deionized water in a 4-liter Florence or Erlenmeyer flask. This medium is heated to a boil, while being stirred with a magnetic stir bar and sparged with oxygen (O_2)-free nitrogen. The O_2-free nitrogen is obtained by passing nitrogen gas through a quartz cylinder filled with copper filings heated to 600 °C. Alternatively, commercial nitrogen free of oxygen may be used.

(B) The flask containing the medium is placed in an ice bath and O_2-free carbon dioxide (CO_2) is introduced into the stream of O_2-free nitrogen to a concentration in the gas stream of 30 percent (v/v).

(C) When the medium has cooled to 35 °C, the flask is removed from the ice bath and the following components added: 4 mL of solution S–4; 40 mL of solution S–5; 10.56 g sodium bicarbonate; and 400 mL of sludge inoculum. The final volume should be approximately 4 liters.

(ii) *Filling test bottles.* (A) One-hundred mL portions of the inoculated medium are transferred anaerobically into serum bottles with a total capacity of about 160 mL. An apparatus suitable for the maintenance of anaerobic conditions during medium preparation and transfer is shown in Figure 1. V1 and V2 are valves that are used to control the transfer of medium to the serum bottles. Inoculated medium is drawn into the pipet by suction, the pipet is moved and the tip inserted into a serum bottle. During these processes the serum bottle and neck of the medium flask are continually sparged with the oxygen-free mixture of N_2 and CO_2.

(B) The medium in the pipet is discharged into the serum bottle.

(C) A new butyl rubber serum-bottle stopper is inserted into the neck of the bottle while the needle being used to sparge the contents with the N_2 and CO_2 mixture is removed.

(iii) *Test and reference chemicals.* (A) Test and reference chemicals are added to serum bottles to yield a final concentration of 50 mg/L as organic carbon. These chemicals may be added to the bottles prior to the addition of inoculated medium or following medium addition, depending upon the nature of the test or reference substance and whether or not it must be added to the bottles dissolved in a volatile solvent.

(B) Test or reference chemicals with sufficiently high water solubility may be added to test bottles from a neutralized stock solution. The stock solution should be prepared so that a minimal volume is needed to yield 50 mg/L as organic carbon in the medium.

(C) Suitable liquid test or reference chemicals may be added directly by injection from a calibrated syringe.

(D) Test or reference chemicals with relatively low water solubility may be added to test bottles by a direct addition of weighed amounts or by using an organic solvent. Direct addition is recommended. If a volatile organic solvent is used, a suitable procedure is to dissolve the chemical in the solvent, pipet an appropriate amount into the bottle, allow the solvent to evaporate, and then add the inoculated medium. Diethyl ether is a suitable solvent for many organics but it must be completely removed from the bottle because it will adversely affect methanogenesis. If an organic solvent is used without removal of the solvent before the test, the solvent must neither significantly inhibit nor contribute to apparent gas production. Acetonitrile, dioxane and pyridine have been found acceptable for this purpose.

(E) Bottles containing inoculated medium but no test or reference chemical are employed in each test. These are the blank controls.

(F) If an organic solvent is used to add chemical to test bottles without evaporation of the solvent before the test, bottles containing inoculated medium and an equivalent amount of the organic solvent, but no test or reference chemical, must be employed for each solvent used. These are the solvent controls.

(G) The substance bottles, blank controls and solvent controls, should be prepared in triplicate.

(iv) *Incubation.* (A) At the start of the incubation, pressure in each bottle must be released.

(B) Bottles are incubated in the dark at 35±1 °C for 8 weeks or until biodegradation is complete. Bottles containing oxidized (pink) resazurin should be discarded.

(3) *Analytical measurements.* (i) A sufficient number of measurements of gas pressure or gas volume should be made to establish the pattern of gas production with time. Measurements are generally made weekly for up to 8 weeks. The frequency of measurements may be varied by the investigator as deemed appropriate to match the degradation rate of the chemical.

(ii) Gas production is measured for each bottle, using a pressure transducer, syringe or other suitable apparatus.

(iii) The use of a pressure transducer is recommended. The ohmmeter should be calibrated by injecting known volumes of gas into bottles containing medium and a standard curve of gas volume vs. meter reading plotted. Excess pressure should be vented after each measurement so that all bottles will have the same internal pressure following each measurement time.

(iv) If a syringe is used to measure gas volume, the following procedure is recommended. The syringe is flushed with 30 percent (v/v) CO_2 in O_2-free nitrogen. The syringe is held in a horizontal position during the measurement, taking care to keep the needle within the gas space of the serum bottle. Gas production is determined by allowing the syringe plunger to move freely to equalize the vessel and atmospheric pressures.

(v) Methane and carbon dioxide may be determined using analytical methods suitable for the detection and quantification of those compounds.

(c) *Data and reporting*—(1) *Treatment of results.* (i) Cumulative average gas volume from the anaerobic biodegradation of test or reference substances is calculated by subtracting the cumulative average gas volume production for triplicate blank controls (or solvent controls, if an organic solvent was included) from the average value for triplicate test or reference substance bottles at the same incubation time. The percent of theoretical gas volume produced is calculated by dividing cumulative average gas volume from test or reference chemical by the theoretical maximum gas production and multiplying by 100.

(ii) The maximum methane plus carbon dioxide production theoretically obtainable from an organic chemical in this test is 10.5 mL, if the starting concentration is 50 mg/L as organic carbon. This can be calculated as shown below, using benzoic acid as an example.

$$C_7H_6O_2 + 12\ H_2O \rightarrow 7CO_2 + 15\ H_2$$
$$7\ CO_2 + 15\ H_2 \rightarrow 3.75\ CH_4 + 3.25\ CO_2 + 7.5\ H_2O$$
$$C_7H_6O_2 + 4.5\ H_2O \rightarrow 3.75\ CH_4 + 3.25\ CO_2$$

(iii) At a concentration of 50 mg/L as organic carbon in a 100 mL aqueous phase at 35 °C, the maximum volume of gas produced is calculated as follows:

At 50 mg/L as carbon, in 100 mL there are 7.27 mg benzoic acid.

7.27 mg benzoic acid = 0.0595 m moles.
$0.0595 \times 3.75 = 0.2232$ m moles CH_4
$0.0595 \times 3.25 = 0.1934$ m moles CO_2

= 0.4167 m moles total gas production.

At 35° and atmospheric pressure, one mole of gas occupies approximately 25.25 liters.

Thus, 0.4167 m moles at 35° will occupy 10.5 mL.

(iv) Likewise, any test compound added at a concentration that provides 5 mg of organic carbon to the test bottle will have a theoretical maximum gas production of 10.5 mL.

(2) *Test report.* (i) Information on the inoculum including information on the source, retention time, percent volatile solids, date of collection, storage, handling and adaption possibilities (i.e., information on the possibility that the inoculum was exposed to the test chemical or related chemicals before the test). Retention time and percent volatile solids of the sludge are generally obtainable from the waste treatment plant operator.

(ii) Average cumulative gas production (mL) from blank control bottles, solvent control bottles, test substance bottles and reference compound bottles at each measurement time.

(iii) Percent of theoretical anaerobic biodegradation for each test substance and reference compound at each measurement time.

(iv) The standard deviation for each replicate set of bottles at the final measurement time.

(v) A plot of the percent of theoretical anaerobic biodegradation vs. time for each test substance and reference compound.

(vi) A description of any deviations from this test guideline, such as variations in the medium or the concentration of test substance, test conditions, and analytical techniques.

(d) *References.* For additional background information on this test guideline, the following references should be consulted:

(1) Gossett, J.M., Healy, J.B., Stuckey, D.C., Young, L.Y., and McCarty, P.L. "Heat treatment of refuse for increasing

anaerobic biodegradability." *Stanford University Civil Engineering Technical Report No. 205,* (1976).

(2) Healy, J.B., Owen, W.F., Stuckey, D.C., Young, L.Y., and McCarty, P.L. "Heat treatment of organics for increasing anaerobic biodegradability," *Stanford University Civil Engineering Technical Report No. 222,* (1977).

(3) Healy, J.B. and Young, L.Y. "Degradation of simple aromatic compounds under methanogenic conditions," *Abstracts—Annual Meeting American Society for Microbiology,* Vol. 013 (1977), p. 263.

(4) U.S. Environmental Protection Agency. Healy, J.B. and Young, L.Y. "Methanogenic biodegradation of aromatic compounds," Workshop: Microbial Degradation of Pollutants in Marine Environments, EPA Report No. 600/9-79-012 (Gulf Breeze, Florida, 1978).

(5) Owen, W.F., Stuckey, D.C., Healy, J.B., Jr., Young, L.Y. and McCarty, P.L. "Bioassay for Monitoring Biochemical Methane Potential and Anaerobic Toxicity," *Water Research,* 13:485-492 (1979).

(6) Healy, J.B., Jr., and Young, L.Y. "Anaerobic Biodegradation of Eleven Aromatic Compounds to Methane," *Applied Environmental Microbiology,* 38:84-89 (1979).

(7) Miller, T.C., and Wolin, M.J. "A Serum Bottle Modification of the Hungate Technique for Cultivating Obligate Anaerobes," *Applied Microbiology,* 27:985-987 (1974).

§ 796.3500 Hydrolysis as a function of pH at 25 °C.

(a) *Introduction—(1) Background and purpose.* (i) Water is one of the most widely distributed substances in the environment. It covers a large portion of the earth's surface as oceans, rivers, and lakes. The soil also contains water, as does the atmosphere in the form of water vapor. As a result of this ubiquitousness, chemicals introduced into the environment almost always come into contact with aqueous media. Certain classes of these chemicals, upon such contact, can undergo hydrolysis, which is one of the most common reactions controlling chemical stability and is, therefore, one of the main chemical degradation paths of these substances in the environment.

(ii) Since hydrolysis can be such an important degradation path for certain classes of chemicals, it is necessary, in assessing the fate of these chemicals in the environment, to know whether, at what rate, and under what conditions a substance will hydrolyze. Some of these reactions can occur so rapidly that there may be greater concern about the products of the transformation than about the parent compounds. In other cases, a substance will be resistant to hydrolysis under typical environmental conditions, while, in still other instances, the substance may have an intermediate stability that can result in the necessity for an assessment of both the original compound and its transformation products. The importance of transformation of chemicals via hydrolysis in aqueous media in the environment can be determined quantitatively from data on hydrolysis rate constants. This hydrolysis Test Guideline represents a test to allow one to determine rates of hydrolysis at any pH of environmental concern at 25 °C.

(2) *Definitions and units.* (i) "Hydrolysis" is defined as the reaction of an organic chemical with water, such that one or more bonds are broken and the reaction products of the transformation incorporate the elements of water (H_2O).

(ii) "Elimination" is defined in this Test Guideline to be a reaction of an organic chemical (RX) in water in which the X group is lost. These reactions generally follow the same type of rate laws that hydrolysis reactions follow and, thus, are also covered in this Test Guideline.

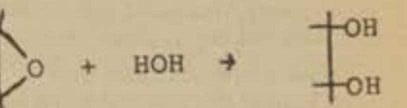
(iii) A "first-order reaction" is defined as a reaction in which the rate of disappearance of the chemical substance being tested is directly proportional to the concentration of the chemical substance and is not a function of the concentrations of any other substances present in the reaction mixture.

(iv) The "half-life" of a chemical is defined as the time required for the concentration of the chemical substance being tested to be reduced to one-half its initial value.

(v) "Hydrolysis" refers to a reaction of an organic chemical with water such that one or more bonds are broken and the reaction products incorporate the elements of water (H_2O). This type of transformation often results in the net exchange of a group X, on an organic chemical RX, for the OH group from water. This can be written as:

$RX + HOH \rightarrow ROH + HX$.

(A) Another result of hydrolysis can be the incorporation of both H and OH in a single product. An example of this is the hydrolysis of epoxides, which can be represented by



(B) The hydrolysis reaction can be catalyzed by acidic or basic species, including OH^- and H_3O^+ (H^+). The promotion of the reaction by H_3O^- or OH^- is called specific acid or specific base catalysis, respectively, as contrasted with general acid or base catalysis encountered with other cationic or anionic species. Usually, the rate law for chemical RX can be written as:

Equation 1

$$-d[RX]/_{dt} = k_h[RX] = k_A[H^+][RX] + k_B[OH^-][RX] + k'_N[H_2O][RX],$$

where K_A, k_B and k'_N are the second-order rate constants for acid and base catalyzed and neutral water processes, respectively. In dilute solutions, such as are encountered in following this Test Guideline, water is present in great excess and its concentration is, thus, essentially constant during the course of the hydrolysis reaction. At fixed pH, the reaction, therefore, becomes pseudo first-order, and the rate constant (k_h) can be written as:

Equation 2

$$k_h = k_A[H^+] + k_B[OH^-] + k_N,$$

where k_N is the first-order neutral water rate constant. Since this is a pseudo first-order process, the half-life is independent of the concentration and can be written as:

Equation 3

$$t_{1/2} = 0.693/k_h.$$

At constant pH, Equation 1 can be integrated to yield the first order rate expression

Equation 4

$$\log_{10} C = -(k_h t/_{2.303}) + \log_{10} C_o,$$

where C is the concentration of the test chemical at time t and C_o is the initial chemical concentration (t=0).

(C) At a given pH, Equation 2 under paragraph (a)(2)(v)(B) of this section contains three unknowns, k_A, k_B, and k_N. Therefore, three equations (i.e., measurements at three different pH's at a fixed temperature) are required if one wishes to solve for these quantities. Making suitable approximations for quantities that are negligible, the expressions for k_A, k_B, and k_N using values of k_h measured at pH 3, 7, and 11 are:

Equation 5

$$k_A = 10^3 [k_h (3) - k_h (7) + 10^{-4} k_h (11)]$$
$$k_B = 10^3 [k_h (11) - k_h (7) + 10^{-4} k_h (3)]$$
$$k_N = k_h (7) - 10^{-4} [k_h (3) + k_h (11)]$$

The calculated rate constants from equation 5 under this paragraph can be employed in equation 2 under paragraph (a)(2)(v)(B) of this section to calculate the hydrolysis rate of a chemical at any pH of environmental concern.

(D) The equations under paragraph (a)(2) of this section apply whether the test chemical has one or more hydrolyzable groups. In the latter case, the rate may be written as:

Equation 6

$$-d[RX]/_{dt} = [RX] + k_2 [RX] + \ldots + k_n [RX] = (k_1 + k_2 + \ldots k_n) [RX] = k_h [RX].$$

Equation 6 applies to the hydrolysis rate of a molecule having n hydrolyzable groups, each of which follows first-order reaction kinetics. The measured k_h is now the sum of the individual reaction rates and is the only rate constant required in this section.

(3) *Principle of the test method.* Procedures described in this section enable sponsors to obtain quantitative information on hydrolysis rates through a determination of hydrolysis rate constants and half-lives of chemicals at pH 3.00, 7.00, and 11.00 at 25 °C. The three measured rate constants are used to determine the acidic, basic, and neutral rate constants associated with a hydrolytic reaction. The latter constants can then be employed in determining the hydrolysis rates of chemicals at any pH of environmental concern at 25 °C.

(4) *Applicability and specificity.* There are several different common classes of organic chemicals that are subject to hydrolysis transformation, including esters, amides, lactones, carbamates, organophosphates, and alkyl halides. Processes other than nucleophilic displacement by water can also take place. Among these are elimination reactions that exhibit behavior similar to hydrolysis and, therefore, are also covered in this section.

(b) *Test procedures*—(1) *Test conditions*—(i) *Special laboratory equipment.* (A) A thermostatic bath that can be maintained at a temperature of 25±1 °C.

(B) A pH meter that can resolve differences of 0.05 pH units or less.

(C) Stoppered volumetric flasks (no grease) or glass ampoules that can be sealed.

(ii) *Purity of water.* Reagent-grade water (e.g., water meeting ASTM Type IIA standards or an equivalent grade) is highly recommended to minimize biodegradation. ASTM Type IIA water is described in ASTM D 1193–77, "Standard Specification for Reagent Water." ASTM D 1193–77 is available for inspection at the Office of the Federal Register, Rm. 8401, 1100 L St., NW., Washington, DC. This incorporation by reference was approved by the Director of the Office of the Federal Register. This material is incorporated as it exists on the date of approval and a notice of any change in this material will be published in the **Federal Register.** Copies of the incorporated material may be obtained from the Document Control Officer (TS–793), Office of Toxic Substances, EPA, Rm 107, 401 M St., SW., Washington, DC 20460, and from the American Society for Testing and Materials (ASTM), 1916 Race Street, Philadelphia, PA 19103.

(iii) *Sterilization.* It is extremely important to sterilize all glassware and to use aseptic conditions in the preparation of all solutions and in carrying out all hydrolysis experiments to eliminate or minimize biodegradation. Glassware can be sterilized in an autoclave or by any other suitable method.

(iv) *Precautions for volatility.* If the chemical is volatile, it is extremely important that the reaction vessels be almost completely filled and sealed.

(v) *Temperature controls.* It is important that all hydrolysis reactions be carried out at 25 °C and the temperature is controlled to ±1°C.

(vi) *pH conditions.* It is recommended that all hydrolysis experiments be performed at pH 3.00, 7.00, and 11.00 ± 0.05 using the appropriate buffers described in paragraph (b)(2)(i)(A) of this section.

(vii) *Concentration of solutions of chemical substances.* It is extremely imprtant that the concentration of the test chemical be less than one-half the chemical's solubility in water and not greater than 10^{-3} M.

(viii) *Effect of acidic and basic groups.* Complications can arise upon measuring the rate of hydrolysis of chemicals that reversibly ionize or are protonated in the pH range 3.00 to 11.00. Therefore, for these chemicals, it is recommended that these hydrolysis tests be performed at pH 5.00, 7.00, and 900±0.05 using the appropriate buffers described in paragraph (b)(2)(i)(A) and (B) of this section. If a test chemical reversibly ionizes or protonates in the pH range 5.00 to 9.00, then it is recommended that additional hydrolysis tests should be carried out at pH 6.00 and 8.00±0.05 using the buffers described in paragraph (b)(2)(i)(B) of this section.

(ix) *Buffer catalysis.* For certain chemicals, buffers may catalyze the hydrolysis reaction. If this is suspected, it is extremely important that hydrolysis rate determinations be carried out with the appropriate buffers and that the same experiments be repeated at buffer concentrations lowered by at least a factor of five. If the hydrolysis reaction produces a change of greater than 0.05 pH units in the lower concentration buffers at the end of the measurement time, then it is extremely important that the test chemical concentrations also be lowered by at least a factor of five. Alternatively, test chemical concentrations and buffer concentrations may both be lowered simultaneously by a factor of five. A sufficient criterion for minimization of buffer catalysis is an observed equality in the hydrolysis rate constant of two different solutions differing in buffer or test chemical concentration by a factor of five.

(x) *Photosensitive chemicals.* The solution absorption spectrum can be employed to determine whether a particular chemical is potentially subject to photolytic transformation upon exposure to light. For chemicals that absorb light of wavelengths greater than 290 nm, it is important that the hydrolysis experiment be carried out in the dark, under amber or red safelights, in amber or red glassware, or employing other suitable methods for preventing photolysis. The absorption spectrum of the chemical in aqueous solution can be measured under § 796.1050.

(xi) *Chemical analysis of solutions.* In determining the concentrations of the test chemicals in solution, any suitable analytical method may be employed, although methods which are specific for the compound to be tested are preferred. Chromatographic methods are recommended because of their compound specificity in analyzing the parent chemical without interferences from impurities. Whenever practicable, the chosen analytical method should have a precision within ±5 percent.

(2) *Preparation*—(i) *Reagents and solutions*—(A) *Buffer solutions.* Prepare buffer solutions using reagent-grade chemicals and reagent-grade water as follows:

(*1*) pH 3.00 use 250 mL of 0.100M potassium hydrogen phthalate; 111 mL of 0.100M hydrochloric acid; and adjust volume to 500 mL with reagent-grade water.

(*2*) pH 7.00 use 250 mL of 0.100M potassium dihydrogen phosphate; 145 mL of 0.100M sodium hydroxide; and adjust volume to 500 mL with reagent-grade water.

(*3*) pH 11.00 use 250 mL of 0.0500M sodium bicarbonate; 113 mL of 0.100M sodium hydroxide; and adjust volume to 500 mL with reagent-grade water.

(B) *Additional buffer solutions.* For chemicals that ionize or are protonated as discussed in paragraph (b)(1)(viii) of this section, prepare buffers using reagent-grade water and reagent-grade chemicals as follows:

(*1*) pH 5.00 use 250 mL of 0.100M potassium hydrogen phthalate; 113 mL of 0.100M sodium hydroxide; and adjust volume to 500 mL with reagent-grade water.

(*2*) pH 6.00 use 250 mL of 0.100M potassium dihydrogen phosphate; 28 mL of 0.100M sodium hydroxide; and adjust volume to 500 mL with reagent-grade water.

(*3*) pH 8.00 use 250 mL of 0.100M potassium dihydrogen phosphate; 234 mL of 0.100M sodium hydroxide; and adjust volume to 500 mL with reagent-grade water.

(*4*) pH 9.00 use 250 mL of 0.0250M borax ($Na_2B_4O_7$); 23 mL of 0.100M hydrochloric aid; and adjust volume to 500 mL with reagent-grade water.

(C) *Adjustment of buffer concentrations.* (*1*) The concentrations of all the above buffer solutions are the maximum concentrations to be employed in carrying out hydrolysis measurements. If the initial concentration of the test chemical is less than 10^{-3}M, it is extremely important that the buffer concentrations be lowered by a corresponding amount; e.g., if the initial test chemical concentration is 10^{-4}M, then reduce the concentration of the above buffers by a factor of 10. In addition, for those reactions in which an acid or base is not a reaction product, then employ the minimum buffer concentration necessary for maintaining the pH within ±0.05 units.

(*2*) Check the pH of all buffer solutions with a pH meter at 25 °C and adjust the pH to the proper value, if necessary.

(D) *Preparation of test solution.* (*1*) If the test substance is readily soluble in water, prepare an aqueous solution of the chemical in the appropriate buffer and determine the concentration of the chemical. Alternatively, a solution of the chemical in water may be prepared and added to an appropriate buffer solution and the concentration of the chemical then determined. In the latter case, it is important that the aliquot be small enough so that the concentration of the buffer in the final solution and the pH of the solution remain essentially unchanged. Do not employ heat in dissolving the chemical. It is extremely important that the final concentration not be greater than one-half the substance's solubility in water and not greater than 10^{-3}M.

(*2*) If the test chemical is too insoluble in pure water to permit reasonable handling and analytical procedures, it is recommended that the chemical be dissolved in reagent-grade acetonitrile and buffer solution then added to an aliquot of the acetonitrile solution. Do not employ heat to dissolve the chemical in acetonitrile. It is extremely important that the final concentration of the test substance not be greater than one-half the chemical's solubility in water and not greater than 10^{-3}M. In addition, it is extremely important that the final concentration of the acetonitrile be one volume percent or less.

(3) *Performance of the test.* Carry out all hydrolysis experiments by employing one of the procedures described in this paragraph. Prepare the test solutions as described in paragraph (b)(2)(i) of this section at pH 3.00, 7.00, and 11.00±0.05, and determine the initial test chemical concentration (C_o) in triplicate. Analyze each reaction mixture in triplicate at regular intervals, employing one of the following procedures:

(i) *Procedure 1.* Analyze each test solution at regular intervals to provide a minimum of six measurements with the extent of hydrolysis between 20 and 70 percent. Rates should be rapid enough so that 60 to 70 percent of the chemical is hydrolyzed in 672 hours.

(ii) *Procedure 2.* If the reaction is too slow to conveniently follow hydrolysis to high conversion in 672 hours but still rapid enough to attain at least 20 percent conversion, take 15 to 20 time points at regular intervals after 10 percent conversion is attained.

(iii) *Procedure 3.* (A) If chemical hydrolysis is less than 20 percent after 672 hours, determine the concentration (C) after this time period.

(B) If the pH at the end of concentration measurements employing any of the above three procedures has changed by more than 0.05 units from the initial pH, repeat the experiment using a solution having a test chemical concentration lowered sufficiently to keep the pH variation within 0.05 pH units.

(iv) *Analytical methodology.* Select an analytical method that is most applicable to the analysis of the specific chemical being tested under paragraph (b)(1)(xi) of this section.

(c) *Data and reporting*—(1) *Treatment of results.* (i) If Procedure 1 or 2 were employed in making concentration measurements, use a linear regression analysis with Equation 4 under paragraph (a)(2)(v)(B) of this section to calculate k_h at 25 °C for each pH employed in the hydrolysis experiments. Calculate the coefficient of determination (R^2) for each rate constant. Use Equation 3 under paragraph (a)(2)(v)(B) of this section to calculate the hydrolysis half-life using k_h.

(ii) If Procedure 3 was employed in making rate measurements, use the mean initial concentration (C_o) and the mean concentration of chemical (C) in Equation 4 under paragraph (a)(2)(v)(B) of this section to calculate k_h for each pH used in the experiments. Calculate the hydrolysis half-life using k_h in Equation 3 under paragraph (a)(2)(v)(B) of this section.

(iii) For each set of three concentration replicates, calculate the mean value of C and the standard deviation.

(iv) For test chemicals that are not ionized or protonated between pH 3 and 11, calculate k_A, k_B, and k_N using Equation 5.

(2) *Specific analytical and recovery procedures.* (i) Provide a detailed description or reference for the analytical procedure used, including the calibration data and precision.

(ii) If extraction methods were used to separate the solute from the aqueous solution, provide a description of the extraction method as well as the recovery data.

(3) *Test data report.* (i) For procedures 1 and 2, report k_h, the hydrolysis half-life ($t_{1/2}$), and the coefficient of determination (R^2) for each pH employed in the rate measurements. In addition, report the individual values, the mean value, and the standard deviation for each set of replicate concentration measurements. Finally, report k_A, k_B, and k_N.

(ii) For Procedure 3, report k_h and the half-life for each pH employed in the rate measurements. In addition, report the individual values, the mean value, and the standard deviation for each set of replicate concentration measurements. Finally, report k_A, k_B, and k_N.

(iii) If, after 672 hours, the concentration (C) is the same as the initial concentration (C_o) within experimental error, then k_h cannot be calculated and the chemical can be reported as being persistent with respect to hydrolysis.

§ 796.3700 Photolysis in aqueous solution in sunlight.

(a) *Introduction*—(1) *Background and purpose.* Numerous chemicals enter natural aquatic systems from a variety of sources. For example, chemical wastes are discharged directly into natural water bodies, and chemicals leach into natural water bodies from landfills. Pesticides are applied directly into water bodies, and are applied to soils and vegetation, and subsequently leach into water bodies. Pollutants present in aqueous media can undergo photochemical transformation in the environment (i.e., in sunlight by direct photolysis or by sensitized photolysis). As a result, there is considerable interest in photolysis in solution, especially the photolysis of pesticides. However, most of these studies have been qualitative in nature and involved the identification of photolysis products. Quantitative data in the form of rate constants and half-lives are needed to determine the importance of photochemical transformation of pollutants in aqueous media. This test method describes a two-tiered screening level approach for determining direct photolysis rate constants and half-lives of chemicals in water in sunlight.

(2) *Definitions and units.* (i) "Radiant energy," or radiation, is defined as the energy traveling as a wave unaccompanied by transfer of matter. Examples include x-rays, visible light, ultraviolet light, radio waves, etc.

(ii) "Absorbance (A_λ)" is defined as the logarithm to the base 10 of the ratio of the initial intensity (I_o) of a beam of radiant energy to the intensity (I) of the same beam after passage through a sample at a fixed wavelength λ. Thus, $A_\lambda = \log_{10}(I_o/I)$.

(iii) The "Beer-Lambert law" states that the absorbance of a solution of a given chemical species, at a fixed wavelength, is proportional to the thickness of the solution (l), or the light pathlength, and the concentration of the absorbing species (C).

(iv) "Molar absorptivity (ϵ_λ)" is defined as the proportionality constant in the Beer-Lambert law when the concentration is given in terms of moles per liter (i.e., molar concentration). Thus, $A_\lambda = \epsilon_\lambda Cl$, where A_λ and ϵ_λ represent the absorbance and molar absorptivity at wavelength λ and *l* and C are defined in (3). The units of ϵ_λ are $molar^{-1} cm^{-1}$. Numerical values of molar absorptivity depend upon the nature of the absorbing species.

(v) A "first-order reaction" is defined as a reaction in which the rate of disappearance of a chemical is directly proportional to the concentration of the chemical and is not a function of the concentration of any other chemical present in the reaction mixture.

(vi) The "half-life ($t_{1/2}$)" of a chemical is defined as the time required for the concentration of the chemical being tested to be reduced to one-half its initial value.

(vii) The "sunlight direct aqueous photolysis rate constant (k_{pE})" is the first-order rate constant in the units of day^{-1} and is a measure of the rate of disappearance of a chemical dissolved in a water body in sunlight.

(viii) The "solar irradiance in water (L_λ)" is related to the sunlight intensity in water and is proportional to the average light flux (in the units of 10^{-3} einsteins $cm^{-2} day^{-1}$) that is available to cause photoreaction in a wavelength interval centered at λ over a 24-hour day at a specific latitude and season date.

(ix) "The Grotthus-Draper law," the first law of photochemistry, states that only light which is absorbed can be effective in producing a chemical transformation.

(x) The "Stark-Einstein law," the second law of photochemistry, states that only one molecule is activated to an excited state per photon or quantum of light absorbed.

(xi) The "reaction quantum yield (ϕ_λ)" for an excited-state process is defined as the fraction of absorbed light that results in photoreaction at a fixed wavelength λ. It is the ratio of the number of molecules that photoreact to the number of quanta of light absorbed or the ratio of the number of moles that photoreact to the number of einsteins of light absorbed at a fixed wavelength λ.

(xii) "Direct photolysis" is defined as the direct absorption of light by a chemical followed by a reaction which transforms the parent chemical into one or more products.

A glossary of symbols can be found under paragraph (c)(5) of this section.

(3) *Principle of the test method.* (i) This test method is based on the principles developed by Zepp and Cline (1977) under paragraph (d)(8) of this section, Zepp (1978) under paragraph (d)(11) of this section, Mill et al. (1981, 1982) under paragraph (d)(4), (5), and (6) of this section, and Dulin and Mill (1982) under paragraph (d)(2) of this section.

(ii) Zepp and Cline (1977), under paragraph (d)(8) of this section published a paper on the rates of direct photolysis in aquatic environments. The rates of all photochemical processes in a water body are affected by solar spectral irradiance at the water surface, radiative transfer from air to water, and the transmission of sunlight in the water body. It has been shown that for photolysis of a chemical in an optically thin aqueous solution, the kinetics of direct photolysis can be described by the following equations:

Equation 1

$$\ln (C_o/C_t) = k_{pE}t$$

Equation 2

$$t_{1/2E} = 0.693/k_{pE}$$

Equation 3

$$k_{pE} = \phi_E k_a,$$

where ϕ_E is the reaction quantum yield of the chemical in dilute solution and is independent of the wavelength, $k_a = \Sigma k_{a\lambda}$, the sum of $k_{a\lambda}$ values of all wavelengths of sunlight that are absorbed by the chemical (i.e., the light absorption rate constant), t is the time, C_o and C_t are the concentrations of chemical at t=0 and t, and $t_{1/2E}$ represents the half-life. The term k_{pE} represents the first-order photolysis rate constant for a water body in sunlight in the units of reciprocal time.

(iii) Furthermore, under the same conditions cited above, the first-order direct photolysis rate constant, k_{pE}, is given by the equation—

Equation 4

$$k_{pE} = \phi_E \Sigma \epsilon_\lambda L_\lambda,$$

where ϕ_E is the reaction quantum yield, $\epsilon\lambda$ is the molar absorptivity in the units $molar^{-1} cm^{-1}$, L_λ is the solar irradiance in water in the units of 10^{-3} einsteins $cm^{-2} day^{-1}$ [Mill et al. (1982) under paragraph (d)(5) of this section], and the summation is taken over the range λ = 290 to 800 nm. L_λ is the solar irradiance at shallow depths for a water body under clear sky conditions and is a function of latitude and season of the year.

(iv) The method of Zepp and Cline (1977) under paragraph (d)(8) of this section and the method of Mill et al. (1982) under paragraph (d)(5) of this section are applicable to sunlight incident on a water surface such as natural water body. However, the method developed in this guideline measures rate constants in tubes (e.g., 13×100 mm) and the rate is faster in tubes. This is discussed in more detail in paragraph (b)(2)(i)(J) of this section. Thus, equations 1 and 2 have to be modified to take this into account. For simplicity, the following nomenclature is used. For water bodies, the rate constant is designated as k_{pE} with the subscript E designating rates in the environment in water bodies. For tubes, the rate constant is designated as k_p. The corresponding half-lives for water bodies and tubes are $t_{1/2E}$ and $t_{1/2}$, respectively. Thus, for tubes, equations 1 and 2 can be written as:

Equation 5

$$\ln(C_o/C_t) = k_pt$$

Equation 6

$$t_{1/2} = \frac{0.693}{k_p}.$$

(v) A simple first-tier screening test has been developed using Equation 4 under paragraph (a)(3)(iii) of this section. As an approximation, it is assumed that the reaction quantum yield ϕ_E is equal to one, the maximum value. As a result, the upper limit for the direct photolysis sunlight rate constant in aqueous solution is obtained and Equation 4 under paragraph (a)(3)(iii) of this section becomes

Equation 7

$(k_{pE})_{max} = \Sigma\epsilon_\lambda L_\lambda$.

Using equation 7 in equation 2 under paragraph (a)(3)(ii) of this section, the lower limit for the half-life is then given by

Equation 8

$$(t_{1/2E})_{min} = \frac{0.693}{(k_{pE})_{max}}.$$

The molar absorptivity can be determined experimentally by the method outlined in paragraph (b)(1) of this section and values of L_λ are given in Tables 3 to 6 as a function of latitude and season of the year under paragraph (c)(3) of this section. These data can then be used in equation 7 to calculate $(k_{pE})_{max}$. Finally, $(k_{pE})_{max}$ can then be substituted in Equation 8 to calculate $(t_{1/2E})_{min}$.

(vi) In a second-tier test method, an aqueous photolysis screening test has been developed to determine rate constants and half-lives in the presence of sunlight using Equations 1, 2, 4, 5, and 6 [Mill et al. (1981, 1982, under paragraph (d) (4), (5) and (6) of this section, and Dulin and Mill (1982) under paragraph (d)(2) of this section]. The second-tier test method is divided into two phases. In phase one, the test chemical is photolyzed in sunlight in order to obtain an approximate rate constant, k^c_p. This method only gives an approximate rate constant since it fails to measure sunlight intensities incident on the sample during photolysis.

(vii) In phase two, a standard p-nitroacetophenone-pyridine actinometer (PNAP/PYR) is used to measure sunlight intensities incident on the sample during photolysis [Mill et al. (1982) under paragraph (d)(6) of this section and Dulin and Mill (1982) under paragraph (d)(2) of this section]. The rate constant for this actinometer, k^a_p, can be adjusted to match the approximate rate constant of the test chemical by adjusting the concentration of pyridine. Since the rate constant is a function of the reaction quantum yield of the actinometer, the rate constant can be adjusted according to the equation

Equation 9

$\phi^a_E = 0.0169[PYR]$,

where [PYR] is the molar concentration of pyridine for a p-nitroacetophenone (PNAP) concentration of 1.00×10^{-5} M. The reaction quantum yield for the test chemical, ϕ^c_E, is given by

Equation 10

$$\phi^c_E = \frac{k^c_p \, \Sigma\phi^a_\lambda L_\lambda}{k^a_p \, \Sigma\phi^c_\lambda L_\lambda} \phi^a_E.$$

The reaction quantum yield of the test chemical, ϕ^c_E, can be determined in the following way. By measuring the concentration of test chemical and actinometer (PNAP) as a function of time t in sunlight, the ratio of rate constants, (k^c_p/k^a_p), can be determined using equation 5 under paragraph (b)(2)(i)(H) of this section. The reaction quantum yield ϕ^a_E can be determined from Equation 9 at the molar concentration of pyridine used in the standard actinometer. The term $\Sigma\epsilon^a_\lambda L_\lambda$ for the actinometer has been tabulated as a function of latitude and season of the year in Table 2 under paragraph (c)(3) of this section. The term $\Sigma\epsilon^c_\lambda L_\lambda$ for the test chemical can be obtained from the experimentally measured molar absorptivities under paragraph (b)(1) of this section and the values of L_λ listed in Tables 3 to 6, as a function of latitude and season of the year under paragraph (c)(3) of this section.

(viii) With the values of ϕ^c_E, ϵ^c_λ, and the appropriate L_λ values, k_{pE} for the test chemical can be calculated as a function of latitude and season of the year in the United States using Equation 4 under paragraph (a)(3)(iii) of this section. The corresponding half-life can be calculated using k_{pE} in Equation 2 under paragraph (a)(3)(ii) of this section.

(4) *Applicability and specificity.* (i) This test method is applicable to all chemicals which have UV-visible absorption maxima in the range of 290 to 800 nm. Some chemicals have absorption maxima significantly below 290 nm and consequently cannot undergo direct photolysis in sunlight (e.g., chemicals such as alkanes, alkenes, alkynes, saturated alcohols, and saturated acids). This is a direct consequence of the Grotthus-Draper law of photochemistry. Some chemicals have absorption maxima significantly below 290 nm but have measurable absorption tails above the baseline in their absorption spectrum at wavelengths greater than 290 nm. Photolysis experiments should be carried out for these chemicals.

(ii) These test methods are only applicable to pure chemicals and not to the technical grade.

(iii) The first-tier screening test can be employed to estimate $(k_{pE})_{max}$ and $(t_{1/2E})_{min}$. If these data indicate that aqueous photolysis is an important process relative to other transformation processes (e.g., biodegradation, hydrolysis, oxidation, etc.), then it is recommended that the second-tier photolysis tests be carried out to determine environmentally relevant rate constants and half-lives in sunlight. The data obtained from this test can be used to determine k_{pE} for the test chemical as a function of latitude and season of the year anywhere in the United States. These rate constants are in a form suitable for preliminary mathematical modeling for environmental fate of a test chemical.

(iv) The second-tier screening test is applicable to the direct photolysis of chemicals in a homogeneous dilute solution with absorbance less than 0.05 in the reaction cell at all wavelengths greater than 290 nm and at shallow depths (less than 0.5 m). These results are applicable to direct sunlight photolysis for water bodies and clear sky conditions. In addition, these experiments are limited to the direct photolysis of chemicals in air-saturated pure water.

(v) This screening test has been designed to determine the molar absorptivity of a test chemical, ϵ_λ^c, and its reaction quantum yield, ϕ^c_E. These parameters can be used to determine environmentally relevant rate constants at low absorbance and shallow depths in pure water as a function of latitude and season of the year. Tables of solar irradiance (Tables 3 to 6) under paragraph (c)(3) of this section have been included in this test method to carry out all the calculations. However, the method is really very general and can be extended to determine the rates of photolysis over a range of other environmental conditions using a computer program. Zepp and Cline (1977) under paragraph (d)(8) of this section have written a computer program to calculate the rates of photolysis as a function of depth in water, as a function of the attenuation coefficient of the water (α_λ) for natural water bodies, the average ozone layer thickness that pertains to the seasons and location of interest, and as a function of latitude and season of the year. This program has been recently updated with the best available solar irradiance data and is called the GC SOLAR program. The GC SOLAR computer program is available on request as referenced under paragraph (d)(10) of this section.

(b) *Test procedures*—(1) *Tier 1 Test: UV-Visible Absorption Spectra-Estimation of Aqueous Photolysis Maximum Rate Constant and Minimum Half-Life in Sunlight.* The uv-visible absorption spectra in aqueous solution can be determined by the methods described in § 796.1050. It is recommended that the following additional procedures be followed:

(i) For chemicals which ionize or protonate (e.g., carboxylic acids, phenols, amines), carry out uv-visible absorption studies at pHs at least two orders or magnitude above the pK_a and at least two orders of magnitude below the pK_a. Prepare buffer solutions at 25 °C using reagent grade chemicals and distilled water as follows:

pHs in the range 3–6[1]: NaH_2PO_4HCl;
pHs in the range 6–8[1]: KH_2PO_4 NaOH;
pHs in the range >8: Prepare buffers as described in the Handbook of Chemistry and Physics.

Check the pH of all the buffer solutions with a pH meter at 25 °C and adjust to the proper pH, if necessary. These buffer solutions can then be added to the test chemical solution until the desired pH is obtained. If these buffers are inadequate, then adjust the pH of the test chemical solution with 1 M HCl or NaOH at 25 °C.

(ii) (A) Measure the absorbance, A_λ, as a function of wavelength in the range of 290 to 800 nm in duplicate. If applicable, measure A_λ at each experimental pH. Record, in duplicate, the baseline when both the sample and reference cells are filled with blank solutions. These data will be used to calculate the molar absorptivities for the appropriate wavelength intervals and wavelength centers in Table 1 under paragraph (c)(3) of this section, where the test chemical absorbs light. The wavelength center is defined as the midpoint of the interval range.

(B) It must be emphasized that the molar absorptivities of the test chemical must be carefully determined, especially in the tails of the absorption bands at λ >290 nm. Large errors will be encountered in calculating photolysis rate constants and half-lives if these measurements are not carefully carried out.

(2) *Tier 2 Test: Aqueous Photolysis in Sunlight*—(i) *Test conditions*—(A) *Special laboratory equipment.* It is recommended that quartz tubes be used for the photolysis of chemicals with appreciable absorption at wavelengths below 340 nm. Chemicals that absorb appreciably at wavelengths greater than 340 nm may be tested in borosilicate tubes. Thin-walled borosilicate or quartz tubes are recommended. Disposable culture tubes (13 x 100 mm) with Teflon-lined screw caps or quartz tubes with quartz or borosilicate stoppers, Teflon-lined, may be used as reaction vessels. Tubes of 11 mm i.d. are recommended. For some chemicals, it may be difficult to determine the concentration of the test chemical in reaction tubes of small volume. For these chemicals, larger volume reaction vessels are recommended provided that the cell walls are thin and the pathlength of radiation through the vessel is less than 0.5 meter.

(B) *Purity of water.* Reagent grade water, e.g., water meeting ASTM Type II A standards, or an equivalent grade, is highly recommended to minimize biodegradation. ASTM Type II A water is described in ASTM D 1193–77, "Standard Specification for Reagent Water." Air-saturated water can be easily prepared by allowing the water to equilibrate in a vessel plugged with sterile cotton. ASTM D 1193–77 is available for inspection at the Office of the Federal Register, Rm. 8401, 1100 L St., NW., Washington, DC. This incorporation by reference was approved by the Director of the Office of the Federal Register. This material is incorporated as it exists on the date of approval and a notice of any change in this material will be published in the Federal Register. Copies of the incorporated material may be obtained from the Document Control Officer (TS–793), Office of Toxic Substances, EPA, Rm. 107, 401 M St., SW., Washington, DC 20460, and from the American Society for Testing and Materials (ASTM), 1916 Race Street, Philadelphia, PA 19103.

(C) *Sterilization.* It is extremely important to sterilize all glassware and to use aseptic conditions in the preparation of all solutions and in carrying out all photolysis experiments to eliminate or minimize biodegradation. Glassware can be sterilized in an autoclave or by any other suitable non-chemical method.

(D) *pH effects.* It is recommended that all photolysis experiments be carried out at pHs at least two orders of magnitude above the pK_a and at least two orders of magnitude below the pK_a for any chemical which ionizes or protonates (e.g., carboxylic acids, phenols, and amines). Buffers described in paragraph (b)(2)(ii)(B) of this section should be used.

(E) *Volatile chemical substances.* Special care should be taken when testing a volatile chemical so that the chemical substance is not lost due to volatilization during the course of the photolysis experiment. Thus, it is important to effectively seal the reaction vessels. Disposable culture tubes with Teflon-lined screw caps or quartz tubes with quartz or borosilicate stoppers, Teflon-lined, are recommended. Volatile compounds can be conveniently studied in culture tubes equipped with Mininert® valves. Samples can be introduced into or removed from the tubes through the septum in these valves with no loss of substrate. As an alternative, the tubes can be sealed with a torch. In addition, the reaction vessels should be as completely filled as is possible to prevent volatilization to any air space.

(F) *Control solution.* It is extremely important to take certain precautions to prevent loss of chemical from the reaction vessels by processes other than photolysis. For example, biodegradation and volatilization can be eliminated or minimized by use of sterile conditions and minimal airspace in sealed vessels. Hydrolysis is a process which cannot be minimized by such techniques. Thus, control vessels containing test substances which are not exposed to sunlight are required. In this way, the loss of test chemical for processes other than photolysis may be determined and eliminated. For simplicity, if the loss of chemical in the control is small (i.e., approximately 10 percent or less), one can calculate a first-order loss, k_{loss}, and subtract it from $(k_p)_{obs}$ to give the corrected direct photolysis rate constant k_p. If hydrolysis is found to be significant (i.e., greater than 10 percent), hydrolysis studies should be carried out first under § 796.3500.

(G) *Absorption spectrum as a criterion for performing the Aqueous Photolysis Test.* This aqueous photolysis screening test is applicable to all chemicals which have UV-visible absorption maxima in the range 290 to 800 nm. Some chemicals have absorption maxima significantly below 290 nm but have measurable absorption tails above the baseline in their absorption spectrum at wavelengths greater than 290 nm. Photolysis experiments should be carried out for these chemicals. The absorption spectrum of the chemical in aqueous solution can be measured by § 796.1050.

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[1] Use the minimum concentration of buffers to attain the desired pH.

(H) *Sunlight Actinometer.* (*1*) In order to quantify the rate of photolysis more precisely, it is necessary to measure the sunlight intensity incident on the sample during photolysis. A standard p-nitroacetophenone-pyridine actinometer (PNAP/PYR) has been developed [Mill et al. (1981, 1982) under paragraph (d)(4) and (6) of this section; Dulin and Mill (1982) under paragraph (d)(2) of this section] to measure the sunlight intensity incident on the sample during photolysis and this actinometer has been incorporated in this section. According to Equation 4 under paragraph (a)(3)(iii) of this section, the rate constant is a function of the reaction quantum yield. Furthermore, the reaction quantum yield can be adjusted by varying the molar concentration of the pyridine according to Equation 9 under paragraph (a)(3)(vii) of this section. Hence, by varying the pyridine concentration, the actinometer photolysis rate constant can be adjusted so that the half-life can range from several hours to several weeks. The initial concentration of PNAP is set at 1.00×10^{-5} M.

(*2*) Using the test chemical photolysis rate constant, k_p^c, determined in Tier 2, Phase 1, and the variable $k_a^a(=\Sigma\epsilon_\lambda^a L_\lambda)$, listed in Table 2 under paragraph (c)(3) of this section the molar concentration needed to adjust the rate of disappearance of PNAP in PNAP/PYR to match the rate of disappearance of the test chemical is given by

Equation 11

$$[PYR]=26.9\,(k_p^c/k_a^a).$$

(*3*) Experiments are carried out by simultaneously photolyzing the test chemical and actinometer solutions. The concentrations of test chemical and actinometer are measured periodically as a function of time. These data are then used to determine the ratio of the rate constants, k_p^c/k_p^a, using linear regression analysis on the following equation:

Equation 12

$$\ln(C_o/C_t)^c=(k_p^c k_p^a)\ln(C_o/C_t)^a,$$

with $\ln(C_o/C_t)^a$ as the independent variable and $\ln(C_o/C_t)^c$ as the dependent variable. The slope of the best straight line is the ratio of the rate constants, k_p^c/k_p^a.

(I) *Solar irradiance data.* In order to calculate the reaction quantum yield of the test chemical, ϕ_E^c, and then calculate k_{pE}^c and $t_{1/2E}$, it is $t_{1/2E}$ necessary to use the solar irradiance parameter L_λ• L_λ values are proportional to the average light flux that is available to cause photolysis in a wavelength interval centered at λ over a 24-hour day at a specific latitude and season date. The L_λ values are defined by the angle of declination of the sun at −20° for winter, −10° for fall, +10° for spring, and +20° for summer. The actual dates for 1982 that correspond to these angles of declination are January 21, April 16, July 24, and October 20, for winter, spring, summer, and fall, respectively [AA (1982) under paragraph (d)(1) of this section]. The L_λ values for these season dates are listed in Tables 3 to 6 under paragraph (c)(3) of this section as a function of latitude and are applicable to clear sky conditions, water bodies, shallow depths, and for chemicals whose absorbance is less than 0.05 in pure water [Mill et al. (1984) under paragraph (d)(7) of this section].

(J) *Geometry of the reaction vessel.* The method of Zepp and Cline (1977) under paragraph (d)(8) of this section and the method of Mill et al., (1982) under paragraph (d)(5) of this section are applicable to sunlight incident on a water surface such as a natural water body while the method developed in this test method measures rate constants (k_p) in tubes (e.g., 13 × 100 mm). However, rates in tubes are faster than in water bodies and it has been experimentally observed [Mill et al. (1982) under paragraph (d)(6) of this section] that

Equation 13

$$k_p=2.2k_{pE}.$$

Because tubes are the simplest and easiest reaction vessels to use, this test method recommends the use of tubes as reaction vessels and the method has been modified to take into account the increased rate in tubes (equation 13).

(K) *Chemical analysis of solution.* (*1*) In determining the concentration of the chemical in solution, an analytical method should be selected which is most applicable to the analysis of the specific chemical substance. Chromatographic methods are generally recommended because of their chemical specificity in analyzing the parent chemical substance without interference from impurities. Whenever practicable the chosen analytical method should have a precision of ±5 percent or better.

(*2*) The p-nitroacetophenone in the chemical actinometer solution is conveniently analyzed by high-pressure liquid chromatography using a 30 cm C_{18} reverse-phase column and a uv detector set at 280 nm. The mobile phase in volume percent is 2.5 percent acetic acid, 50 percent acetonitrile, and 47.5 percent water which is passed through the column at a flow rate of 2 mL/minute.

(ii) *Preparations*—(A) *Preparation of test chemical solution.* Prepare homogeneous solutions with the chemical at less than one-half of its solubility in water and at a concentration such that the absorbance is less than 0.05 in the photolysis reaction vessel at wavelengths greater than 290 nm. For very hydrophobic chemicals, it is difficult and time consuming to prepare aqueous solutions. To facilitate the preparation of aqueous solutions containing very hydrophobic chemicals and to allow for easier analytical measurement procedures, the following procedure may be used to aid in the dissolution of the chemical. Dissolve the pure chemical in reagent grade acetonitrile. Add pure water as described under Test Conditions, in paragraph (b)(2)(i)(B) of this section, or buffer solution as described under Preparations, in paragraph (b)(2)(ii)(B) of this section, for chemical substances which ionize or protonate, to an aliquot of the acetonitrile solution. Do not exceed one volume-percent of acetonitrile in the final solution. Place the reaction solution in the appropriate photolysis reaction tubes as described in paragraph (b)(2)(i)(A) of this section.

(B) *Preparation of buffer solutions.* Prepare buffer solutions according to the procedures outlined in paragraph (b)(1)(i) of this section using reagent grade chemicals and pure water as described under Test Conditions, in paragraph (b)(2)(i)(B) of this section.

(C) *Preparation of actinometer solution.* (*1*) Using the test chemical photolysis rate constant, k_p^c, determined in Tier 2, Phase 1, and the variable k_a^a listed in Table 2 under paragraph (c)(3) of this section, the molar concentration of pyridine needed to adjust the rate of disappearance of p-nitroacetophenone (PNAP) to match the rate of disappearance of the test chemical can be obtained from equation 11 under paragraph (b)(2)(i)(H)(*2*) of this section. The variable $k_a^a\ (=\Sigma\epsilon_\lambda^a L_\lambda)$ is equal to the day-average rate constant for sunlight absorption by PNAP which changes with season and latitude. The value of k_a^a is selected from Table 2 under paragraph (c)(3) of this section for the season nearest the mid-experiment date of the Tier 2, Phase 1, studies and the decadic latitude nearest the latitude of the experimental site.

(*2*) Once the molar concentration of pyridine [PYR] has been determined, an actinometer solution can be prepared as follows. Dissolve 0.165 gm. of PNAP in 100 mL of acetonitrile (0.01 M). Add 1 mL of this solution to a one liter volumetric flask. Add to the volumetric flask the mass in grams, or the volume (V) of pyridine at 20° C, obtained from the equations

Equation 14

$mass(grams) = 79.1\ [PYR]$

$V(mL) = 80.6\ [PYR]$.

Fill the volumetric flask with pure water in paragraph (b)(2)(i)(B) of this section to give 1 liter of solution and shake vigorously to make sure that the solution is homogeneous. The PNAP/PYR solution should be wrapped with aluminum foil and kept from bright light.

(iii) *Performance of the tests*—(A) *Phase 1 experiments.* (*1*) For all experiments, prepare an aqueous solution of the chemical substance, as described in paragraph (b)(2)(ii)(A) of this section, and a sufficient number of samples in quartz or borosilicate glass tubes to perform all the required tests. Fill the tubes as completely as possible and seal them. Prepare two control samples in the absence of ultraviolet light and totally exclude light by wrapping the tubes with aluminum foil or by other suitable methods. These samples are analyzed for the chemical substance immediately after completion of the experiment to measure the loss of chemical in the absence of light. Place the samples, including the controls, outdoors in an area free of shade and reflections of sunlight from windows and buildings. Place the samples on a black, nonreflective background and inclined at approximately 30° from the horizontal with upper end pointing due north (in the northern hemisphere). Conduct the photolysis experiments during a frost-free time of year (e.g., May, June, July, August, or September in the northern hemisphere—weather permitting) and start the experiments initially at noon (1,200 hours). Record the date and time the experiment was begun, the date and time completed, the time of sunrise and sunset on all days when photolysis experiments were performed, the times exposure was stopped and restarted for intermittent exposure, the weather conditions during the period, and the latitude of the site. For chemical substances that ionize or protonate, carry out photolysis experiments at the required pHs as described under Test Conditions under paragraph (b)(2)(i)(D) of this section.

(*2*) If a significant loss of test chemical has occurred in the control samples, determine the cause and eliminate or minimize the loss. If hydrolysis is found to be significant, hydrolysis studies should be carried out first under paragraph (b)(2)(i)(F) of this section.

(*3*) Use one of the following procedures, depending on how fast the chemical substance photolyzes.

(*i*) *Procedure 1.* If the chemical substance transforms 50 to 80 percent within 28 days, measure the concentration of the chemical substance, in duplicate, at time $t=0$ and periodically (at least four data points at approximately equal time intervals) at noon (1200 hours) until at least 50 percent of the substance has been consumed. As a simplification, the sampling times can be estimated as the photolysis experiments progress. Determine the concentration of test chemical from two, freshly opened, reaction tubes for each time point. Determine the concentration in each of the two control solutions as soon as the photolysis experiments are completed.

(*ii*) *Procedure 2.* If the chemical substance transforms in the range of 20 to 50 percent in 28 days, determine the concentration of the chemical substance, in duplicate, at time $t=0$. Determine the concentration of the chemical in the two separate reaction tubes and the two control tubes after 28 days of photolysis.

(*iii*) *Procedure 3.* For chemical substances that transform in sunlight 50–80 percent within one or two days, place the samples outside at noon (1200 hours) and analyze two samples for the concentration of the chemical substance at $t=0$, and in two, freshly opened, reaction tubes at noon (1200 hours) the next day, and again, in two, freshly opened, reaction tubes at noon (1200 hours) the second day. Determine the concentration of the test chemical in each of the two control solutions after the first day of photolysis and as soon as the photolysis experiments have been completed on the second day.

(*iv*) *Analytical methodology.* Select an analytical method which is most applicable to the analysis of the specific chemical being tested under paragraph (b)(2)(i)(K) of this section.

(B) *Phase 2 experiments.* (*1*) Using the test chemical photolysis rate constant, k^{c}_{p}, determined in Tier 2, Phase 1, prepare an actinometer solution, as described in paragraph (b)(2)(ii)(C) of this section and a sufficient number of samples in quartz tubes to perform all the required tests. Fill all the tubes as completely as possible, seal them, and cover them with aluminum foil as soon as possible after preparation. Prepare an aqueous solution of test chemical, as described in paragraph (b)(2)(ii)(A) of this section, and a sufficient number of samples in quartz or borosilicate tubes to perform all the required tests. Fill these tubes as completely as possible, seal them, and cover them with aluminum foil as soon as possible after preparation. Place all the samples outdoors in an area free of shade and reflections of sunlight from windows and buildings. Place the samples on a black, nonreflective background and inclined at approximately 30° from the horizontal with the upper end pointing due north (in the northern hemisphere). Remove the foil from all samples except for the test chemical control solutions and the actinometer control solutions at noon (1200 hours). Based on the results of the Phase 1 experiments, determine the concentration of test chemical and actinometer (PNAP), in triplicate, at time $t=0$ and periodically (at least five data points at approximately equal time intervals). Determine the concentration of PNAP in the three actinometer control solutions and the concentration of test chemical in the three control solutions for each time point.

(*2*) Select an analytical method which is most applicable to the analysis of the specific chemical tested, in paragraph (b)(2)(i)(K) of this section and follow the procedure given in paragraph (b)(2)(i)(K) of this section for the analysis of PNAP.

(c) *Data and reporting*—(1) *Tier 1 Test: UV-Visible Absorption Spectra—Estimation of Aqueous Photolysis Maximum Rate Constant and Minimum Half-Life in Sunlight*—(i) *Treatment of results.* (A) The molar absorptivity can be determined from the absorption spectra using the expression.

Equation 15

$\epsilon_{\lambda}^{c} = A_{\lambda}/Cl$,

where A_{λ} is the absorbance at wavelength λ, C is the molar concentration of test chemical, and l is the cell pathlength in centimeters. The molar absorptivity of the chemical should be determined for the wavelengths listed in Table 1 under paragraph (c)(3) of this section for a solution of concentration C and in a cell with pathlength, *l*. If the absorption curve is flat within the interval around the wavelength $_{\lambda}$center, ϵ_{λ} may be determined from the absorbance A_{λ} at $_{\lambda}$center using equation 15. If a large change in absorbance occurs within this interval, obtain an average absorbance A_{λ} at $_{\lambda}$center based on the absorbances at the two boundaries of the interval. Calculate an average ϵ_{λ} using the average value of A_{λ} in equation 15. Determine the molar absorptivity for each replicate and calculate a mean value.

(B) Using the molar absorptivities obtained from the spectra and the values of the L_{λ} from Tables 3 to 6 under paragraph (c)(3) of this section, the maximum rate constant $(k_{pE})_{max}$, can be calculated at a specific latitude and season of the year using equation 7 under paragraph (a)(3)(v) of this section. The minimum half-life, $(t_{1/2E})_{min}$, can then be calculated using this $(k_{pE})_{max}$, in equation 8 under paragraph (a)(3)(v) of this section.

(C) Two hypothetical examples are presented in paragraph (c)(4)(i) of this section to illustrate how the test data obtained in the first-tier screening test can be used.

(ii) *Test data report.* (A) Submit the original chart, or photocopy, containing a plot of absorbance of test chemical vs. wavelength plus the baseline. Spectra should include a readable wavelength scale, preferably marked at 10 nm intervals. Each spectrum should be clearly marked with the test conditions.

(B) Report the concentration of the test chemical solution, the type of absorption cell used (quartz or borosilicate glass) and the pathlength.

(C) Report A_λ and ϵ_λ at $_\lambda$center for each replicate and the mean value.

(D) Report $(k_{pE})_{max.}$ and $(t_{1/2E})_{min.}$ for the summer and winter solstices using the appropriate L_λ values from Tables 3–6 closest to the latitude of the chemical manufacturing site.

(E) Report the identity and compositon of the solvent used in the spectral absorption study.

(F) For ionizable chemicals, report its pK_a. Report the type and concentration of the buffers employed for each pH. Report the pHs in which the photolysis experiments were carried out.

(G) Describe the method employed in determining the test chemical's concentration.

(H) Report the name, structure, and purity of the test chemical.

(I) Submit a recent test spectrum on appropriate reference chemicals for photometric and wavelength accuracy.

(J) Report the name and model of the spectrophotometer used.

(K) Report the various control settings employed with the spectrophotometer. These might include scan speed, slit width, gain, etc.

(2) *Tier 2 Test: Aqueous Photolysis in Sunlight*—(i) *Phase 1 experiments*—(A) *Treatment of results.* (*1*) If a small loss of test substance in the control tubes has occurred, use this data to make corrections to the measured photolysis rate in paragraph (b)(2)(i)(F) of this section. Note the site of photolysis and its latitude and the weather conditions. For procedures 1 and 2 note the dates and times of actual exposure including times of sunrise and sunset and, in case the cells are moved to prevent freezing or for other reasons, make sure that these times are recorded and that the cells are kept in a dark place when exposure is not in progress.

(*i*) For chemical substances which transform 50 to 80 percent within 28 days, use a concentration C_t, which corresponds to less than 50 percent of the initial concentration of chemical substance remaining, and the corresponding time t, in days, along with the initial molar concentration C_o, in Equation 5 to calculate k_p in days^{-1}. From the analysis of the two samples at time t=0 and t, calculate a mean value of C_o and C_t, respectively, and a value of k_p. If a slight loss of chemical has been detected in the controls, then calculate a rate constant as follows: Calculate an average concentration C_t, based on the duplicate measurements of concentration in the controls. Use this concentration along with the average initial concentration in Equation 5 and calculate a rate constant k_{loss}. Using this rate constant along with the observed rate constant, the corrected rate constant is then

Equation 16

$$k_p=(k_p)_{obs.}-k_{loss.}$$

Calculate the half-life, $t_{1/2}$ using the corrected k_p value in Equation 6 under paragraph (a)(3)(iv) of this section.

(*ii*) For chemical substances which transform 20–50 percent in 28 days, use the mean concentration C_t remaining at t=28 days along with the mean value of C_o to calculate k_p. Use the same procedure as described above to calculate the value of k_p and $t_{1/2}$. If less than 20 percent of the chemical substance degrades in 28 days, report the mean concentration of C_t and C_o. In this case the apparent half-life is reported as greater than 3 months.

(*iii*) For chemical substances which transform 50 percent or more in the first day, as described in procedure 3, calculate a full day k_p value using the mean concentration C_t of chemical substance remaining at noon (1200 hours) after the first day along with the mean value of C_o using Equation 5 under paragraph (a)(3)(iv) of this section. For chemical substances which degrade less than 50 percent at noon (1200 hours) after the first day but 50 percent or more at noon (1200 hours) the second day, calculate k_p using the mean concentration of chemical substances remaining at noon (1200 hours) the second day. Calculate the half-life, $t_{1/2}$, using the mean value of k_p in Equation 6 under paragraph (a)(3)(iv) of this section. If a small loss of test substance in the control tubes has occurred, use this data to make corrections to the measured photolysis rate as described. Note the dates of photolysis, the latitude, and the site.

(*2*) A hypothetical example is presented in paragraph (c)(4)(ii) of this section, to illustrate how the test data obtained in the Tier 2, Phase 1, test method can be used.

(B) *Specific analytical and recovery procedures.* (*1*) Provide a detailed description or reference for the analytical procedures used, including the calibration data and precision.

(*2*) If extraction methods were used to separate the solute from the aqueous solution, provide a description of the extraction method as well as the recovery data.

(C) *Other test conditions.* (*1*) Report the size, approximate cell wall thickness, and type of glass used for the reaction tubes.

(*2*) Report the initial pH of all test solutions, if appropriate.

(*3*) For all procedures, report the dates of photolysis, the time of sunrise and sunset on each photolysis day, the site of photolysis and its latitude, and the weather conditions. For Procedures 1 and 2 submit the dates and times of actual exposure, and the duration of exposure, and, for intermittent exposure, the fraction of each day during which photolysis occurred.

(*4*) If acetonitrile was used to solubilize the test substance, report the percent, by volume.

(*5*) If a significant loss of test chemical occurred in the control solution, indicate the causes and how they were eliminated or minimized.

(D) *Test data report.* (*1*) For each photolysis experiment, report:

(*i*) The initial molar concentration of test chemical (C_o) of each replicate and the mean value.

(*ii*) The molar concentration of test chemical for each replicate and the mean value for each time point t.

(*iii*) The molar concentration of each replicate control sample and the mean value after completion of the photolysis experiments.

(*2*) For procedure 1, 2, or 3, report the value of k_p. If small losses of chemical are observed, report $(k_p)_{obs.}$, k_{loss} and k_p. Report the half-life ($t_{1/2}$) calculated using the value of k_p.

(ii) *Phase 2 experiments.*—(A) *Treatment of results.* (*1*) The objectives of this set of experiments is to determine the sunlight reaction quantum yield, ϕ^c_E, for a specific test chemical. ϕ^c_E can be calculated using equation 10 under paragraph (a)(3)(vii) of this section,

$$\phi^c_E=\frac{k^c_p}{k^a_p}\frac{\Sigma\epsilon^a_\lambda L_\lambda}{\Sigma\epsilon^c_\lambda L_\lambda}\phi^a_E$$

by the following steps:

(*i*) Determine the ratio of the rate constants, k^a_p/k^c_p, as described in paragraph (b)(2)(i)(H) of this section using Equation 12. If a slight loss of test chemical or actinometer (PNAP) was detected in the controls at any time t, then employ the following procedure

Consider, as an example, the loss of test chemical in the control at time t. Using the average concentration of the test chemical in the controls from the replicates at time t and the average initial concentration, calculate $\ln(C_o/C_t)^c_{loss}$. Using the average concentration of test chemical from the replicates after photolysis time t, calculate $\ln(C_o/C_t)^c_{obs.}$. The corrected term is then

Equation 17

$$\ln(C_o/C_t)^c_{corr} = \ln(C_o/C_t)^c_{loss}$$

The same procedure can be applied to obtain a corrected term from the actinometer (PNAP). Using the corrected terms for test chemical and/or actinometer in equation 12 under paragraph (b)(2)(i)(H)(*3*) of this section, determine the ratio of the rate constants (k^a_p/k^c_p) as described in paragraph (b)(2)(i)(H) of this section.

(*ii*) Determine the quantum yield of the actinometer, ϕ^a_E, using equation 9 and the molar concentration of pyridine [PYR] present in the actinometer.

(*iii*) Determine the value of $\Sigma\epsilon^c_\lambda L_\lambda$ for the test chemical as follows: the molar absorptivities, ϵ^c_λ, have been determined by the procedure given in paragraph (b)(1) of this section and the results have been tabulated according to paragraph (c)(1)(ii) of this section. Choose the appropriate L_λ values (Tables 3 to 6 under paragraph (c)(3) of this section) that correspond to the season closest to the season in which the Phase 2 experiments were performed and to the latitude nearest the latitude of the experimental site. Calculate the product of ϵ^c_λ and L_λ for each wavelength interval where ϵ_λ has a nonzero value. Sum the products of $\epsilon^a_\lambda L_\lambda$ over all wavelength intervals.

(*iv*) Determine the value of $\Sigma\epsilon_\lambda{}^a L_\lambda$, for the actinometer, as follows: These values have been calculated and are given in Table 2 under paragraph (c)(3) of this section. Choose the appropriate value that corresponds to the season closest to the season in which the Phase 2 experiments were performed and to the latitude nearest the latitude of the experimental site.

(*v*) Substitute the values of k^a_p/k^c_p, ϕ^a_E, $\Sigma\epsilon^a_\lambda L_\lambda$, and $\Sigma\epsilon^c_\lambda L_\lambda$ in Equation 10 under paragraph (a)(3)(iii) of this section and calculate ϕ^c_E, the quantum yield of the test chemical in the environment (i.e., in sunlight).

(*2*) Once ϕ^c_E has been determined, equation 4 under paragraph (a)(3)(iii) of this section can be used to calculate k_{pE} at any season of the year and latitude using the measured values of the molar absorptivities, ϵ^c_λ, and the appropriate L_λ values (Tables 3 to 6 under paragraph (c)(3) of this section). The half-life can then be calculated using k_{pE} in Equation 2 under paragraph (a)(3)(ii) of this section.

(*3*) A hypothetical example is presented in paragraph (c)(4)(iii) of this section, to illustrate how the test data obtained in the Tier 2, Phase 1, test method can be used.

(B) *Other test conditions.* (*1*) Report the size, approximate cell wall thickness, and type of glass used for tubes to hold the test chemical and actinometer solutions.

(*2*) Report the initial pH of all test chemical solutions, if appropriate, and the type and concentration of the buffers employed for each pH.

(*3*) If acetonitrile was used to solubilize the test chemical, report the percent, by volume, of the acetonitrile, which was used.

(*4*) If significant loss of test chemical occurred in the control solution, indicate the causes and how they were eliminated or minimized.

(C) *Test data report.* (*1*) Report the initial molar concentration of chemical (C_o) of each replicate and the mean value.

(*2*) Report the initial molar concentration of PNAP and the molar concentration of pyridine used in the actinometer.

(*3*) Report the time and date the sunlight photolysis experiments were started, the time and date the experiments were completed, and the elapsed photolysis time in days.

(*4*) For each time point, report the three separate values for the molar concentration of test chemical and PNAP and the mean values.

(*5*) For each time point, report the three separate values of the molar concentration of test chemical and PNAP for the controls and the mean values.

(*6*) Tabulate and report the following data: t, $\ln(C_o/C_t)^c$, and $\ln(C_o/C_t)^a$. From the linear regression analysis, report the ratio of the rate constants, $k_p{}^c/k_p{}^a$, and the correlation coefficient.

(*7*) If loss of test chemical and/or actinometer was observed during photolysis, then report the data $\ln(C_o/C_t)_{corr.}$, $\ln(C_o/C_t)_{obs.}$, $\ln(C_o/C_t)_{loss}$ for the test chemical and/or actinometer at each time t. From the linear regression analysis of $\ln(C_o/C_t)^c_{corr.}$ and $\ln(C_o/C_t)^a_{corr.}$, report the ratio of the rate constants, $k_p{}^c/k_p{}^a$ and the correlation coefficient.

(*8*) Report the reaction quantum yield of the actinometer (ϕ^a_E).

(*9*) Report the value of $k_a{}^a$ for the actinometer corresponding to the season closest to the season in which the photolysis experiments were carried out and to the latitude nearest the latitude of the experimental site.

(*10*) Tabulate the values of λcenter, $\epsilon_\lambda{}^c$, L_λ, and $\epsilon_\lambda{}^c L_\lambda$ for the test chemical corresponding to the season closest to the season in which the photolysis experiments were carried out and to the latitude nearest the latitude of the experimental site.

(*11*) Report the value $-\Sigma\epsilon^c_\lambda L_\lambda$ for the test chemical from step 10.

(*12*) Report the reaction quantum yield of the test chemical.

(*13*) Report k_{pE} and $t_{1/2E}$ for the summer and winter seasons using the appropriate L_λ values from Tables 3–6 under paragraph (c)(3) of this section closest to the latitude of the chemical manufacturing site.

(*14*) For chemicals that ionize, report the data for steps 1–13 for the experiments at the required pHs.

(3) *Tables of solar irradiance and related tables.*

TABLE 1—WAVELENGTH CENTER AND INTERVALS FOR L_λ

λ center (nm)	Interval from (nm)	Range to (nm)	Δλ (nm)
297.5	296.2	298.7	2.5
300.0	298.7	301.2	2.5
302.5	301.2	303.7	2.5
305.0	303.7	306.2	2.5
307.5	306.2	308.7	2.5
310.0	308.7	311.2	2.5
312.5	311.2	313.7	2.5
315.0	313.7	316.2	2.5
317.5	316.2	318.7	2.5
320.0	318.7	321.2	2.5
323.1	321.2	325.0	3.8
330.0	325.0	335.0	10.0
340.0	335.0	345.0	10.0
350	345.0	355.0	10.0
360	355.0	365.0	10.0
370	365.0	375.0	10.0
380	375.0	385.0	10.0
390	385.0	395.0	10.0
400	395.0	405.0	10.0
410	405.0	415.0	10.0
420	415.0	425.0	10.0
430	425.0	435.0	10.0
440	435.0	445.0	10.0
450	445.0	455.0	10.0
460	455.0	465.0	10.0
470	465.0	475.0	10.0
480	475.0	485.0	10.0
490	485.0	495.0	10.0
500	495.0	505.0	10.0
525	512.5	537.5	25
550	537.5	562.5	25
575	562.5	587.5	25
600	587.5	612.5	25
625	612.5	637.5	25
650	637.5	662.5	25
675	662.5	687.5	25
700	687.5	712.5	25
750	725.0	775.0	50
800	775.0	825.0	50

TABLE 2—DAY AVERAGED RATE CONSTANT (K^a_a)[1] FOR SUNLIGHT ABSORPTION BY PNAP AS A FUNCTION OF SEASON AND DECADIC LATITUDE

Latitude (degrees north)	Season			
	Spring	Summer	Fall	Winter
20	515	551	409	327
30	483	551	333	233

TABLE 2—DAY AVERAGED RATE CONSTANT (k^a_λ)[1] FOR SUNLIGHT ABSORPTION BY PNAP AS A FUNCTION OF SEASON AND DECADIC LATITUDE—Continued

Latitude (degrees north)	Season			
	Spring	Summer	Fall	Winter
40	431	532	245	139
50	362	496	154	64

[1] $k^a_\lambda = \Sigma \epsilon^a_\lambda L_\lambda$ in day^{-1}.

TABLE 3—L_λ VALUES FOR LATITUDE 20° N.[1][2][3]

λ center (nm)	Spring	Summer	Fall	Winter
297.5	1.10(-4)	1.52(-4)	7.77(-5)	3.71(-5)
300.0	4.06(-4)	5.26(-4)	2.96(-4)	1.62(-4)
302.5	1.10(-3)	1.35(-3)	8.21(-4)	4.99(-4)
305.0	2.37(-3)	2.79(-3)	1.79(-3)	1.17(-3)
307.5	4.24(-3)	4.86(-3)	3.24(-3)	2.25(-3)
310.0	6.65(-3)	7.45(-3)	5.13(-3)	3.72(-3)
312.5	9.42(-3)	1.04(-2)	7.33(-3)	5.47(-3)
315.0	1.24(-2)	1.35(-2)	9.68(-3)	7.40(-3)
317.5	1.54(-2)	1.66(-2)	1.21(-2)	9.38(-3)
320.0	1.82(-2)	1.96(-2)	1.44(-2)	1.13(-2)
323.1	3.23(-2)	3.45(-2)	2.55(-2)	2.04(-2)
330.0	1.10(-1)	1.17(-1)	8.75(-2)	7.06(-2)
340.0	1.37(-1)	1.45(-1)	1.10(-1)	9.02(-2)
350.0	1.52(-1)	1.60(-1)	1.22(-1)	1.01(-1)
360.0	1.67(-1)	1.76(-1)	1.35(-1)	1.12(-1)
370.0	1.78(-1)	1.88(-1)	1.45(-1)	1.21(-1)
380.0	1.89(-1)	2.00(-1)	1.55(-1)	1.30(-1)
390.0	1.79(-1)	1.89(-1)	1.46(-1)	1.22(-1)
400.0	2.57(-1)	2.71(-1)	2.09(-1)	1.75(-1)
410.0	3.38(-1)	3.57(-1)	2.76(-1)	2.31(-1)
420.0	3.47(-1)	3.67(-1)	2.84(-1)	2.38(-1)
430.0	3.35(-1)	3.54(-1)	2.74(-1)	2.30(-1)
440.0	3.95(-1)	4.18(-1)	3.25(-1)	2.72(-1)
450.0	4.45(-1)	4.70(-1)	3.65(-1)	3.07(-1)
460.0	4.50(-1)	4.75(-1)	3.70(-1)	3.11(-1)
470.0	4.65(-1)	4.91(-1)	3.83(-1)	3.22(-1)
480.0	4.76(-1)	5.03(-1)	3.92(-1)	3.31(-1)
490.0	4.50(-1)	4.76(-1)	3.72(-1)	3.13(-1)
500.0	4.59(-1)	4.85(-1)	3.80(-1)	3.20(-1)
525.0	1.21	1.28	1.00	8.48(-1)
550.0	1.26	1.33	1.05	8.83(-1)
575.0	1.27	1.35	1.06	8.92(-1)
600.0	1.29	1.36	1.07	9.05(-1)
625.0	1.29	1.37	1.08	9.15(-1)
650.0	1.30	1.38	1.09	9.24(-1)
675.0	1.30	1.38	1.09	9.27(-1)
700.0	1.29	1.36	1.08	9.21(-1)
750.0	2.48	2.62	2.08	1.78
800.0	2.38	2.51	2.00	1.71

[1] Units of L_λ are 10^{-3} einsteins cm^{-2} day^{-1}. Multiplication of L_λ by ϵ_λ in units of molar^{-1} cm^{-1} gives rate constants in units of day^{-1}.

[2] The second number in the columns in parenthesis is the power of ten by which the first number is multiplied.

[3] Based on the GC SOLAR program.

TABLE 4—L_λ VALUES FOR LATITUDE 30° N.[1][2][3]

λcenter (nm)	Spring	Summer	Fall	Winter
297.5	5.73(-5)	1.09(-4)	3.18(-5)	6.78(-6)
300.0	2.50(-4)	4.11(-4)	1.46(-4)	4.23(-5)
302.5	7.65(-4)	1.14(-3)	4.64(-4)	1.71(-4)
305.0	1.79(-3)	2.46(-3)	1.12(-3)	4.95(-4)
307.5	3.43(-3)	4.45(-3)	2.19(-3)	1.11(-3)
310.0	5.64(-3)	7.02(-3)	3.67(-3)	2.04(-3)
312.5	8.27(-3)	1.00(-2)	5.46(-3)	3.26(-3)
315.0	1.12(-2)	1.32(-2)	7.43(-3)	4.69(-3)
317.5	1.41(-2)	1.64(-2)	9.48(-3)	6.21(-3)
320.0	1.70(-2)	1.95(-2)	1.15(-2)	7.76(-3)
323.1	3.04(-2)	3.46(-2)	2.07(-2)	1.43(-2)
330.0	1.05(-1)	1.18(-1)	7.23(-2)	5.17(-2)
340.0	1.33(-1)	1.48(-1)	9.23(-2)	6.75(-2)
350.0	1.47(-1)	1.63(-1)	1.03(-1)	7.65(-2)
360.0	1.62(-1)	1.80(-1)	1.15(-1)	8.60(-2)
370.0	1.73(-1)	1.91(-1)	1.24(-1)	9.31(-2)
380.0	1.84(-1)	2.04(-1)	1.33(-1)	1.01(-1)
390.0	1.74(-1)	1.93(-1)	1.25(-1)	9.39(-2)
400.0	2.50(-1)	2.77(-1)	1.79(-1)	1.35(-1)
410.0	3.29(-1)	3.64(-1)	2.36(-1)	1.79(-1)
420.0	3.38(-1)	3.74(-1)	2.43(-1)	1.84(-1)
430.0	3.26(-1)	3.61(-1)	2.35(-1)	1.78(-1)
440.0	3.86(-1)	4.26(-1)	2.79(-1)	2.12(-1)

TABLE 4—L_λ VALUES FOR LATITUDE 30° N.[1][2][3]—Continued

λcenter (nm)	Spring	Summer	Fall	Winter
450.0	4.34(-1)	4.79(-1)	3.14(-1)	2.39(-1)
460.0	4.39(-1)	4.85(-1)	3.19(-1)	2.42(-1)
470.0	4.54(-1)	5.01(-1)	3.30(-1)	2.51(-1)
480.0	4.65(-1)	5.13(-1)	3.38(-1)	2.58(-1)
490.0	4.40(-1)	4.85(-1)	3.20(-1)	2.44(-1)
500.0	4.49(-1)	4.95(-1)	3.27(-1)	2.50(-1)
525.0	1.18	1.31	8.67(-1)	6.61(-1)
550.0	1.23	1.36	9.03(-1)	6.87(-1)
575.0	1.24	1.37	9.11(-1)	6.93(-1)
600.0	1.25	1.38	9.24(-1)	7.04(-1)
625.0	1.26	1.39	9.34(-1)	7.15(-1)
650.0	1.27	1.40	9.45(-1)	7.27(-1)
675.0	1.28	1.40	9.48(-1)	7.32(-1)
700.0	1.27	1.39	9.42(-1)	7.31(-1)
750.0	2.44	2.67	1.82	1.41
800.0	2.34	2.57	1.75	1.37

[1] Units of L_λ are 10^{-3} einsteins cm^{-2} day^{-1}. Multiplication of L_λ by ϵ_λ in units of molar^{-1} cm^{-1} gives rate constants in units of day^{-1}.

[2] The second number in the columns in parenthesis is the power of ten by which the first number is multiplied.

[3] Based on the GC SOLAR program.

TABLE 5—L_λ VALUES FOR LATITUDE 40° N.[1][2][3]

λcenter (nm)	Spring	Summer	Fall	Winter
297.5	1.85(-5)	6.17(-5)	7.83(-6)	5.49(-7)
300.0	1.06(-4)	2.70(-4)	4.76(-5)	5.13(-6)
302.5	3.99(-4)	8.30(-4)	1.89(-4)	3.02(-5)
305.0	1.09(-3)	1.95(-3)	5.40(-4)	1.19(-4)
307.5	2.34(-3)	3.74(-3)	1.19(-3)	3.38(-4)
310.0	4.17(-3)	6.17(-3)	2.19(-3)	7.53(-4)
312.5	6.51(-3)	9.07(-3)	3.47(-3)	1.39(-3)
315.0	9.18(-2)	1.22(-2)	4.97(-3)	2.22(-3)
317.5	1.20(-2)	1.55(-2)	6.57(-3)	3.19(-3)
320.0	1.48(-2)	1.87(-2)	8.18(-3)	4.23(-3)
323.1	2.71(-2)	3.35(-2)	1.51(-2)	8.25(-3)
330.0	9.59(-2)	1.16(-1)	5.44(-2)	3.16(-2)
340.0	1.23(-1)	1.46(-1)	7.09(-2)	4.31(-2)
350.0	1.37(-1)	1.62(-1)	8.04(-2)	4.98(-2)
360.0	1.52(-1)	1.79(-1)	9.02(-2)	5.68(-2)
370.0	1.63(-1)	1.91(-1)	9.77(-2)	6.22(-2)
380.0	1.74(-1)	2.04(-1)	1.05(-1)	6.78(-2)
390.0	1.64(-1)	1.93(-1)	9.86(-2)	6.33(-2)
400.0	2.36(-1)	2.76(-1)	1.42(-1)	9.11(-2)
410.0	3.10(-1)	3.64(-1)	1.87(-1)	1.20(-1)
420.0	3.19(-1)	3.74(-1)	1.93(-1)	1.24(-1)
430.0	3.08(-1)	3.61(-1)	1.87(-1)	1.20(-1)
440.0	3.65(-1)	4.26(-1)	2.22(-1)	1.43(-1)
450.0	4.11(-1)	4.80(-1)	2.51(-1)	1.61(-1)
460.0	4.16(-1)	4.85(-1)	2.54(-1)	1.64(-1)
470.0	4.30(-1)	5.02(-1)	2.63(-1)	1.69(-1)
480.0	4.40(-1)	5.14(-1)	2.70(-1)	1.74(-1)
490.0	4.16(-1)	4.86(-1)	2.56(-1)	1.65(-1)
500.0	4.25(-1)	4.96(-1)	2.62(-1)	1.68(-1)
525.0	1.12	1.31	6.93(-1)	4.45(-1)
550.0	1.16	1.36	7.21(-1)	4.61(-1)
575.0	1.17	1.37	7.22(-1)	4.61(-1)
600.0	1.18	1.38	7.39(-1)	4.69(-1)
625.0	1.20	1.40	7.50(-1)	4.82(-1)
650.0	1.21	1.41	7.62(-1)	4.95(-1)
675.0	1.22	1.41	7.68(-1)	5.03(-1)
700.0	1.21	1.40	7.66(-1)	5.05(-1)
750.0	2.33	2.69	1.48	9.84(-1)
800.0	2.25	2.59	1.43	9.56(-1)

[1] Units of L_λ are 10^{-3} einsteins cm^{-2} day^{-1}. Multiplication of L_λ ϵ_λ in the units of molar^{-1} cm^{-1} gives the rate constant in units of day^{-1}.

[2] The second number in the columns in parenthesis is the power of ten by which the first number is multiplied.

[3] Based on the GC SOLAR program.

TABLE 6—L_λ VALUES FOR LATITUDE 50° N.[1][2][3]

λcenter (nm)	Spring	Summer	Fall	Winter
297.5	3.61(-6)	2.86(-5)	9.58(-7)	5.47(-8)
300.0	3.05(-5)	1.50(-4)	8.27(-6)	4.17(-7)
302.5	1.54(-4)	5.33(-4)	4.47(-5)	2.62(-6)
305.0	5.24(-4)	1.39(-3)	1.63(-4)	1.34(-5)
307.0	1.32(-3)	2.89(-3)	4.39(-4)	5.14(-5)
310.0	2.66(-3)	5.05(-3)	9.32(-4)	1.49(-4)
312.5	4.53(-3)	7.75(-3)	1.66(-3)	3.43(-4)
315.0	6.82(-3)	1.06(-2)	2.58(-3)	6.52(-4)

TABLE 6—L_λ VALUES FOR LATITUDE 50° N.[1][2][3]—Continued

λcenter (nm)	Spring	Summer	Fall	Winter
317.5	9.34(-3)	1.40(-2)	3.64(-3)	1.07(-3)
320.0	1.19(-2)	1.71(-2)	4.76(-3)	1.57(-3)
323.1	2.25(-2)	3.12(-2)	9.19(-3)	3.39(-3)
330.0	8.26(-2)	1.10(-1)	3.48(-2)	1.45(-2)
340.0	1.09(-1)	1.40(-1)	4.71(-2)	2.12(-2)
350.0	1.22(-2)	1.57(-1)	5.43(-2)	2.53(-2)
360.0	1.36(-1)	1.74(-1)	6.18(-2)	2.96(-2)
370.0	1.47(-1)	1.86(-1)	6.76(-2)	3.30(-2)
380.0	1.57(-1)	1.99(-1)	7.37(-2)	3.65(-2)
390.0	1.48(-1)	1.87(-1)	6.89(-2)	3.49(-2)
400.0	2.12(-1)	2.69(-1)	9.90(-2)	4.96(-2)
410.0	2.80(-1)	3.55(-1)	1.31(-1)	6.54(-2)
420.0	2.89(-1)	3.65(-1)	1.35(-1)	6.71(-2)
430.0	2.79(-1)	3.52(-1)	1.31(-1)	6.47(-2)
440.0	3.31(-1)	4.17(-1)	1.55(-1)	7.66(-2)
450.0	3.73(-1)	4.69(-1)	1.75(-1)	8.62(-2)
460.0	3.78(-1)	4.75(-1)	1.78(-1)	8.74(-2)
470.0	3.90(-1)	4.91(-1)	1.84(-1)	8.95(-2)
480.0	4.00(-1)	5.03(-1)	1.89(-1)	9.15(-2)
490.0	3.78(-1)	4.76(-1)	1.79(-1)	8.62(-2)
500.0	3.86(-1)	4.85(-1)	1.83(-1)	8.77(-2)
525.0	1.01	1.28	4.84(-1)	2.28(-1)
550.0	1.05	1.33	5.03(-1)	2.32(-1)
575.0	1.05	1.34	5.04(-1)	2.26(-1)
600.0	1.06	1.35	5.13(-1)	2.32(-1)
625.0	1.08	1.37	5.26(-1)	2.42(-1)
650.0	1.10	1.38	5.39(-1)	2.53(-1)
675.0	1.11	1.39	5.47(-1)	2.61(-1)
700.0	1.11	1.38	5.49(-1)	2.66(-1)
750.0	2.15	2.86	2.07	5.22(-1)
800.0	2.08	2.57	1.04	5.11(-1)

[1] Units of L_λ are 10^{-3} einsteins cm^{-2} day^{-1}. Multiplication of L_λ by ϵ_λ in the units of molar^{-1} cm^{-1} gives the rate constant in units of day^{-1}.

[2] The second number in the columns in parenthesis is the power of ten by which the first number is multiplied.

[3] Based on the GC SOLAR program.

(4) Examples of application of methodology—(i) Tier 1 Test: UV-Visible Absorption Spectra—Estimation of Aqueous Photolysis Rate Constant and Minimum Half-life in Sunlight—(A) Illustrative Example 1. A neutral organic chemical A was dissolved in water at a concentration of 1.00×10^{-3} M. UV-visible absorption spectra were obtained in a 10.0 cm quartz absorption cell and no absorbance was detected above the baseline in the region 290 nm and greater (i.e., $A_\lambda = 0$) for $\lambda > 290$ nm). Since $A_\lambda = 0$, then $\epsilon_\lambda^c = 0$ (Equation 15). Using this result in Equation 7, it is found that $(k_{pE})_{max.} = 0$, indicating that no direct photolysis can take place in sunlight at any latitude or season of the year. This example illustrates the principle of the Grotthus-Draper law, the first law of photochemistry. That is, in order for direct photolysis to take place in sunlight, the chemical must absorb sunlight in the region $\lambda > 290$ nm.

(B) *Illustrative example 2. (1)* Consider a plant located in Columbus, Georgia on the Chattahoochee River which produces an organic chemical B which is not an acid or a base. The waste effluent passes through a primary and secondary treatment plant and is then discharged directly into the river. The plant produces chemical B continuously every day of the year. The plant is located at 32.5° north latitude. Estimate the maximum sunlight direct photolysis rate constant and the corresponding minimum half-life for this chemical in the river for the winter and summer seasons under clear skies.

(*2*) Laboratory experiments, data, and calculations: (*i*) The water solubility of chemical B is 1.00 × 10^{-3} M at 25 °C. Chemical B was dissolved directly in water and a 1.00 × 10^{-4} molar solution was prepared at 25 °C. The uv-visible absorption spectra were obtained according to the Tier 1 procedure in a 10.0 cm quartz absorption cell in duplicate. Using the wavelength interval range (from Table 1 under paragraph (c)(3) of this section), the average absorbance of the duplicate runs at λcenter was obtained and the results are summarized in the following Table 7:

TABLE 7—SUMMARY OF PHOTOLYSIS DATA FOR CHEMICAL B

SPECTRAL DATA

λ center (nm)	A_λ	ϵ_λ ($M^{-1}cm^{-1}$)
297.5	1.684	1684
300.0	1.434	1434
302.5	1.221	1221
305.0	0.919	919
307.5	0.742	742
310.0	0.208	208
312.5	0.138	138
315.0	0.094	94
317.5	0.057	57
320.0	0.009	9
323.1	0.002	2
330.0	0.000	0

PHOTOLYSIS DATA

λ center (nm)	Summer		Winter	
	L_λ[1]	$\epsilon^c_\lambda L_\lambda$ (day^{-1})	L_λ[1]	$\epsilon^c_\lambda L_\lambda$ (day^{-1})
297.5	1.00(−4)	0.18	6.78(−6)	0.01
300.0	4.11(−4)	0.59	4.23(−5)	0.06
302.5	1.14(−3)	1.39	1.71(−4)	0.21
305.0	2.46(−3)	2.26	4.95(−4)	0.45
307.5	4.45(−3)	3.30	1.11(−3)	0.82
310.0	7.02(−3)	1.46	2.04(−3)	0.42
312.5	1.00(−2)	1.38	3.26(−3)	0.45
315.0	1.32(−2)	1.24	4.69(−3)	0.44
317.5	1.64(−2)	0.94	6.21(−3)	0.35
320.0	1.95(−2)	0.18	7.76(−3)	0.07
323.1	3.46)−2)	0.07	1.43(−2)	0.03
330.0	1.18(−1)	0.00	5.17(−2)	0.00
		$\Sigma\epsilon^c_\lambda L_\lambda$ = 12.99		$\Sigma\epsilon^c_\lambda L_\lambda$ = 3.31

[1] The units of L_λ are in 10^{-3} einsteins cm^{-2} day^{-1}. The second number in the columns in parenthesis is the power of ten by which the first number is multiplied.

(*ii*) From the above data and Equation 15 under paragraph (c)(1)(i)(A) of this section, the average molar absorptivity is

Equation 18

$\epsilon_\lambda = 1000\ A_\lambda$.

From the average A_λ value at λ center, the average molar absorptivity can be obtained from Equation 18 and the results are summarized in Table 7 under paragraph (c)(4)(i)(B)(*2*)(*i*) of this section. Since the plant is located at 32.5° north latitude, the closest L_λ values are at 30° north latitude. These values are obtained from Table 4 under paragraph (c)(3) of this section and are summarized in Table 8 under paragraph (c)(4)(i)(B)(*2*)(*i*) of this section for the summer and winter seasons. Using the data from Table 7 under paragraph (c)(4)(B)(*2*)(*i*) of this section and Equations 7 and 8 under paragraph (a)(3)(v) of this section, the following results are obtained.

Summer

$(k_{pE})_{max} = \Sigma\epsilon_\lambda^c L_\lambda = 13.0\ day^{-1}$

$(t_{1/2E})_{min} = 0.053\ day$

Winter

$(k_{pE})_{max} = \Sigma\epsilon^c L_\lambda = 3.31\ day^{-1}$

$(t_{1/2E})_{min} = 0.21\ day$

Since the chemical transforms rapidly for the summer and winter seasons, it is necessary to carry out Tier 2 experiments to more accurately define direct photolysis rates in aqueous media as a function of the season of the year.

(ii) *Tier 2 Phase 1: Aqueous Photolysis in Sunlight—Illustrative Example 3.* (A) Consider the same scenario as described in illustrative example 2, under paragraph (c)(4)(i)(B) of this section. Using the Tier 2, Phase 1 Procedure, carry out experiments to estimate the rate of direct photolysis and half-life in aqueous solution in the spring for water bodies.

(B) Photolysis experiments and calculations: Since chemical B absorbs appreciably below 340 nm, 11 mm i.d. quartz tubes were used (note: this tube has an approximate pathlength of 1 cm). Chemical B was dissolved directly in pure water and a 1.00 x 10^{-5} molar solution was prepared at 25°C. Since the water solubility is 1.00 x 10^{-3} M at 25°C, this sample solution was well below one-half its water solubility. The uv spectrum of this solution in a one cm absorption cell indicated that A_λ was less than 0.05 at 290 nm. Hence, under these conditions, first-order kinetics are applicable.

(C) A series of quartz tubes were filled with this aqueous solution, sealed, and photolysis experiments were carried out in sunlight according to the appropriate procedure described in paragraph (b)(2)(iii)(A) of this section. The experiments were started at noon (1200 hours) on May 8, 1982. The weather conditions are summarized for this period of time and the concentration data given represent the mean of duplicate determinations.

(*1*) May 2, 1982: at t = 0 (noon—1200 hours) C_0 = 1.00 x 10^{-5} M.

(*2*) May 2, 1982: Noon to sunset—clear and sunny.

(*3*) May 3, 1982: Noon (1200 hours). C_t = 0.840 x 10^{-5} M.

(*4*) May 3, 1982: at 1400 hours the weather conditions were cloudy with rain. The rain and cloudy weather continued until 2200 hours.

(*5*) From sunrise, May 4, 1982 through 1200 hours May 8, 1982, the weather was clear and sunny. At 1200 hours, May 8, 1982, analysis of the samples gave an average concentration of C_t = 0.400 x 10^{-5} M. Since 60 percent of chemical B transformed, the photolysis experiments were terminated and the control samples were analyzed. The average concentration of the control samples was 0.997 x 10^{-5} M which was essentially the same as C_o. Hence, no adventitious processes occurred and the loss of chemical was only due to sunlight photolysis.

(D) Listed in the following Table 8 are the times of sunrise and sunset for the dates sunlight photolysis experiments were carried out along with the total number of hours of sunlight.

TABLE 8—SUMMARY OF TIMES FOR SUNRISE AND SUNSET FOR THE PERIOD MAY 2–8, 1982

Date (1982)	Sunrise (a.m.)	Sunset (p.m.	Total sunlight hours
5/2	0600	2010	14.2
5/3	0559	2011	14.2
5/4	0558	2012	14.2
5/5	0557	2013	14.3
5/6	0556	2014	14.3
5/7	0555	2015	14.3
5/8	0554	2016	14.4

(E) The following data summarizes the dates photolyzed, the times exposed to sunlight, the total sunlight photolysis time for each date in days, the total number of days of sunlight photolysis, and the calculation of k^c_p and $t_{1/2}$.

Date	Times photolyzed	Sunlight photolysis time for each (days)
5/2	1200 hrs. to 2010 hrs. (8.2/14.2)	0.58
5/3	0559 hrs. to 1200 hrs. (6.0/14.2)	0.42
5/3	1200 hrs. to 1400 hrs. (2.0/14.2)	0.14
5/4	0558 hrs. to 2012 hrs.	1.00
5/5	0557 hrs. to 2013 hrs.	1.00
5/6	0556 hrs. to 2014 hrs.	1.00
5/7	0555 hrs. to 2015 hrs.	1.00
5/8	0554 hrs. to 1200 hrs. (6.1/14.4)	0.42

[1] Total hours.

$t = 5.6\ days;\ C_0 = 1.00\times10^{-5};\ C_t = 0.400\times10^{-5}$

$\ln(C_0/C_t) = k^c_p t$

$k^c_p = (1/t)\ \ln(C_0/C_t) = (1/5.6)\ln(1.00\times10^{-5}/0.400\times10^{-5})$

$k^c_p = 0.16\ days^{-1}$

$t_{1/2} = 0.693/0.16\ days^{-1} = 4.3\ days$

Therefore, the rate constant for direct photolysis of chemical B in tubes in pure water is 0.16 days^{-1} and the corresponding half-life is 4.3 days for the period of photolysis May 2–8, 1982 at 32.5° north latitude. Using equation 13, under paragraph (b)(2)(i)(J) of this section, the direct photolysis rate constant (k_{pE}) for water bodies is 0.073 days^{-1} and the corresponding half-life ($t_{1/2E}$) is 9.5 days.

(iii) *Tier 2, Phase 2: Aqueous Photolysis in Sunlight—Illustrative Example* 4. (A) Consider the same scenario as described in illustrative examples 2 and 3. Using the Tier 2, Phase 2, procedure, carry out experiments to determine the sunlight reaction quantum yield and estimate the rate constant for direct photolysis in aqueous solution and the half-life for water bodies and clear sky conditions for the summer and winter seasons.

(B) Photolysis Experiments and Calculations: The sunlight photolysis experiments were carried out in the beginning of May 1982, at 32.5° north latitude.

(C) Preparation of the Actinometer Solution: (*1*) The results from the Tier 2, Phase 1, experiments indicated that K^{c}_{p} for the test chemical was 0.16 days^{-1}. Since the experiments were carried out in early May at 32.5° north latitude, the value of K^{a}_{p} was chosen from Table 2 which corresponds to the spring season and at 30° north latitude; and the value is 483 days^{-1}. Using Equation 11 under paragraph (b)(2)(i) (H) of this section, the molar concentration of pyridine required to adjust the actinometer rate to match the rate of disappearance of the test chemical is

$$[PYR] = 26.9\,(0.16/483) = 8.91 \times 10^{-3} \text{ molar.}$$

Using this concentration of pyridine, an actinometer solution was (ii)(C) prepared according to the procedure described in paragraph (b)(2) of this section. The quantum yield for this actinometer is calculated using equation 9 under paragraph (a)(3)(vii) of this section.

$$\phi^{a}_{E} = 0.0169[PYR] = 0.0169(8.91 \times 10^{-3}) = 1.51 \times 10^{-4}$$

(*2*) Procedures for Tier 2, Phase 2 experiments (under paragraph (b)(2)(iii) of this section) were followed and sunlight experiments were initiated at 1200 hours on May 9, 1982. The mean initial concentration of test chemical was 1.00×10^{-5} molar and the mean initial concentration of PNAP was 1.00×10^{-5} molar. Samples of the chemical and actinometer and the controls were analyzed in triplicate periodically at 1200 hours on May 10, 11, 13, 15, and 16. On May 16, the photolysis experiments were terminated. The mean concentrations of all samples are summarized as follows:

Date	Concentration of chemical (M)	Concentration of actinometer (M)	Concentration of chemical control (M)	Concentration of actinometer control (M)
May 9	1.00×10^{-5}	1.00×10^{-5}	1.00×10^{-5}	1.00×10^{-5}
May 10	0.820×10^{-5}	0.855×10^{-5}	0.997×10^{-5}	1.00×10^{-5}
May 11	0.654×10^{-5}	0.710×10^{-5}	1.00×10^{-5}	0.997×10^{-5}
May 13	0.440×10^{-5}	0.515×10^{-5}	0.996×10^{-5}	0.999×10^{-5}
May 15	0.299×10^{-5}	0.383×10^{-5}	0.999×10^{-5}	0.998×10^{-5}
May 16	0.233×10^{-5}	0.304×10^{-5}	0.997×10^{-5}	0.996×10^{-5}

Since no significant loss of PNAP or test chemical was observed in the control samples, no adventitious processes occurred and the loss of test chemical and PNAP was only due to sunlight photolysis.

(*3*) Using the above data, $\ln(C_o/C_t)$ for the test chemical and actinometer can be calculated and the results are summarized as follows:

t (days)	Chemical		Actinometer	
	$C_t \times 10^{5}$ (M)	$\ln(C_o/C_t)^{c}$	$C_t \times 10^{5}$ (M)	$\ln(C_o/C_t)^{a}$
0	1.00	0.000	1.00	0.000
1	0.820	0.199	0.855	0.157
2	0.654	0.425	0.710	0.343
4	0.440	0.821	0.515	0.664
6	0.299	1.21	0.383	0.960
7	0.233	1.46	0.304	1.19

(*4*) The ratio of the rate constants, k^{c}_{p}/k^{a}_{p}, is defined by equation 12 under paragraph (b)(2)(i)(H)(3) of this section.

Equation 12

$$\ln(C_o/C_t)^{c} = (k^{c}_{p}/k^{a}_{p})\ln(C_o/C_t)^{a}.$$

(*5*) Using all the data (including the time point t=0) and linear regression analysis, the slope is found to be 1.237 with a correlation coefficient of 0.9998. Therefore

$$(k^{c}_{p}/k^{a}_{p}) = 1.24.$$

(*6*) Using the molar absorptivities obtained in example 2 under paragraph (c)(4)(i)(B) of this section and the L_λ values for spring at 30° north latitude in Table 4 under paragraph (c)(3) of this section, the value of $\sum \epsilon^{c}_{\lambda} L_\lambda$ can be calculated as follows:

λ center (nm)	$\epsilon^{c}_{\lambda}(M^{-1}cm^{-1})$	L^{1}_{λ}	$\epsilon^{c}_{\lambda}L_\lambda$ (days⁻¹)
297.5	1684	5.73 (−5)	0.10
300.0	1434	2.50 (−4)	0.36
302.5	1221	7.65 (−4)	0.93
305.0	919	1.79 (−3)	1.65
307.5	742	3.43 (−3)	2.55
310.0	208	5.64 (−3)	1.17
312.5	138	8.27 (−3)	1.14
315.0	94	1.12 (−2)	1.05
317.5	57	1.41 (−2)	0.80
320.0	9	1.70 (−2)	0.15
323.1	2	3.04 (−2)	0.06
330.0	0	1.05 (−1)	0.00

[1] The units of L_λ are 10^{-3} einsteins cm^{-2} day^{-1}.

$$\Sigma\epsilon^{c}_{\lambda}L_\lambda = 9.96 \text{ days}^{-1}.$$

For this experiment, $k^{a}_{a}(\Sigma = \epsilon^{a}_{\lambda}L_\lambda)$ is 483 days^{-1} (Table 2 under paragraph (c)(3) of this section). All the pertinent data are summarized as follows:

$$k^{c}_{p}/k^{a}_{p} = 1.24;\ \Sigma\epsilon^{c}_{\lambda}L_\lambda = 9.96 \text{ days}^{-1};$$
$$\Sigma\epsilon^{a}_{\lambda}L_\lambda = 483 \text{ days}^{-1};\ \phi^{a}_{E} = 1.51 \times 10^{-4}.$$

Substituting these results into equation 10 under paragraph (a)(3)(vii) of this section yields

$$\phi^{c}_{E} = (1.24)(483/9.96)(1.51 \times 10^{-4})$$
$$\phi^{c}_{E} = 9.08 \times 10^{-3}.$$

(*7*) The rate constants for direct photolysis of test chemical in aqueous media and the half-life for water bodies and clear sky conditions for the winter and summer seasons can be calculated as follows: The values of $\sum \epsilon_\lambda^{c} L_\lambda$ have been calculated from example under paragraph (c)(3)(i)(A) of this section. For summer $\sum \epsilon_\lambda^{c} L_\lambda = 13.0$ days^{-1}; for winter $\Sigma\epsilon_\lambda^{c} L_\lambda = 3.31$ days^{-1}. The reaction quantum yield for the chemical is 9.08×10^{-3}. Using these data in equation 4 under paragraph (a)(3)(iii) of this section yields

Summer

$$k_{pE} = 9.08 \times 10^{-3}(13.0) = 0.118 \text{ days}^{-1}$$

Winter

$$k_{pE} = 9.08 \times 10^{-3}(3.31) = 0.0301 \text{ days}^{-1}.$$

These values can be substituted into equation 2 under paragraph (a)(3)(ii) of this section to obtain the half-lives for these two seasons.

Summer:

$$t_{1/2E} = (0.693/0.118) = 5.9 \text{ days}$$

Winter:

$$t_{1/2E} = (0.693/0.0301) = 23 \text{ days}.$$

(5) *Glossary of important symbols*

PYR—Pyridine.
PNAP—p-Nitroacetophenone.
λ—Wavelength λ.
A_λ—Absorbance at wavelength λ.
a—Actinometer (composed of PNAP/PYR).
ϵ^{c}_{λ}—Molar absorptivity of a chemical C.
ϵ^{a}_{λ}—Molar absorptivity of the actinometer.
l—light pathlength; the distance traveled by a beam of light passing through the system.
ϕ^{c}_{E}—Sunlight reaction quantum yield of chemical c in water.

ϕ^a_E—Sunlight reaction quantum yield of the actinometer in water. Since the reaction quantum yield is independent of λ, $\phi^a_E = \phi^a$ (i.e., the reaction quantum yield of the actinometer measured in the laboratory).

[C]—Molar concentration of chemical c.

[PYR]—Molar concentration of pyridine.

$-d[C]/dt$—Direct photolysis rate of chemical c.

k_{pE}—Direct photolysis sunlight rate constant in water bodies in the environment.

$(k_{pE})_{max}$—Maximum direct photolysis sunlight rate constant in water bodies in the environment.

k^c_p—Direct photolysis sunlight rate constant of chemical c in water in tubes.

k^a_p—Direct photolysis sunlight rate constant of the actinometer in water in tubes.

$k_{a\lambda}$—Specific light absorption of a photoreactive chemical at a low concentration and at wavelength λ.

k_a—Specific light absorption rate constant integrated over all wavelengths absorbed by the chemical.

k^a_a—Specific light absorption rate constant integrated over all wavelengths absorbed by the actinometer.

$t_{1/2}$—Sunlight half-life of a chemical in water in tubes.

$(t_{1/2E})_{min}$—The minimum sunlight half-life of a chemical in water bodies in the environment.

I—The numbers of photons of light of wavelength λ in the system per cm^2 per second.

L_λ—Solar irradiance in water in the units 10^{-3} einsteins cm^{-2} day^{-1}.

γ—The geometry factor which represents the ratio of the rate constants in tubes (k_p) to the rate constant in water bodies in the environment (k_{pE}).

(d) *References.* For additional background information on this test guideline the following references should be consulted:

(1) *Astronomical Almanac* (1982).

(2) Dulin, D. and Mill, T. "Development and application of solar actinometers." *Environmental Science and Technology* 16:815 (1982).

(3) *Handbook of Chemistry and Physics.* (Chemical Rubber Co., Cleveland, Ohio)

(4) Mill, T., Davenport, S.E., Dulin D.E., Mabey, W.R., and Bawol, R. *Evaluation and Optimization of Photolysis Screening Protocols.* EPA Report No. 560/5–81–003 (1981).

(5) Mill, T., Mabey, W.R., Bomberger, D.C., Chou T-W., Hendry, D.G., and Smith, J.H. *Laboratory Protocols for Evaluating the Fate of Organic Chemicals in Air and Water.* EPA Report No. 600/3–82–022 (1982).

(6) Mill, T., Mabey, W.R., Hendry, D.G. Winterle, J., Davenport, J., Barich, V., Dulin, D., and Tse, D. *Design and Validation of Screening and Detailed Methods for Environmental Processes.*

(7) U.S. Environmental Protection Agency, Mill, T., Davenport, J.E., Winterle, J.S., Mabey, W.R., Drossman, H., Tse, D., and Liu, A. "Toxic substances process data generation and protocol development." Draft final report, EPA Contract No. 68–03–2981 with EPA Athens Research Laboratory, Office of Research and Development (1984).

(8) Zepp, R.G., and Cline, D.M. "Rates of direct photolysis in aquatic environment" *Environmental Science and Technology* 11:359 (1977).

(9) Zepp, R.G."Quantum yields for reaction of pollutants in dilute aqueous solution" *Environmental Science and Technology*, 12:327 (1978).

(10) Zepp, R.G. Environmental Research Laboratory, U.S. Environmental Protection Agency, College Station Road, Athens, Georgia 30601.

§ 796.3780 Laboratory determination of the direct photolysis reaction quantum yield in aqueous solution and sunlight photolysis.

(a) *Introduction*—(1) *Background and purpose.* (i) Numerous chemicals enter natural aquatic water bodies from a variety of sources. Some pollutants present in aqueous media can undergo photochemical transformation in sunlight by direct photolysis. Therefore, quantitative data in the form of rate constants and half-lives are needed to determine the importance of direct photochemical transformation of pollutants in aqueous media.

(ii) Section 796.3700, the first in a series of aqueous photolysis test methods, was designed to determine the molar absorptivity and reaction quantum yield of a test chemical in aqueous solution. These parameters can be combined with solar irradiance data to determine environmentally relevant rate constants and half-lives in aqueous solutions as a function of latitude and season of the year anywhere in the United States.

(iii) Section 796.3700 was developed as a screening test to obtain the direct photolysis reaction quantum yield of a chemical in aqueous solution by carrying out photolysis experiments in sunlight. This method does not require sophisticated and expensive photochemical equipment and therefore the reaction quantum yield can be easily determined and at a modest cost. However, there are circumstances when this method may not be applicable. For example, this procedure is not applicable for determining the reaction quantum yield of a test chemical when the temperature outdoors falls below zero degrees Centigrade. Furthermore, depending upon the status of a risk assessment for a specific chemical, a more precise value of the reaction quantum yield may be required. Thus, a more comprehensive procedure is needed to determine the direct photolysis reaction quantum yield in the laboratory using specialized photochemical equipment and monochromatic (or narrow band) light. This section describes laboratory procedures for determining the direct photolysis reaction quantum yield in aqueous solution.

(iv) The reaction quantum yield obtained in this test method can be combined with molar absorptivity data and solar irradiance data to determine environmentally relevant rate constants and half-lives in aqueous solution as a function of latitude and season of the year anywhere in the United States.

(v) The procedures described in this test method are very detailed, and the theory of photolysis in aqueous solution is relatively complicated. In order to follow these procedures, it is recommended that the Technical Support Document in the public record for this section should first be studied carefully.

(2) *Definitions and units.* (i) "Radiant energy", or radiation, is defined as the energy traveling as a wave unaccompanied by transfer of matter. Examples include x-rays, visible light, ultraviolet light, radio waves, etc.

(ii) "Absorbance (A_λ)" is defined as the logarithm to the base 10 of the ratio of the initial intensity (I_o) of a beam of radiant energy to the intensity (I) of the same beam after passage through a sample at a fixed wavelength λ. Thus, $A_\lambda = \log_{10}(I_o/I)$.

(iii) The "Beer-Lambert law" states that the absorbance of a solution of a given chemical species, at a fixed wavelength, is proportional to the thickness of the solution (l), or the light pathlength, and the concentration of the absorbing species (C).

(iv) "Molar absorptivity (ϵ_λ)" is defined as the proportionality constant in the Beer-Lambert law when the concentration is given in terms of moles per liter (i.e., molar concentration). Thus, $A_\lambda = \epsilon_\lambda Cl$, where A_λ and ϵ_λ represent the absorbance and molar absorptivity at wavelength λ and l and C are defined in paragraph (a)(2)(iii) of this section. The units of ϵ_λ are $molar^{-1}$ cm^{-1}. Numerical values of molar absorptivity depend upon the nature of the absorbing species.

(v) A "first-order reaction" is defined as a reaction in which the rate of disappearance of a chemical is directly proportional to the concentration of the chemical and is not a function of the concentration of any other chemical present in the reaction mixture.

(vi) A "zero-order reaction" is defined as a reaction in which the rate of disappearance of a chemical is independent of the concentration of the chemical or the concentration of any other chemical present in the reaction mixture.

(vii) The "first-order half-life ($t_{1/2}$)" of a chemical is defined as the time required for the concentration of the chemical to be reduced to one-half its initial value.

(viii) The "sunlight direct aqueous photolysis rate constant (k_{PE})" is the first-order direct photolysis rate constant in the units of day^{-1} and is a measure of the rate of disappearance of a chemical dissolved in a water body in sunlight.

(ix) The "solar irradiance in water (L_λ)" is related to the sunlight intensity in water at shallow depths and is proportional to the average light flux (in the units of 10^{-3} einsteins $cm^{-2}\,day^{-1}$) that is available to cause photoreaction in the wavelength interval centered at λ over a 24-hour day at a specific latitude and season date.

(x) "The Grotthus-Draper law", the first law of photochemistry, states that only light which is absorbed can be effective in producing a chemical transformation.

(xi) The "Stark-Einstein law", the second law of photochemistry, states that only one molecule is activated to an excited state per photon or quantum of light absorbed.

(xii) The "reaction quantum yield (ϕ_λ)" for an excited state process is defined as the fraction of absorbed light that results in photoreaction at a fixed wavelength λ. It is the ratio of the number of molecules that photoreact to the number of quanta of light absorbed or the ratio of the number of moles that photoreact to the number of einsteins of light absorbed at a fixed wavelength λ.

(xiii) "Direct photolysis" is defined as the direct absorption of light by a chemical followed by a reaction which transforms the parent chemical into one or more products.

A glossary of symbols can be found under paragraph (c)(4) of this section.

(3) *Principle of the test method.* (i) This test method is based on the principles developed by Zepp (1978) under paragraph (d)(13) of this section, the use of low optical density actinometers developed by Mill et al. (1981, 1982) under paragraph (d) (7) and (8) of this section , and Dulin and Mill (1982) under paragraph (d)(4) of this section, and the high optical density ferrioxalate actinometer developed by Parker (1953) under paragraph (d)(12) of this section, and Hatchard and Parker (1956) under paragraph (d)(6) of this section.

(ii) Zepp (1978), under paragraph (d)(13) of this section, published a paper on the determination of the reaction quantum yield for the reaction of pollutants in dilute aqueous solution in the laboratory. Based on this work, two procedures are described to determine the reaction quantum yield of a test chemical at low optical density in the laboratory. These procedures involve the use of: low-optical density test chemical and actinometer; and low-optical density test chemical and high-optical density actinometer.

(A) *Procedure One: Determination of the Reaction Quantum Yield by the Low-Optical Density Test Chemical and Actinometer Method.* (*1*) For a low optical density test chemical and actinometer in which the absorbance of aqueous solutions is less than 0.02, the reaction quantum yield of a test chemical θ_c at wavelength λ is given by equation

Equation 1

$$\phi_c = (k_{pc}/k_{pa})(\epsilon_{\lambda a}/\epsilon_{\lambda_c})\phi_a.$$

where ϵ_a and ϵ_{λ_c} are molar absorptivities of the actinometer and test chemical, respectively, at wavelength λ, ϕ_a is the reaction quantum yield of the actinometer at wavelength λ, and k_{pa} and k_{pc} are the first-order direct photolysis rate constant for actinometer and test chemical, respectively. These rate constants are defined by the equations

Equation 2

$$\ln(C_o/C_t)_a = k_{pa}t$$

Equation 3

$$\ln(C_o/C_t)_c = k_{pc}t.$$

where $(C_o)_a$ and $(C_t)_a$ are the molar concentrations of actinometer at time t=0 and t and $(C_o)_c$ and $(C_t)_c$ are the molar concentrations of test chemical at time t=0 and t. Since k_{pa} and k_{pc} are first-order rate constants, the half-lives of actinometer and test chemical are:

Equation 4

$$(t_{1/2})_a = 0.693/k_{pa}$$

Equation 5

$$(t_{1/2})_c = 0.693/k_{pc}.$$

(*2*) If both the actinometer and test chemical solutions are photolyzed in identical cells, equation 3 under paragraph (a)(3)(ii)(A)(*1*) of this section can be divided into equation 2 under paragraph (a)(3)(ii)(A)(*1*) of this section. Carrying out this operation and rearranging the resultant equation yields

Equation 6

$$\ln(C_o/C_t)_c = (k_{pc}/k_{pa})\ln(C_o/C_t)_a.$$

(*3*) Procedure One involves the simultaneous photolysis of test chemical and actinometer in an Ace-type photochemical "merry-go-round" reactor (PMGRR) using monochromatic light of wavelength λ. Two low optical density actinometers have been developed by SRI International for the Office of Toxic Substances/U.S. Environmental Protection Agency to measure the light intensity incident on the sample during photolysis [Mill et al. (1981, 1982) under paragraph (d)(7) and (8) of this section and Dulin and Mill (1982) under paragraph (d)(4) of this section]. These actinometers are: (*i*) p-nitroacetophenone-pyridine actinometer (PNAP/PYR); and (*ii*) p-nitroanisole-pyridine actinometer (PNA/PYR). The rate constant of each of these actinometers can be adjusted to match the rate constant of the test chemical by adjusting the concentration of pyridine. Since the rate constant is a function of the reaction quantum yield of the actinometer, the rate constant can be adjusted according to the following equations:

Equation 7

PNAP/PYR actinometer $\phi_a = 0.0169[PYR]$

Equation 8

PNA/PYR actinometer $\phi_a = 0.437[PYR] + 0.000282,$

where [PYR] is the molar concentration of pyridine for a PNAP or PNA concentration of approximately 1×10^{-5} M.

(*4*) The laboratory procedure for determining the reaction quantum yield of a test chemical in aqueous solution has been divided into three phases using a uv-visible absorption spectrophotometer, an Ace-type PMGRR, and a 450 watt medium pressure mercury lamp with appropriate filters to isolate the monochromatic wavelength λ. In Phase 1, the molar absorptivities of a test chemical, ϵ_{313_c} and ϵ_{366_c}, are determined with a UV-visible absorption spectrophotometer using procedures outlined in §§796.1050 and 796.3700. Based on these results, photolysis experiments are carried out at 313 or 366 nm corresponding to the higher value of ϵ_{313_c} or ϵ_{366_c}.

(*5*) The Phase 2 procedure is composed of trial photolysis experiments at the chosen wavelength λ (313 or 366 nm) in an Ace-type PMGRR to determine the approximate rate constant and half-life of the test chemical and to choose the appropriate actinometer which has a rate constant approximately the same as the rate constant of the test chemical. First, an aqueous solution of test chemical at low optical density is photolyzed in the PMGRR at the chosen wavelength λ to determine K_{pc} and $(t_{1/2})_c$ using equations 3 and 5, respectively under paragraph (a)(3)(ii)(A)(*1*) of this section. If $(t_{1/2})_c$ is less than 12 hours, use the PNA/PYR actinometer. If $(t_{1/2})_c$ is greater than 12 hours, then use the PNAP/PYR actinometer. Trial photolysis experiments are then carried out at wavelength λ with the chosen low optical density actinometer to determine the molar concentration of pyridine needed to make the rate constant of the actinometer approximately match the rate constant of the test chemical.

(*6*) In the Phase 3 procedure, low-optical density aqueous solutions of test chemical and actinometer (at a fixed molar concentration of pyridine [PYR]) are photolyzed in identical tubes in the PMGRR at wavelength λ. Concentrations of test chemical and actinometer are measured as a function of time. These data are used in Equation 6 under paragraph (a)(3)(ii)(A)(*2*) of this section to determine the ratio of the rate constants (k_{pc}/k_{pa}). The reaction quantum yield of the actinometer ϕ_a, employed in these experiments, can be determined at the molar concentration of pyridine [PYR] using Equation 7 or 8 under paragraph (a)(3)(ii)(A)(*3*) of this section. These data, along with the molar absorptivity of the test chemical (ϵ_{313_c} or ϵ_{366_c}) and the actinometer (ϵ_{313_a} or ϵ_{366_a}) are substituted in equation 1 under paragraph (a)(3)(ii)(A)(*1*) of this section to determine the reaction quantum yield of the test chemical ϕ_c.

(B) *Procedure Two: Determination of the Reaction Quantum Yield by the Low-Optical Density Test Chemical and High Optical Density Actinometer Method.* (*1*) For a low-optical density test chemical (absorbance <0.02) and for a high optical density actinometer (absorbance >2), such as the ferrioxalate actinometer, the reaction quantum yield of the test chemical ϕ_c at wavelength λ is given by the equation

Equation 9

$$\phi_c = \phi_a (k_{pc}/k_{pa})(2.303\epsilon_{\lambda_c} l)^{-1},$$

where ϕ_a is the reaction quantum yield of the ferrioxalate actinometer at wavelength λ, ϵ_{λ_c} is the molar absorptivity of the test chemical at wavelength λ, l is the cell pathlength, k_{pc} is the first-order direct photolysis rate constant for the test chemical, and k_{pa} is the zero-order direct photolysis rate constant for the ferrioxalate actinometer. These rate constants are defined by the equations.

Equation 10

$$\ln(C_o/C_t)_c = k_{pc}t$$

Equation 11

$$(C_t)_a = k_{pa}t.$$

where $(C_o)_c$ and $(C_t)_c$ are the molar concentrations of the test chemical at time t=o and t and $(C_t)_a$ is the molar concentration of the ferrous ion formed at time t.

(*2*) The laboratory procedure for determining the reaction quantum yield of the test chemical is divided into two phases using a UV-visible absorption spectrophotometer and a photochemical optical bench (POB) or a PMGRR containing a 450-watt medium pressure lamp with appropriate filters to isolate the monochromatic wavelength λ. In Phase 1, the molar absorptivities of test chemical (ϵ_{313_c} and ϵ_{366_c}) are determined using spectroscopic procedures outlined in §§ 796.1050 and 796.3700. Based on these results, photolysis experiments are carried out at 313 or 366 nm corresponding to the higher value of ϵ_{313_c} or ϵ_{366_c}.

(*3*) In the Phase 2 procedure, aqueous low-optical density test chemical solution (absorbance <0.02) and aqueous high-optical density solution of ferrioxalate actinometer (absorbance >2) are photolyzed sequentially in identical cells in POB or PMGRR at wavelength λ. In the first and third set of experiments, the ferrioxalate actinometer is photolyzed for a few minutes and the molar concentration of ferrous ion formed $(C_t)_a$ is measured as a function of time t. These data are used in equation 11 under paragraph (a)(3)(ii)(B)(*1*) of this section to determine an average value of the actinometer rate constant $(k_{pa})_{ave}$. The second series of experiments involves the photolysis of the aqueous solution of test chemical in the POB or PMGRR in identical cells to those used in the actinometer experiments and the molar concentration of test chemical $(C_t)_c$ is measured as a function of the time t. These data are used in equation 10 under paragraph (a)(3)(ii)(B)(*1*) of this section to determine k_{pc}. Using k_{pc} and $(k_{pa})_{ave}$, the molar absorptivity of the test chemical (ϵ_{313_c} or ϵ_{366_c}), the pathlength of the cell l, and the reaction quantum yield of the ferrioxalate actinometer ϕ_a at wavelength λ in equation 9 under paragraph (a)(3)(ii)(B)(*1*) of this section, the reaction quantum yield of the test chemical ϕ_c can be determined.

(4) As described in detail in § 796.3700 and the public record for that test guideline, and briefly in the public record for this section, the direct sunlight photolysis of a chemical in an optically thin aqueous solution can be described by the following equations:

Equation 12

$$\ln(C_o/C_t) = k_{pE}t$$

Equation 13

$$t_{1/2_E} = 0.693/k_{pE}$$

Equation 14

$$k_{pE} = \phi_E k_a$$

where ϕ_E is the reaction quantum yield of the chemical in dilute solution and is independent of the wavelength, $k_a = \Sigma k_{a\lambda}$, the sum of $k_{a\lambda}$ values for all wavelengths of sunlight that are absorbed by the chemical (i.e., the light absorption rate constant), t is the time, C_o and C_t are the molar concentrations of chemical at t=0 and t, and $t_{1/2_E}$ represents the half-life. The term k_{pE} represents the first-order direct photolysis rate constant for a chemical in a water body in sunlight in the units of reciprocal time. In general, the reaction quantum yield ϕ_E is equivalent to the reaction quantum yield ϕ_c determined in the laboratory.

(*5*) Furthermore, under the same conditions cited above, the first-order direct photolysis rate constant, k_{pE}, is given by the equation.

Equation 15

$$k_{pE} = \phi_E \Sigma \epsilon_{\lambda_c} L_\lambda,$$

where ϕ_E is the reaction quantum yield, ϵ_{λ_c} is the molar absorptivity in the units molar^{-1} cm^{-1}, L_λ is the solar irradiance in water in the units 10^{-3} einsteins cm^{-2} day^{-1}, and the summation is taken over the range λ=290 to 800 nm. L_λ is the solar irradiance at shallow depths for a water body under clear sky conditions and is a function of latitude and season of the year. Solar irradiance data are tabulated in § 796.3700(c)(3), Tables 3–6 as a function of latitude and season of the year.

(*6*) A simple screening test has been developed in § 796.3700(c)(1)(i)(A) equation 15. As an approximation, it has been assumed that the reaction quantum yield ϕ_E is equal to one, the maximum value. As a result, the upper limit for the direct photolysis sunlight rate constant in aqueous solution is obtained and equation 15 becomes

Equation 16

$$(k_{pE})_{max} = \Sigma \epsilon_{\lambda_c} L_\lambda.$$

Using equation 16 in equation 13 under paragraph (a)(3)(ii)(B)(4) of this section, the lower limit for the half-life is then given by

Equation 17

$(t_{1/2E})_{\min.} = 0.693/(k_{pE})_{\max.}$

The molar absorptivity can be determined experimentally by the method outlined in paragraph (b)(2)(i) of this section and the solar irradiance data are tabulated in § 796 (c)(3), Tables 3–6. These data can then be used in equation 16 to calculate $(k_{pE})_{\max.}$. Finally, $(k_{pE})_{\max.}$ can be substituted in equation 17 to calculate $(t\frac{1}{2}_E)_{\min.}$. Based on these data, a decision can be made whether to determine the reaction quantum yield of the test chemical in the laboratory.

(*7*) Once the reaction quantum yield has been determined in the laboratory by Procedures One or Two under paragraphs (b) (2) and (3) of this section, it can be combined with the molar absorptivity date $\epsilon_{\lambda c}$ and the appropriate L_λ values to calculate k_{pe} as a function of latitude and season of the year anywhere in the United States, using Equation 15 under paragraph (a)(3)(ii)(B)(*5*) of this section. The corresponding half-life can be calculated using k_{pe} in equation 13 under paragraph (a)(3)(ii)(B)(*4*) of this section.

(4) *Applicability and specificity.* (i) For environmental photochemistry, the general procedures outlined in this test method are applicable to all chemicals which have UV-visible absorption maxima in the range 290 to 800 nm. Some chemicals have absorption maxima significantly below 290 nm and consequently cannot undergo direct photolysis in sunlight (e.g., chemicals such as alkanes, alkenes, alkynes, saturated alcohols, and saturated acids). This is a direct consequence of the Grotthus-Draper law of photochemistry. Some chemicals have absorption maxima significantly below 290 nm but have measurable absorption tails above the baseline in their absorption spectrum at wavelengths greater than 290 nm. Photolysis experiments should be carried out for these chemicals.

(ii) These test methods are applicable to pure chemicals and not to technical grade chemicals.

(iii) The molar absorptivity data ($\epsilon_{\lambda c}$) obtained in paragraph (b)(2)(i) of this section can be combined with the appropriate L_λ data to estimate $(k_{pE})_{\max.}$ and $(t\frac{1}{2}_E)_{\min.}$ at shallow depths in water bodies as a function of latitude and season of the year in the U.S. If these data indicate that aqueous photolysis is an important process relative to other transformation processes (e.g., biodegradation, hydrolysis, oxidation, etc.), then it is recommended that the reaction quantum yield ϕ_c be determined in the laboratory by Procedures One or Two outlined under paragraph (b) (2) and (3) of this section. Once ϕ_c has been determined, it can be combined with the molar absorptivity data $\epsilon_{\lambda c}$ and the appropriate L_λ data to calculate k_{pE} at shallow depths in water bodies as a function of latitude and season of the year in the United States. The corresponding half-life can then be calculated from k_{pE}.

(iv) Procedure One under paragraph (b)(2) of this section is only applicable to solutions of test chemicals and actinometers which have low optical densities (i.e., absorbance <0.02). Procedure Two under paragraph (b)(3) of this section is only applicable to solutions of test chemicals with low optical densities and actinometers with high optical densities (i.e., absorbance >2).

(v) Procedure One, as described in detail under paragraph (B)(2) of this section, is limited to the spectral region 290–400 nm because the recommended low optical density actinometers are only sensitive to light in this region. However, Procedure Two, under paragraph (b)(3) of this section using the ferrioxalate actinometer is useful in the spectral region 290–500 nm. This procedure can be extended up to approximately 750 nm using the Reinicke's salt actinometer [de Mayō and Shizuka (1976) under paragraph (d)(3) of this section].

(vi) There is a third procedure which could be used to determine ϕ_c in the laboratory. This procedure involves the determination of ϕ_c under the conditions when the test chemical and actinometer solutions both have high optical density. However, in general, this method is limited to only very soluble or strongly absorbing organic compounds. Thus, this method is not described in detail as a procedure in this test method. However, this method is briefly described in the public record for this section.

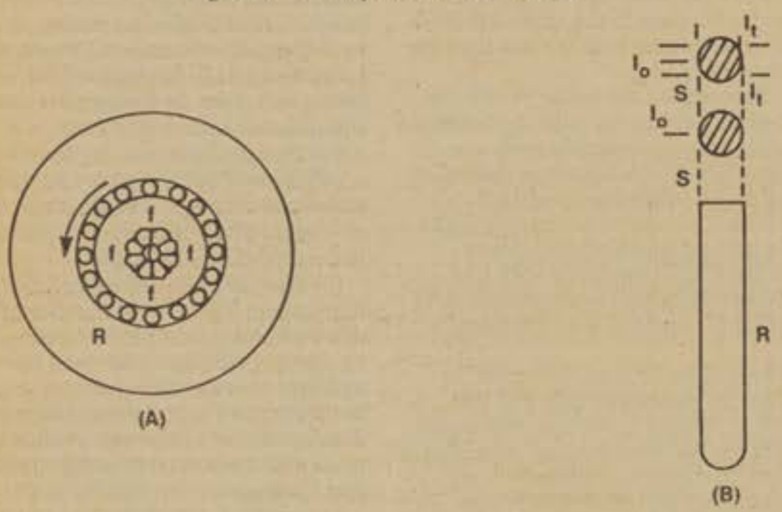
(vii) This test method has been designed to determine the molar absorptivity of a test chemical, $\epsilon_{\lambda c}$, and its quantum yield, ϕ_c. These parameters can be used to determine environmental rate constants at low absorbance and shallow depths in pure water as a function of latitude and season of the year. Tables 3 to 6 containing solar irradiance data have been included in § 796.3700 to carry out all the calculations. However, the method is really very general and can be extended to determine the rates of photolysis over a range of other environmental conditions using a computer program. Zepp has written a GC SOLAR computer program to calculate the rates of photolysis as a function of depth in water, as a function of the attenuation (or absorption) coefficient of the water ($\alpha\lambda$) for natural water bodies, the average ozone layer thickness that pertains to the seasons and location of interest, and as a function of latitude and season of the year. The computer program is available on written request to R. Zepp, Environmental Research Laboratory, U.S. Environmental Protection Agency, College Station Road, Athens, Georgia 30601.

(b) *Test procedures*—(1) *Test conditions*—(i) *UV-visible absorption spectrophotometer.* The recommended uv-visible absorption spectrophotometer is described in § 796.1050.

(ii) *Special photochemical laboratory Equipment.* There are a number of different designs of photochemical equipment which can be used to measure the reaction quantum yield in the laboratory and the one chosen will depend on the equipment available and to a certain extent on the light source and filter system chosen for the measurement. The apparatus chosen must contain a light source, appropriate filters, sample holders, and cells which allow solutions of test chemical and actinometer to be reproducibly irradiated with a uniform and constant amount of light at a discrete or narrow band wavelength λ. The temperature of the reaction cells must be at reasonably constant temperature $t_1 \pm 2$ °C in the range 20 to 30 °C. The apparatus should be housed in a separate part of a laboratory and properly shielded so that laboratory personnel are not exposed to uv light and to exclude extraneous light that could contribute to the photoreaction of chemicals. If the ferrioxalate actinometer is used, then actinometry experiments must be carried out in a darkroom with photographic "safelights". Excellent reviews and descriptions of a wide variety of photochemical equipment used to measure the reaction quantum yield in the laboratory are given by Calvert and Pitts (1966) under paragraph (d)(2) of this section, de Mayo and Shizuka (1976) under paragraph (d)(3) of this section, Murov (1973) under paragraph (d)(10) of this section, and Mill et al. (1982) under paragraph (d)(9) of this section, and these references are highly recommended.

(A) *Design of the apparatus.* (*1*) *Photochemical "Merry-Go-Round" Reactor.* (*i*) The design of the photochemical "merry-go-round" reactor (PMGRR) has been described in the literature [Moses et al. (1969) under paragraph (d)(11) of this section, Murov (1973) under paragraph (d)(10) of this section, de Mayo and Shizuka (1976) under paragraph (d)(3) of this section]. In the design of a PMGRR, the light source is in the center of the apparatus with reaction tubes arranged in a ring around the light source. The ring rotates around the light source to give a uniform irradiation of the reaction tubes, as depicted in the following Figure 1:

Figure 1—"Merry-Go-Round" reactor



(A) "MERRY-GO-ROUND" APPARATUS

(B) CYLINDRICAL CELL (R). I_o = INCIDENT LIGHT; I_t = TRANSMITTED LIGHT PASSED THROUGH CELL; S = SLIT

(a) "Merry-go-round" Apparatus;

(b) Cylindrical cell (R). I_o = incident light;

I_t = transmitted light passed through cell;

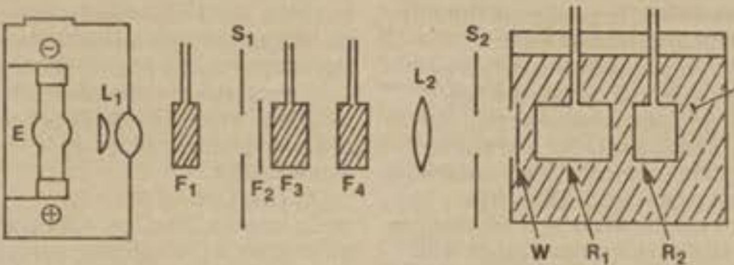
S = slit.

Glass filters (f) may be inserted between the light source and the reaction tubes. Filter solutions (f) may also be contained in the immersion well holding the light source or in a glass donut that surrounds the light source. In order to dissipate the heat generated by the light source, the PMGRR may be immersed in a water bath or a stream of air may be passed through the space between the light source and the filters. The filter solutions can be circulated to an external cooling source. There are two types of PMGRR equipment: the Ace-type and the Moses-type and both are very similar. The Ace-type PMGRR is designed so that the entire reaction cell is irradiated while the Moses-type PMGRR [Moses et al. (1969) under paragraph (d)(11) of this section] is designed with windows (which act as slits with a fixed aperture) so that only a narrow portion of the reaction cell is irradiated. Since the Ace-type PMGRR is the only one commercially available, this test method has been specifically designed to use this PMGRR. However, if the Moses-type PMGRR is available, this test method has to be slightly modified as described in public record for this section.

(*ii*) Small cylindrical tubes are used in the PMGRR to hold small volumes of test chemical and actinometer solutions. One tube is used for each datum point measured.

(*2*) *Photochemical optical bench.* The photochemical optical bench (POB) is composed of a light source, a lens, glass or chemical solution filters, and a reaction vessel. The component parts are mounted on a rail in the optical bench. A typical POB apparatus is depicted in the following Figure 2:

Figure 2—A typical optical train



A TYPICAL OPTICAL TRAIN, WHERE E: POINT SOURCE ARC; L: LENS; F: SOLUTION AND GLASS FILTERS; R_1: REACTION CELL; R_2: ACTINOMETER CELL; V_a: THERMOSTAT VESSEL; W: QUARTZ WINDOW

A typical optical train, where E: point source arc;

L: lens; F: solution and glass filters; R_1: reaction cell;

R_2: actinometer cell; V_a: thermostat vessel; W: quartz window.

The light source is located at the end of the bench and contains a housing around a light source and a lens to collimate the beam. The light passes through glass filters and/or through cells containing filter solutions to transmit wavelength. Filter solutions in the cell may be circulated through a cooling system and the glass filters should be cooled with a stream of air to prevent heat buildup. The photochemical reaction vessel containing the test chemical solution or actinometer solution is mounted coaxially with the lamp on the bench so that the filtered light enters the window of the reaction vessel. The reaction vessel should be temperature controlled by circulating constant temperature water through side walls. The light flux is usually measured before and after the photolysis of the test chemical solution. Commercially available optical benches are highly recommended but simple "home-made" benches can be used [Andre (1977) under paragraph (d)(1) of this section]. If a "home-made" bench is used, it must be completely described.

(B) *Light sources.* There are a number of light sources which are available for use in photochemical studies: for example, low, medium, and high pressure mercury lamps; xenon lamps; and lasers. The characteristics and application of these lamps are described in detail by Calvert and Pitts (1966) under paragraph (d)(3) of this section, Murov (1973) under paragraph (d)(10) of this section, and de Mayo and Shizuka (1976) under paragraph (d)(3) of this section. The use of a POB or PMGRR will often narrow the choice of lamp with regard to use of a point source or a tube-type lamp, since the former can be focused to give a collimated beam while the latter cannot. The light source should emit light at a constant and high intensity. The 450 watt medium pressure mercury lamp is highly recommended for use in this test method.

(C) *Light filtering systems.* (*1*) Monochromatic or narrow band wavelength light is essential for the accurate determination of the reaction quantum yield of a test chemical. Various systems for isolating monochromatic or narrow band wavelengths are described by Calvert and Pitts (1966) under paragraph (d)(2) of this section, Murov (1973) under paragraph (d)(10) of this section, and de Mayo and Shizuka (1976) under paragraph (d)(3) of this section. Two filter systems commonly used to isolate the 313 and 366 bands from a 450 watt medium pressure mercury lamp are highly recommended and have been incorporated in this test method. These two filter systems are described as follows:

(*i*) 313 nm filtering system: Corning Glass CS-754 filter (if the test chemical does not absorb light at wavelengths greater than 400 nm, this filter is not needed. The 754 filter is designed to block out visible light) with 0.005 M potassium chromate solution containing 3 percent sodium carbonate.

(*ii*) 366 nm filtering system: Corning Glass CS 0-52 and CS 7-60 filters.

(*2*) Reagent-grade chemicals should be used to prepare the chemical filter solution. Since this filter solution (and in general any filter solution) degrades slowly over prolonged periods of photolysis, the solution should be carefully monitored. Even when tap water is used to cool the lamp, the buildup of solid material or algae may reduce the light intensity; and this must be checked repeatedly.

(D) *Reaction cells.* (*1*) In general, reaction cells of large volume are appropriate for POB equipment, while small reaction cells are used for PMGRR equipment. These cells should be constructed of borosilicate glass or quartz. Actinometer and test chemical solutions must be photolyzed in identical cells and should contain the same volumes of actinometer and test chemical solution.

(*2*) For the PMGRR equipment, disposable culture tubes (13 × 100 mm) with Teflon-lined screw caps or quartz tubes (13 × 100 mm) with quartz ground glass stoppers or borosilicate screw caps, Teflon-lined, are recommended for use as reaction tubes. Grease should be avoided since hydrophobic chemicals might adsorb to it. In carrying out the photolysis experiments, one tube is used for each datum point measured. The pathlength of these cells is discussed in paragraph (b)(1)(iii) of this section.

(*3*) For the POB equipment, the most common and functional design for reaction cells is a cylindrical shape with optically flat circular windows fused to each end of the cylinder and at right angles to its axis. The windows should be made of material that will transmit 100 percent of the light at the desired wavelengths. Optically flat quartz windows are recommended. The size of the cylindrical reaction cell and windows should be consistent with the dimensions of the light beam used in the equipment. The reaction cell should be of sufficient volume to permit removal of samples for analysis without significantly altering the volume of the reaction solution in the cell. The cell pathlength can be measured directly or it can be determined by the procedure discussed in paragraph (b)(1)(iii) of this section. Details for the construction of these reaction cells may be found in Calvert and Pitts (1966) under paragraph (d)(2) of this section and de Mayo and Shizuka (1976) under paragraph (d)(3) of this section.

(iii) *Cell pathlength.* Zepp (1978) under paragraph (d)(b) of this section, described an experimental method using an isolated wavelength band to determine the effective pathlength of any cell. This procedure is described in the public record for this section. This procedure has been used to measure the effective pathlength of Corning Glass culture tubes 13 × 100 mm and it was found that l was 11.2 mm [Mill et al. (1982) under paragraph (d)(9) of this section]. In a similar manner, reaction tubes made from borosilicate glass stock of O.D. 12 mm had an effective pathlength of 10.0 mm [Mill et al. (1982) under paragraph (d)(9) of this section]. This procedure can be used to measure the pathlength of rectangular cells designed for the POB apparatus.

However, it is recommended that the pathlength of the rectangular cells be measured directly with a precise centimeter ruler or an equivalent measuring device.

(iv) *Solvents.* (A) If the half-life of an aqueous solution of test chemical in the photochemical equipment is less than 24 hours, then distilled water meeting ASTM Type II standards, or an equivalent grade, is recommended for use in this test method. If the half-life of an aqueous solution of test chemical in the photochemical equipment is greater than 24 hours, then water meeting ASTM type IIA standards, or an equivalent grade, is highly recommended for use in this test method to minimize biodegradation. ASTM Type II and IIA water are described in ASTM D 1193–77. Air saturated water is required for photolysis of test chemical solutions. Air saturated water can be easily prepared by allowing the ASTM Type II water to equilibrate in a vessel plugged with cotton or ASTM Type IIA water to equilibrate in a vessel plugged with sterile cotton. ASTM D 1193–77 is available for inspection at the Office of the Federal Register, Rm. 8401, 1100 L St., NW., Washington, DC. This incorporation by reference was approved by the Director of the Office of the Federal Register. This material is incorporated as it exists on the date of approval and a notice of any change in this material will be published in the **Federal Register.** Copies of the incorporated material may be obtained from the Document Control Officer (TS–793), Office of Toxic Substances, EPA, Rm. 107, 401 M St., SW., Washington, DC 20460, and from the American Society for Testing and Materials (ASTM), 1916 Race Street, Philadelphia, PA 19103.

(B) Reagent grade acetonitrile is recommended as the organic cosolvent with water in photochemical studies. Spectrograde acetonitrile or methanol is recommended for spectroscopic studies to determine the molar absorptivity of the test chemical.

(v) *Sterilization.* If the half-life of an aqueous solution of test chemical in the photochemical equipment is greater than 24 hours, then it is important to sterilize all glassware and to use aseptic conditions in the preparation of all solutions for photolysis studies in order to eliminate or minimize biodegradation. Glassware can be sterilized in an autoclave or by any other suitable non-chemical method.

(vi) *pH effects.* It is recommended that all photolysis and molar absorptivity experiments be carried out at pHs at least two pH units above the pK_a and at least two pH units below the pK_a for any chemical that ionizes or protonates (e.g., carboxylic acids, phenols, and amines). Buffers described in paragraph (b)(2)(i) of this section should be used.

(vii) *Volatile chemical substances.* Special care should be taken when testing a volatile chemical so that the chemical substance is not lost due to volatilization during the course of the photolysis experiment. Thus, it is important to effectively seal the reaction vessels. Disposable culture tubes with Teflon-lined screw caps or quartz tubes with quartz or borosilicate screw caps, Teflon-lined, are recommended. Grease should not be used. Volatile compounds can be conveniently studied in culture tubes equipped with gas-tight Mininert® valves. Samples can be introduced into or removed from the tubes through the septum in these valves with no loss of substrate. As an alternative, the tubes can be sealed with a torch. In addition, the reaction vessels should be as completely filled as is possible to minimize volatilization to any air space.

(viii) *Control solution.* It is extremely important to take certain precautions to prevent loss of chemical from the reaction vessels by processes other than photolysis. For example, biodegradation and volatilization can be eliminated or minimized by use of sterile conditions and minimal air space in sealed vessels. Hydrolysis is a process which cannot be minimized by such techniques. Thus, control vessels containing test substance which are not exposed to light are required. In this way, the loss of test chemical in processes other than photolysis may be determined and eliminated or minimized. For simplicity, if the loss of chemical in the control is small (i.e., approximately 10 percent or less), one can calculate a first-order loss, k_{loss}, and subtract it from $(k_p)_{obs}$ to give the corrected direct photolysis rate constant K_p. If hydrolysis is found to be significant (greater than 10 percent), hydrolysis studies should be carried out first under § 796.3500.

(ix) *Absorption Spectrum as a criterion for performing the reaction quantum yield experiments.* This aqueous photolysis test is applicable to all chemicals which have UV-visible absorption maxima in the range 290–800 nm. Some chemicals have absorption maxima significantly below 290 nm but have measurable absorption tails above the baseline in their absorption spectrum at wavelengths greater than 290 nm. Photolysis experiments should be carried out for these chemicals to determine the reaction quantum yield. The absorption spectrum of the chemical in aqueous solution can be measured by the procedures given in § 796.1050.

(x) *Actinometers.* Chemical actinometers are used in reaction quantum yield experiments to measure the integrated light intensity incident on the sample during photolysis. Chemical actinometers have photochemical reactions which have well-defined reaction quantum yields ϕ_a at wavelength λ.

(A) *Low Optical Density Actinometers.* Two low optical density actinometers (absorbance <0.02) are described which can be used to determine the reaction quantum yield of a test chemical at low optical density (absorbance < 0.02) in Procedure One, under paragraph (b)(2) of this section. These actinometers are: p-nitroacetophenone-pyridine actinometer (PNAP/PYR); and p-nitroanisole-pyridine actinometer (PNA/PYR). The rate constants and half-lives of these actinometers can be adjusted to match the rate constant and half-life of the test chemical by adjusting the concentration of pyridine. With a 450-watt medium pressure mercury lamp in an Ace-type PMGRR, the PNAP/PYR actinometer can be adjusted with pyridine to have half-lives that range from greater than 12 hours to several weeks while the PNA/PYR actinometer can be adjusted with pyridine to have half-lives that range from approximately 15 minutes to approximately 12 hours (if a Moses-type PMGRR is used, then new criteria have to be defined to determine which of these two actinometers should be used. Details in the public record for this section).

(*1*) *p-Nitroacetophenone-Pyridine Actinometer (PNAP/PYR).* (*i*) The reaction quantum yield of the PNAP/PYR actinometer is a function of the molar concentration of pyridine [PYR] and this relationship is given by equation 7 under paragraph (a)(3)(ii)(A)(*3*) of this section. This equation is valid up to 0.2 M pyridine. The initial concentration of PNAP (C_o) is precisely set in the range of approximately 1×10^{-5} M. The molar absorptivities of PNAP at 313 and 366 nm are: $\epsilon_{313_a} = 2{,}056\ M^{-1}\ cm^{-1}$; and $\epsilon_{366_a} = 160\ M^{-1}\ cm^{-1}$. The chemical analysis of PNAP is given in paragraph (b)(1)(xi)(B) of this section.

(*ii*) Trial photolysis experiments are required to determine the concentration of pyridine needed to adjust the rate constant of the actinometer to approximately equal the rate constant of the test chemical (within ± 50 percent). In the Phase 2 experiments of Procedure 1 under paragraph (b)(2)(ii) of this section, the rate constant of the test chemical k_{pc} is determined in the Ace-type PMGRR with a 450-watt medium pressure mercury lamp. As an approximation, for the PNAP/PYR actinometer in the PMGRR with a 450 watt medium pressure mercury lamp, the concentration of pyridine needed to adjust the rate constant of the actinometer to approximately equal the rate constant of the test chemical at 313 and 366 nm is given by the following equations:

Equation 18

at 313 nm $[PYR] = 1.16\ k_{pc}$

Equation 19

at 366 nm $[PYR] = 5.95k_{pc}$.

where k_{pc} is in the units of $(hours)^{-1}$ and [PYR] is the molar concentration of pyridine.

(*iii*) A trial experiment is then carried out in the Phase 2 procedure under paragraph (b)(2)(ii) of this section by preparing the actinometer with the molar concentration of pyridine [PYR] estimated from equations 18 or 19 under paragraph (b)(1)(X)(A)(*1*)(*ii*) of this section and photolyzing the actinometer solution at a fixed molar concentration of PNAP (C_o) in the PMGRR at 313 or 366 nm. After approximately 50 percent transformation, the concentration of PNAP (C_t) is recorded at time t and an approximate rate constant k_{pa} is calculated using these data in Equation 2 under paragraph (a)(3)(ii)(A)(*1*) of this section. This trial experiment is repeated by adjusting the molar concentration of pyridine [PYR] until the rate constant of the test chemical and actinometer are approximately equal.

(*2*) *p-Nitroanisole-Pyridine Actinometer (PNA/PYR).* (*i*) The reaction quantum yield of the PNA/PYR actinometer is a function of the molar concentration of pyridine [PYR] and this relationship is given by equation 8 under paragraph (a)(3)(ii)(A)(*B*) of this section. This equation is valid up to 0.02 M pyridine. The initial concentration of PNA (C_o) is precisely set in the range of approximately 1×10^{-5} M at 366 nm and at approximately 0.4×10^{-5} M at 313 nm. The molar absorptivities of PNA at 313 and 366 nm are: $\epsilon_{313_a} = 10{,}300\ M^{-1}\ cm^{-1}$; and $\epsilon_{366_a} = 1{,}990\ M^{-1}\ cm^{-1}$. The chemical analysis for PNA is given in paragraph (b)(1)(xi)(C) of this section.

(*ii*) Trial photolysis experiments are required to determine the concentration of pyridine needed to adjust the rate constant of the actinometer to approximately equal the rate constant of the test chemical (within ± 50 percent). In the Phase 2 experiments of Procedure 1 under paragraph (b)(2)(ii) of this section, the rate constant of the test chemical k_{pc} is determined in an Ace-type PMGRR with a 450-watt medium pressure mercury lamp. As an approximation, for the PNA/PYR actinometer in the PMGRR with a 450 watt medium pressure mercury lamp, the concentration of pyridine needed to adjust the rate constant of the actinometer to equal the rate constant of the test chemical at 313 and 366 nm is given by the following equations:

Equation 20

at 313 nm $[PYR] = 8.93 \times 10^{-3} (k_{pc} - 0.0722)$

Equation 21

at 366 nm $[PYR] = 1.65 \times 10^{-3} (k_{pc} - 0.0349)$

where k_{pc} is in the units of $(hours)^{-1}$ and [PYR] is the molar concentration of pyridine.

(*iii*) A trial experiment is then carried out in the Phase 2 procedure under paragraph (b)(2)(ii) of this section by preparing the actinometer with the molar concentration of pyridine [PYR] estimated from equation 20 or 21 under paragraph (b)(1)(X)(A)(*2*)(*ii*) of this section and photolyzing the actinometer solution at a fixed molar concentration of PNA (C_o) in the Ace-type PMGRR at 313 or 366 nm. After approximately 50 percent transformation, the concentration of PNA (C_t) is recorded at time t and an approximate rate constant k_{pa} is calculated using these data in equation 2 under paragraph (a)(3)(ii)(A)(*1*) of this section. This trial experiment is repeated by adjusting the molar concentration of pyridine [PYR] until the rate constant of the test chemical and actinometer are approximately equal.

(B) *High Optical Density Actinometer (Ferrioxalate Actinometer).* The ferrioxalate actinometer at high optical density (absorbance >2) is a widely used actinometer by photochemists and has been recommended for use in Procedure Two under paragraph (b)(3) of this section [Murov (1973) under paragraph (d)(9) of this section, de Mayo and Shizuka (1976) under paragraph (d)(2) of this section, Calvert and Pitts (1966) under paragraph (d)(1) of this section]. This actinometer is applicable over the environmentally relevant range 290–500 nm. The net photochemical reaction is

Equation 22

$Fe^{3+} + (C_2O_4{}^{2-})/2 \xrightarrow{h\gamma} Fe^{2+} + CO_2$.

At high optical density, the reaction kinetics are zero-order and the kinetics are followed by measuring the molar concentration of Fe^{2+} formed as a function of time t using equation 11 under paragraph (a)(3)(ii)(B)(1) of this section. The Fe^{2+} formed is measured by procedures outlined in paragraph (b)(1)(xi)(D) of this section. Murov (1973) under paragraph (d)(10) of this section gives a detailed procedure for using this actinometer and it is highly recommended. This procedure should be modified slightly to use the ferrioxalate at 0.15 M. The irradiated solution is diluted 100-fold prior to analysis for ferrous ion, which must not be allowed to exceed 0.005 M. Table 1 of the associated Technical Support Document, which is part of the public record for this section, lists the reaction quantum yield as a function of wavelength. At 0.15 M, the quantum yield (ϕ_a) at 313 and 366 nm is 1.20 and 1.18, respectively. All ferrioxalate actinometry experiments must be carried out in a darkroom with photographic "safelights." Ferrioxylate is available from Alfa Inorganics. If the test chemical absorbs light at wavelengths greater than 500 nm, then Reinicke's salt can be used as the actinometer. The use of Reinicke's salt actinometer is described in the public record for this section.

(xi) *Chemical analysis of solutions.* (A) *Chemical analysis of test chemical solutions.* In determining the concentration of the chemical in solution, an analytical method should be selected which is most applicable to the analysis of the specific chemical substance. Chromatographic methods are generally recommended because of their chemical specificity in analyzing the parent chemical substance without interference from impurities. Whenever practicable the chosen analytical method should have a precision of ± 5 percent or better.

(B) *Chemical analysis of p-nitroacetophenone (PNAP).* The p-nitroacetophenone (PNAP) in the chemical actinometer solution is conveniently analyzed by high-pressure liquid chromatography using a 30 cm C_{18} reverse-phase column and a UV detector set at 280 nm. The mobile phase in volume percent is 2.5 percent acetic acid, 50 percent acetonitrile, and 47.5 percent water which is passed through the column at a flow rate of 2 mL/minute. [Mill et al. (1982) under paragraph (d)(8) of this section and Mill and Dulin (1982) under paragraph (d)(4) of this section.

(C) *Chemical analysis of p-nitroanisole (PNA).* The p-nitroanisole (PNA) in the chemical actinometer solution is conveniently analyzed by high-pressure liquid chromatography using a 30 cm C_{18} reverse-phase column and a uv detector set at 280 nm. The mobile phase in volume percent is 50 percent acetonitrile and 50 percent water which is passed through the column at a flow rate of 2 mL/minute [Dulin and Mill (1982) under paragraph (d)(4) of this section].

(D) *Chemical analysis of Ferrous Ion in the Ferrioxalate Actinometer.* The concentration of Fe^{2+} formed in the photolysis of the ferrioxalate actinometer is measured spectrophotometrically via the formation of a red phenanthroline complex and determining the absorbance of the complex at 510 nm. Murov (1973) under paragraph (d)(10) of this section describes a detailed procedure for measuring the molar concentration of ferrous ion formed in the photolysis reaction and this procedure is highly recommended. This procedure has been modified slightly to use the ferrioxalate at 0.15 M. As a result, the irradiated solution has to be diluted 100-fold prior to analysis for ferrous ion, which must not be allowed to exceed 0.005 M.

(2) *Procedure One: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and Actinometer Method*—(i) *Phase 1 Experiments: UV-Visible Absorption Spectra.* The UV-visible absorption spectra in aqueous solution can be determined by the methods described in §§ 796.1050 and 796.3700. It is recommended that the following additional procedures be followed:

(A) For chemicals which ionize or protonate (e.g., carboxylic acids, phenols, amines), carry out uv-visible absorption studies at pHs at least two pH units above the pK_a and at least two pH units below the pK_a. Prepare buffer solutions at 25 °C using reagent grade chemicals and distilled water as follows:

pHs in the range 3–6[1]: NaH_2PO_4, HCl;
pHs in the range 6–8[1]: KH_2PO_4, NaOH;
pHs in the range >8: Prepare buffers as described in the Handbook of Chemistry and Physics.

Check the pH of all the buffer solutions with a pH meter at 25 °C and adjust to the proper pH, if necessary. These buffer solutions can then be added to the test chemical solution until the desired pH is obtained. If these buffers are inadequate, then adjust the pH of the test chemical solution with 1 M HCl or NaOH at 25 °C.

(B) (*1*) Measure the absorbance, A_{λ_c}, as a function of wavelength in the range 290 to 800 nm in duplicate. If applicable, measure A_{λ_c} at each experimental pH under paragraph (b)(i)(vi) of this section. Record, in duplicate, the baseline where both the sample and reference cells are filled with blank solutions. These data will be used to calculate the molar absorptivities for the appropriate intervals and wavelength centers, under 796.3700(c)(3) Table 1, where the test chemical absorbs light. The wavelength center is defined as the midpoint of the interval range listed in §796.(cx3) Table 1.

(*2*) Measure A_{λ_c} at 313.0 nm and 366.0 nm in duplicate. These data will be used to calculate the molar absorptivities of the test chemical, ϵ_{313_c} and ϵ_{336_c}. Photolysis experiments should be carried out at the wavelength corresponding to the higher value of the molar absorptivity.

(*3*) It must be emphasized that the molar absorptivities of the test chemical (ϵ_{λ_c}) must be carefully determined, especially in the tails of the absorption bands at $\lambda > 290$ nm. Large errors will occur when calculating photolysis rate constants and half-lives if these measurements are not carefully carried out.

(ii) *Phase 2 Experiments: Trial Photolysis Experiments*—(A). *Determination of the approximate rate constant of the test chemical*—(*1*) *preparation of buffer solutions.* Prepare buffer solutions according to the procedures outlined in paragraph (b)(2)(i) of this section using reagent grade chemicals and pure water as described under Test Conditions in paragraph (b)(1)(iv) of this section, for chemicals that reversibly ionize or protonate.

(*2*) *Preparation of Test Chemical Solution.* Prepare a homogeneous solution of test chemical below its water solubility and at an absorbance less than 0.02 in the photolysis reaction vessel at 313 or 366 nm. For very hydrophobic chemicals, it is difficult and time consuming to prepare aqueous solutions. To facilitate the preparation of aqueous solutions containing very hydrophobic chemicals and to allow for easier analytical procedures, the following procedure may be used as an aid in the dissolution of the test chemical. Dissolve the pure test chemical in reagent grade acetonitrile. Add pure water as described under Test Conditions in paragraph (b)(1)(iv) of this section, or buffer solution, as described in paragraph (b)(2)(iv) of this section, for chemicals which ionize or protonate, to an aliquot of the acetonitrile solution. Do not exceed one-volume percent of acetonitrile in the final solution.

(*3*) *Performance of the Test.* (*i*) Prepare an aqueous solution of test chemical as described in paragraph (b)(2)(ii)(A)(*2*) of this section and a sufficient number of samples in quartz or borosilicate glass tubes under paragraph (b)(1)(ii)(D) of this section to perform all the required tests. Measure the initial concentration of test chemical (C_o) as described in paragraph (b)(1)(xi)(A) of this section in duplicate. Fill the tubes as completely as possible and seal them. Do not use grease. Prepare two control samples in the absence of ultraviolet light and totally exclude light by wrapping the tubes with aluminum foil or by any other suitable method in paragraph (b)(1)(viii) of this section. Place the samples, including the controls, in the Ace-type PMGRR with a 450 watt medium pressure mercury lamp and appropriate filters as described in paragraph (b)(1)(ii)(B) and (A) of this section. The reaction tubes should be controlled to a temperature of $t_i \pm 2°$ C within the range 20 – 30° C. Photolyze the samples at 313 or 366 nm corresponding to the higher value of the molar absorptivity of the test chemical (ϵ_{313_c} or ϵ_{366_c} determined in the Phase 1 experiments, under paragraph (b)(2)(i) of this section) until approximately 50 percent of the test chemical has transformed. Measure the molar concentration of the test chemical (C_t) in duplicate at time t. For test chemicals that ionize or protonate, carry out the photolysis experiments at the required pHs as described under Test Conditions in paragraph (b)(1)(vi) of this section.

(*ii*) After the photolysis experiments are completed, determine the concentration of test chemical in the controls in duplicate. If a significant loss of test chemical has occurred in the controls, determine the cause and eliminate, or minimize, the loss. If hydrolysis is found to be significant, hydrolysis experiments should be carried out first under paragraph (b)(1)(viii) of this section.

(*iii*) The data obtained in this section will be used to determine an approximate k_{pe} and $(t_{1/2})_c$ using Equations 3 and 5 under paragraph (a)(3)(ii)(A)(*1*) of this section.

(B) *Actinometry Experiments.* These experiments are designed to choose the appropriate low optical density actinometer [i.e., PNAP/PYR under paragraph (b)(1)(x)(A)(*1*) of this section or PNA/PYR under paragraph (b)(1)(x)(A)(*2*) of this section] and to adjust the rate of the chosen actinometer so that the rate constant of the actinometer (k_{pa}) is approximately equal to the rate constant of the test chemical (k_{pc}). Based on the photolysis experiments in paragraph (b)(2)(ii) of this section, if $(t_{1/2})_c$ is less than 12 hours, use the PNA/PYR actinometer; if $(t_{1/2})_c$ is greater than 12 hours, use the PNAP/PYR actinometer.

(*1*) *Preparation of Actinometer Solutions*—(*i*) *PNAP/PYR Actinometer.* Using the test chemical photolysis rate constant k_{pc} determined in paragraph (b)(2)(ii)(A) of this section and equations

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[1] Use the minimum concentration of buffers to attain the desired pH.

18 or 19 under paragraph (b)(1)(x)(A)(*1*)(*ii*) of this section, determine the molar concentration of pyridine needed to adjust the rate constant of the actinometer to approximately equal the rate constant of the test chemical at the appropriate wavelength 313 or 366 nm (chosen based on the results of the Phase 1 experiments). Once the molar concentration of pyridine [PYR] has been estimated, the actinometer solution can be prepared as follows. Dissolve 0.0165 grams of PNAP in 10 mL of acetonitrile (0.01 M). Add 1 mL of this solution to a 1 liter volumetric flask. Add to the volumetric flask the mass in grams, or the volume V of pyridine at 20 °C, obtained from the equations

Equation 23

$$\text{mass (grams)} = 79.1\,[\text{PYR}]$$

Equation 24

$$V(\text{mL}) = 80.6[\text{P\v{Y}R}]$$

Fill the volumetric flask with pure water as described in paragraph (b)(1)(iv) of this section to give 1 liter of solution and shake vigorously to make sure that the solution is homogeneous. The resultant concentration of PNAP is 1.00×10^{-5} M. The PNAP/PYR solution should be wrapped with aluminum foil and kept from bright light.

(*ii*) *PNA/PYR Actinometer.* Using the test chemical photolysis rate constant k_{pc} determined in paragraph (b)(2)(ii)(A) of this section and Equations 20 or 21 under paragraph (b)(1)(4)(A)(2) of this section, determine the concentration of pyridine needed to adjust the rate constant of the actinometer to approximately equal the rate constant of the test chemical at the appropriate wavelength 313 or 366 nm (chosen based on the results of the Phase 1 experiments). Once the molar concentration of pyridine has been determined, an actinometer solution can be prepared as follows. If photolysis experiments are to be carried out at 366 nm, dissolve 0.0153 grams of PNA in 10 mL of acetonitrile (0.01 M). Add 1 mL of this solution to a 1 liter flask. Add to the volumetric flask the mass in grams, or the volume of pyridine at 20 °C as defined by equations 23 and 24 under paragraph (b)(2)(ii)(B)(*1*)(*i*) of this section. Fill the volumetric flask with pure water under paragraph (b)(1)(iv) of this section to give 1 liter of solution and shake vigorously to make sure that the solution is homogeneous. The resultant concentration is 1.00×10^{-5} M. The PNA/PYR solution should be wrapped with aluminum foil and kept from bright light. If photolysis experiments are to be carried out at 313 nm, dissolve 0.00612 grams of PNA in 10 mL of acetonitrile (0.004 M). Then follow the procedure described above. The resultant PNA concentration is 0.400×10^{-5} M. The PNA/PYR solution should be wrapped with aluminum foil and kept from bright light.

(*2*) *Performance of the Test.* Prepare a solution of the appropriate actinometer as described in paragraph (b)(2)(ii)(B)(*1*) of this section. Measure the initial molar concentration of actinometer C_o (PNAP or PNA as described in paragraph (b)(1)(xi) (B) and (C) of this section). Prepare a sufficient number of samples in borosilicate or quartz tubes, under paragraph (b)(1)(ii)(D) of this section, to perform all the required tests. The tubes should be identical to the tubes used to determine k_{pc} in paragraph (b)(2)(ii)(A)(*3*) of this section. Fill the tubes as completely as possible and seal them. It is important that the actinometer tubes contain the same volume as that used in the test chemical tubes, paragraph (b)(2)(ii)(A)(*3*) of this section. Prepare two control samples in the absence of ultraviolet light and totally exclude light by wrapping the tubes with aluminum foil or by any other suitable method under paragraph (b)(1)(viii) of this section. Place the samples, including the controls, in the Ace-type PMGRR with the 450 watt medium pressure mercury lamp and the appropriate filters. The tubes should be controlled to a temperature $t_1 \pm 2$ °C. Photolyze the samples at the chosen wavelength λ (313 or 366 nm) until approximately 50 percent of the actinometer has transformed. Determine the concentration of the actinometer (C_t) in duplicate by the appropriate procedure described in paragraph (b)(1)(xi) (B) and (C) of this section at time t. Calculate a rate constant using Equation 2 under paragraph (a)(3)(ii)(A)(*1*) of this section. If the rate constant k_{pa} is not approximately the same as the rate constant of the test chemical k_{pc}, then repeat the experiment with an appropriate concentration of pyridine until k_{pa} is approximately equal to k_{pc} (within ± 50 percent).

(iii) *Phase 3 Experiments: Determination of the Reaction Quantum Yield of the Test Chemical.* Based on the results of the Phase 1 and 2 experiments, use the appropriate actinometer with the required concentration of pyridine to make the rate constant of the actinometer approximately match the rate constant of test chemical. Follow the procedure outlined in paragraph (b)(2)(ii)(B)(*1*) of this section to prepare the actinometer and a sufficient number of samples in borosilicate glass or quartz tubes as described under paragraph (b)(1)(ii)(D) of this section to perform all the required tests. Determine the initial concentration of the actinometer $(C_o)_a$ in duplicate according to the appropriate procedure in paragraph (b)(1)(xi) of this section. Fill all the tubes as completely as possible, seal them, and cover half of the tubes with aluminum foil as soon as possible after preparation. Prepare an aqueous solution of test chemical as described in paragraph (b)(2)(ii)(A)(*2*) of this section and determine the initial concentration of test chemical $(C_o)_c$ in duplicate according to the procedure described in paragraph (b)(1)(xi)(A) of this section. Prepare a sufficient number of samples in borosilicate or quartz tubes to perform all the required tests. Fill all the tubes as completely as possible, seal them, and cover half of the tubes with aluminum foil as soon as possible after preparation. The reaction tubes for actinometer and test chemical must be identical and contain the same volume of solution. Place the tubes to be photolyzed in the Ace-type PMGRR containing a 450 watt medium pressure mercury lamp and the appropriate filters, paragraph (b)(1)(ii) (B) and (C) of this section, and all the control tubes close to the PMGRR. The tubes should be temperature controlled to a temperature $t_1 \pm 2$ °C. Based on the results of the Phase 2 experiments, determine the concentration of test chemical and actinometer periodically between 10 and 80 percent transformation in duplicate (at least 6 data points at approximately equal times). Determine the concentration of actinometer and test chemical in the controls in duplicate at each time point.

(3) *Procedure Two: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and High Optical Density Actinometer Method*—(i) *Phase 1 Experiments: UV-Visible Absorption Spectra.* Follow the procedure outlined in paragraph (b)(2)(i) of this section and measure the absorbance of the test chemical, $A_{\lambda c}$, as a function of wavelength in the range 290 to 800 nm in duplicate. Measure $A_{\lambda c}$ at 313.0 and 366.0 nm in duplicate. The absorbance data at 313.0 and 366.0 nm will be used to calculate the molar absorptivities of the test chemical, ϵ_{313c} and ϵ_{366c}, and photolysis experiments should be carried out at the wavelength corresponding to the highest value of the molar absorptivity.

(ii) *Phase 2 Experiments: Determination of the Reaction Quantum Yield of the Test Chemical*—(A) *Preparation of buffer solutions.* Prepare buffer solutions according to the procedures outlined in paragraph (b)(2)(ii)(A)(*1*) of this section for a test chemical which ionizes or protonates.

(B) *Preparation of test chemical solution.* Prepare a homogeneous solution of test chemical according to the procedures outlined in paragraph (b)(2)(ii)(A)(*2*) of this section.

(C) *Preparation of ferrioxalate actinometer solution.* Prepare a ferrioxalate actinometer solution at 0.15 M as described in paragraph (b)(1)(x)(B) of this section following the procedure outlined by Murov (1973) under paragraph (d)(10) of this section which has been modified slightly. Since the ferrioxalate actinometer solution is very sensitive to visible light, all these actinometer experiments must be carried out in a darkroom with photographic "safelights."

(D) *Determination of the quantum yield of the test chemical.* (*1*) Prepare a solution of the ferrioxalate actinometer at high optical density (absorbance >2) as described in paragraph (b)(3)(ii)(C) of this section and completely fill the reaction cell described in paragraph (b)(1)(ii)(D) of this section. Place the reaction cell in the photochemical optical bench (POB) as described in paragraph (b)(1)(ii)(A)(*2*) of this section with a 450 watt medium pressure mercury lamp under paragraph (b)(1)(ii)(B) of this section and appropriate filters under paragraph (b)(1)(ii)(C) of this section. The reaction cell should be controlled to a temperature of $t_1 \pm 2$ °C in the range 20 to 30 °C. The filter system used should isolate the wavelength 313 or 366 nm corresponding to the larger value of ϵ_{313_c} or ϵ_{366_c} determined in the Phase 1 experiments. Photolyze the actinometer solution in the reaction cell for 1, 3, 6, 9, 12, and 15 minutes and measure the concentration of Fe^{2+} formed spectrophotometrically by the procedure outlined in paragraph (b)(1)(xi)(D) of this section in duplicate at each of the time points. No time points should be taken when the optical density of the actinometer falls below 2. Only withdraw small volumes for analysis so that the total volume of the actinometer solution in the reaction cell does not change appreciably. This procedure is repeated in a third set of experiments soon after the test chemical photolysis experiments are performed.

(*2*) In the second set of experiments, a solution of test chemical is prepared according to the procedure outlined in paragraph (b)(3)(ii)(B) of this section at low optical density (absorbance <0.02). Measure the initial concentration $(C_o)_c$ in duplicate by the procedure outlined in paragraph (b)(1)(xi)(A) of this section. Fill two reaction cells with the same volume as that used in the actinometer experiments. One of these cells is wrapped with aluminum foil, placed close to the POB and temperature controlled to $t_1 \pm 2$ °C. The second reaction cell (which is the same one used for the actinometry experiments) is placed in the POB and photolyzed at $t_1 \pm 2$ °C at the same wavelength used in the actinometry experiments. Determine the concentration of test chemical periodically between 10 and 80 percent transformation in duplicate [at least 6 data points at approximately equal times]. Only withdraw small volumes for analysis so that the total volume of the test chemical solution in the reaction cell does not change appreciably. Determine the concentration of the control in duplicate at each time point.

(*3*) As an alternative procedure, the same series of experiments described above can be carried out in a PMGRR using individual reaction tubes for each datum point.

(c) *Data and reporting*—(1) *Procedure One: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and Actinometer Method*—(i) *Phase 1 Experiments: UV-Visible Absorption Spectra*—(A) *Treatment of results.*

(*1*) The molar absorptivity ($\epsilon_{\lambda c}$) can be determined from the absorption spectra using the expression

Equation 25

$$\epsilon_{\lambda c} = A_{\lambda c}/Cl.$$

where $A_{\lambda c}$ is the absorbance at wavelength λ, C is the molar concentration of test chemical, l is the cell pathlength in centimeters. The molar absorptivity of the chemical should be determined for the wavelengths listed in § 796.3700(c)(3) Table 1, for a solution of concentration C and in a cell with pathlength l. If the absorption curve is flat within the interval around the wavelength center, $\epsilon_{\lambda c}$ may be determined from the absorbance $A_{\lambda c}$ at λ center using Equation 25. If a large change in absorbance occurs within this interval, obtain an average $A_{\lambda c}$ at λ center based on the two absorbances at the boundary of the interval. Calculate an average $\epsilon_{\lambda c}$ using the average value of $A_{\lambda c}$ in Equation 25. Determine the molar absorptivity for each replicate and calculate a mean value.

(*2*) Determine the molar absorptivity of the test chemical at 313.0 and 366.0 nm for a solution of test chemical at a molar concentration C and in a cell of pathlength l using Equation 25 under paragraph (c)(1)(i)(A)(*1*) of this section. Determine $A_{\lambda c}$ and the molar absorptivity at 313.0 and 366.0 nm for each replicate and calculate a mean value.

(*3*) Using the molar absorptivities obtained from the spectra and the values of L_λ from § 796.3700(c)(3) Tables 3 to 6, calculate the maximum direct photolysis rate constant $(k_{pE})_{max}$, at a specific latitude (corresponding to the manufacturing site) and season of the year using equation 16 under paragraph (a)(3)(ii)(B)(*6*) of this section. The corresponding minimum half-life, $(t_{1/2E})_{min}$, can then be calculated using this $(k_{pE})_{max}$, in equation 17 under paragraph (a)(3)(ii)(B)(*6*) of this section.

(B) *Test data report.* (*1*) Report the name, structure, and purity of the test chemical.

(*2*) Submit a recent test spectrum on appropriate reference chemicals for photometric and wavelength accuracy.

(*3*) Submit the original chart, or photocopy, containing a plot of absorbance of test chemical vs. wavelength plus the baseline. Spectra should include a readable wavelength scale, preferably marked at 10 nm intervals. Each spectrum should be clearly marked with the test conditions.

(*4*) Report the concentration of the test chemical solution, the type of absorption cell used (quartz or borosilicate glass) and the pathlength.

(*5*) Report $A_{\lambda c}$ and $\epsilon_{\lambda c}$ at λ center for each replicate and the mean value.

(*6*) Report the identity and composition of the solvent used in the spectral absorption study.

(*7*) Report ϵ_{313_c} and ϵ_{366_c} along with A_{313_c} and A_{366_c} for each replicate and the mean value.

(*8*) Report $(k_{pE})_{max}$, and $(t_{1/2E})_{min}$, for the summer and winter solstices using the appropriate L_λ values from § 796.3700(c)(3) Tables 3–6, closest to the latitude of the chemical manufacturing site.

(*9*) For chemicals that ionize or protonate, report the data for steps 1 to 8 at the required pHs under paragraph (b)(1)(vi) of this section.

(*10*) For a chemical that ionizes or protonates, report its pK_a. Report the type and concentration of the buffers employed for each pH.

(*11*) Describe the method employed in determining the test chemical's concentration.

(*12*) Report the name and model of the spectrophotometer used.

(*13*) Report the various control settings employed with the spectrophotometer. These might include scan speed, slit width, gain, etc.

(*14*) If a Moses-type PMGRR was used in these experiments, describe it completely and report the aperture in cm^2.

(ii) *Phase 2 Experiments: Trial Photolysis Experiments*—(A)

Determination of the Approximate Rate Constant of the Test Chemical—(*1*) *Treatment of results.* From the photolysis experiments carried out at 313 or 366 nm (photolysis wavelength chosen based on the results of the Phase 1 experiments), use the concentration $(C_t)_c$ corresponding to approximately 50 percent of the initial molar concentration of chemical remaining at time t along with the initial molar concentration $(C_o)_c$ and the time t in hours in equation 3 under paragraph (a)(3)(ii)(A)(*1*) of this section to calculate k_{pc} in hours^{-1}. From the analysis of the two samples at time t=0 and t, calculate a mean value of $(C_o)_c$ and $(C_t)_c$, and a value of k_{pc}. If a slight loss of chemical has been detected in the controls, then calculate a rate constant as follows. Calculate an average concentration $(C_t)_c$ based on duplicate measurements of concentration in the controls at the end of the experiment (time t). Use this concentration along with the average initial concentration $(C_o)_c$ and t in equation 3 under paragraph (a)(3)(ii)(A)(*1*) of this section and calculate a rate constant k_{loss}. Using this rate constant along with the observed rate constant in the photolysis experiments, the corrected rate constant is then

Equation 26

$k_{pc} = (k_{pc})_{obs} - k_{loss}$

Calculate the half-life, $(t_{1/2})_c$ using the corrected value of k_{pc} in equation 5 under paragraph (a)(3)(ii)(A)(*I*) of this section.

(*2*) *Specific analytical and recovery procedures.* (*i*) Provide a detailed description or reference for the analytical procedures used, including the calibration data and precision; and (*ii*) if extraction methods were used to separate the solute from the aqueous solution, provide a description of the extraction method as well as the recovery data.

(*3*) *Other test conditions.* (*i*) Report the size, approximate cell wall thickness, pathlength l, and type of glass used for the test chemical reaction tubes. If the cell pathlength was measured by the procedure described in the Technical Support Document available as part oofthe public record, report all the data obtained in these experiments.

(*ii*) Report the initial pH of all test solutions, if appropriate.

(*iii*) If acetonitrile was used to solubilize the test substance, report the percent, by volume.

(*iv*) If a significant loss of test chemical occurred in the control solution, indicate the causes and how they were eliminated or minimized.

(*4*) *Test data report.*

(*i*) Report the wavelength used to photolyze the test chemical.

(*ii*) Report the initial molar concentration of test chemical $(C_o)_c$ of each replicate and the mean value.

(*iii*) Report the molar concentration of test chemical $(C_t)_c$ for each replicate and the mean value. Report the time t.

(*iv*) Report the molar concentration of each replicate control sample and the mean value after completion of the experiment. Report the time t.

(*v*) Report the value of k_{pc} and $(t_{1/2})_c$. If small losses of chemical were observed, report $(k_{pc})_{obs}$, K_{loss}, and k_{pc}. Report the half-life $(t_{1/2})_c$ calculated using the value of k_{pc}.

(*vi*) For chemicals that ionize or protonate, report the data for steps 1 through 5 for the experiments at the required pHs. Report the initial pH of all test chemical solutions and the type of concentration of the buffers used for each pH.

(B) *Actinometry Experiments*—(*1*) Treatment of results. Follow the same discussion of the treatment of results, paragraph (c)(1)(ii)(A)(*1*) of this section, to determine the rate constant of the actinometer k_{pa} using equation 2 under paragraph (a)(3)(ii)(A)(*1*) of this section. Repeat these calculations for the trial actinometry experiments to determine the molar concentration of pyridine [PYR] needed to adjust the rate constant of the actinometer (k_{pa}) to be approximately equal to the rate constant of the test chemical (k_{pc}).

(*2*) *Test data report.* (*i*) Report the wavelength used to photolyze the actinometer.

(*ii*) Report the size, approximate cell wall thickness, pathlength l, and the type of glass used for the actinometer reaction tubes.

(*iii*) For each trial experiment: report the actinometer used; report the initial concentration of actinometer $(C_o)_a$ for each replicate and the mean value; report the molar concentration of the actinometer $(C_t)_a$ for each replicate and the mean value. Report the time t; report the molar concentration of each replicate control sample and the mean value after the completion of the experiment. Report the time t; and report the value of k_{pa}. If small losses were observed for the actinometer, report $(k_{pa})_{obs}$, k_{loss}, and k_{pa}.

(*iv*) If the Moses-type PMGRR was used, list the criteria and equations used as described in the Technical Support Document available as part of the public record for this section.

(iii) *Phase 3 Experiments: Determination of the Reaction Quantum Yield of the Test Chemical*—(A) *Treatment of results.* The objective of this set of experiments is to determine the reaction quantum yield for a specific test chemical (ϕ_c) at low optical density with a low optical density actinometer. The reaction quantum yield ϕ_c can be calculated using equation 1 under paragraph (a)(3)(ii)(A)(*1*) of this section.

$\phi_c = (k_{pc}/k_{pa})\ \epsilon_{\lambda a}/\epsilon_{\lambda c})\phi_a$,

by the following steps.

(*1*) Photolysis experiments are carried out by simultaneously photolyzing the test chemical and actionometer in the PMGRR at the chosen wavelength λ. The concentration of test chemical and actinometer are measured periodically as a function of time. These data are then used to determine the ratio of the rate constants (k_{pc}/k_{pa}) employing linear regression analyses of the data on equation 6 under paragraph (a)(3)(ii)(A)(*2*) of this section.

$\ln(C_o/C_t)_c = (k_{pc}/k_{pa})\ln(C_o/C_t)_a$

with $\ln(C_o/C_t)_a$ as the independent variable and $\ln(C_o/C_t)_c$ as the dependent variable. The slope of the best straight line is the ratio of the rate constants (k_{pc}/k_{pa}). If a slight loss of test chemical or actinometer was detected in the controls at any time t, then employ the following procedure. Consider, as an example, the loss of test chemical in the control at time t. Using the average concentration of the test chemical in the controls from the replicates at time t and the average initial concentration, calculate $\ln(C_o/C_t)_c^{loss}$. Using the average concentration of test chemical from the replicates after photolysis time t, calculate $\ln(C_o/C_t)_c^{obs}$. The corrected term is then

Equation 27

$\ln(C_o/C_t)_c^{corr} = \ln(C_o/C_t)_c^{obs} - \ln(C_o/C_t)_c^{loss}$.

The same procedure can be applied to obtain a corrected term for the actinometer. Using the corrected terms for test chemical and/or actinometer in equation 6 under paragraph (a)(3)(ii)(A)(*2*) of this section, determine the ratio of the rate constants (k_{pc}/k_{pa}) as described under paragraph (c)(1)(iii)(A)(*1*) of this section.

(*2*) Determine the quantum yield of the actinometer ϕ_a using Equation 7 or 8 under paragraph (a)(3)(ii)(A)(*3*) of this section and the molar concentration of pyridine [PYR] present in the actinometer.

(*3*) Use the molar absorptivities of test chemical $(\epsilon_{\lambda c})$ and actinometer $(\epsilon_{\lambda a})$ at the wavelength the photolysis experiments were carried out (i.e., 313 or 366 nm).

(*4*) (*i*) Substitute the values of (k_{pa}/k_{pc}), $\epsilon_{\lambda a}$, $\epsilon_{\lambda c}$, and ϕ_a in Equation 1 under paragraph (a)(3)(ii)(A)(*1*) of this section and calculate the reaction quantum yield of the test chemical ϕ_c.

(*ii*) A hypothetical example is presented in the paragraph (c)(3) of this section, to illustrate how all the data can be used in Procedure One to determine the reaction quantum yield of the test chemical and to determine (k_{pE}) and $(t_{1/2E})$ as a function of latitude and season of the year.

(B) *Other test conditions.* (*1*) Report the size, approximate cell wall thickness, the pathlength l, and type of glass used for tubes used to hold the test chemical and actinometer solutions.

(*2*) Report the initial pH of all test chemical solutions, if appropriate, and the type and concentration of the buffers employed for each pH.

(*3*) If acetonitrile was used to solubilize the test chemical, report the percent, by volume, of the acetonitrile which was used.

(*4*) If significant loss of test chemical occurred in the control solution, indicate the causes and how they were eliminated or minimized.

(C) *Test data report.* (*1*) Report the wavelength used in the photolysis experiments.

(*2*) Report the actinometer used.

(*3*) Report the initial molar concentration of test chemical $(C_o)_c$ of each replicate and the mean value.

(*4*) Report the initial molar concentration of actinometer $(C_o)_a$ and the molar concentration of pyridine used.

(*5*) For each time point, report the two separate values for the molar concentration of test chemical $(C_t)_c$ and actinometer $(C_t)_a$ and the mean values.

(*6*) For each time point, report the two separate values of the molar concentration of test chemical and actinometer controls and the mean values.

(*7*) Tabulate and report the following data: t, $\ln(C_o/C_t)_c$, and $\ln(C_o/C_t)_a$. From the linear regression analysis, report the ratio of the rate constants (k_{pc}/k_{pa}), and the correlation coefficient.

(*8*) If loss of test chemical and/or actinometer was observed during photolysis, then report the data $\ln(C_o/C_t)^{corr.}$, $\ln(C_o/C_t)^{obs.}$, and $\ln(C_o/C_t)^{loss}$ for the test chemical and/or actinometer for each time point. From the linear regression analysis of test chemical $\ln(C_o/C_t)_c^{corr.}$ and/or actinometer $\ln(C_o/C_t)_a^{corr.}$, report the ratio of the rate constants (k_{pc}/k_{pa}) and the correlation coefficient.

(*9*) Report the reaction quantum yield of the actinometer (ϕ_a).

(*10*) Report the reaction quantum yield of the test chemical (ϕ_c).

(*11*) Report $(k_{pE})_{max.}$, (K_{pE}), $(t_{1/2E})_{min.}$, and $(t_{1/2E})$ for the summer and winter solstices using the appropriate L_λ values from § 796.3700(c)(3) Tables 3 to 6, closest to the latitude of the chemical manufacturing site.

(*12*) For chemicals that ionize or protonate, report the data for steps 1 through 11 for the experiments at the required pHs under paragraph (b)(1)(vi) of this section.

(2) *Procedure Two: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and High Optical Density Actinometer Method*—(i) *Phase 1 Experiments: UV-Visible Absorption Spectra.* The treatment of results and data reporting are exactly the same as described in paragraph (c)(1)(i) of this section.

(ii) *Phase 2 Experiments: Determination of the Reaction Quantum Yield of the Test Chemical*—(A) *Treatment of results.* The objective of this set of experiments is to determine the reaction quantum yield of a test chemical (ϕ_c) at low optical density with the high optical density ferrioxalate actinometer. ϕ_c can be calculated using Equation 9 under paragraph (a)(3)(ii)(B)(*1*) of this section),

$$\phi_c = \phi_a (k_{pc}/k_{pa}) (2.303 \epsilon_{\lambda c} l)^{-1},$$

by the following steps.

(*1*) In the first set of experiments, the ferrioxalate actinometer is photolyzed in a POB at the chosen wavelength λ (313 or 366 nm) and the molar concentration of ferrous ion $(C_t)_a$ is measured as a function of the time t. These data are fitted to Equation 11 under paragraph (a)(3)(ii)(B)(*1*) of this section using linear regression analysis. The slope is equal to K_{pa}. The data obtained from the third set of experiments are used in the same manner as described above to obtain another actinometer rate constant. These two rate constants are used to obtain an average actinometer rate constant $(k_{pa})_{ave.}$.

(*2*) In the second series of experiments, the test chemical is photolyzed in the same cell in the POB and the concentration of test chemical $(C_t)_c$ is measured as a function of the time t. These data are fitted to Equation 3 under paragraph (a)(3)(ii)(A)(*1*) of this section using linear regression analysis. The slope is equal to k_{pc}. If a slight loss of test chemical is detected in the controls, then follow the procedure outlined in paragraph (b)(1)(viii) of this section to calculate a corrected test chemical rate constant.

(*3*) Use the appropriate quantum yield of the ferrioxylate actinometer (at 0.15 M) at the chosen wavelength: at 313 nm, $\phi_a = 1.20$; at 366 nm, $\phi_a = 1.18$.

(*4*) Use the molar absorptivity of the test chemical $(\epsilon_{\lambda c})$ at the appropriate photolysis wavelength (313 or 366 nm) determined in paragraph (c)(2)(i) of this section.

(*5*) Use the known pathlength of the reaction cell *l* under paragraph (b)(1)(iii) of this section.

(*6*) Substitute the values of k_{pc}, $(k_{pa})_{ave.}$, ϕ_a, $\epsilon_{\lambda c}$, and *l* in Equation 9 under paragraph (a)(3)(ii)(B)(*1*) of this section and calculate the reaction quantum yield of the test chemical ϕ_c.

(*7*) If the PMGRR equipment was used in these experiments, then repeat the calculations for Steps 1 through 6 obtained in this equipment.

(B) *Specific analytical and recovery procedures.* (*1*) Provide a detailed description or reference for the analytical procedures used, including the calibration data and precision; and

(*2*) If extraction methods were used to separate the solute from the aqueous solution, provide a description of the extraction method as well as the recovery data.

(C) *Other test conditions.* (*1*) Describe the equipment used (i.e., the type of PMGRR or POB). If a Moses-type PMGRR was used, report the aperture in cm^2.

(*2*) If the POB equipment was used, describe in detail the photolysis reaction cell. Report the volume, pathlength *l*, and a description of the optical windows used.

(*3*) If the PMGRR equipment was used, report the size, approximate cell wall thickness, and type of glass used for the reaction tubes.

(*4*) If the pathlength of the cell was measured by the procedure outlined in the Technical Support Document, available as part of the public record for this section, then report all the data obtained in determining the cell pathlength.

(*5*) If acetonitrile was used to solubilize the test chemical, report the percent by volume.

(*6*) Report the pH of all test chemical solutions, if appropriate.

(*7*) If a significant loss of test chemical occurred in the control samples, indicate the causes and how they were eliminated.

(D) *Test data report.* (*1*) Report the wavelength used in the photolysis experiments.

(*2*) For the actinometry experiments 1 and 3; (*i*) report for each time point the two values of the molar concentration of ferrous ion formed and the mean value; (*ii*) report the actinometer rate constant k_{pa} and correlation coefficient for each experiment; and (*iii*) report the average actinometer rate constant $(k_{pa})_{ave.}$.

(*3*) For the test chemical photolysis experiment; (*i*) report the initial molar concentration of chemical $(C_o)_c$ of each replicate and the mean value; (*ii*) report the two separate values of the molar concentration of test chemical $(C_t)_c$ and the mean value at time t. Report the time t; (*iii*) report the two separate values of the molar concentration of the test chemical controls and the mean value for each time point. Report t for each time point; and (*iv*) report from the linear regression analyses of the data, the rate constant of the test chemical (k_{pc}), and the correlation coefficient.

(*4*) Report the reaction quantum yield of the actinometer and (ϕ_a).

(*5*) Report the reaction quantum yield of the test chemical (ϕ_c).

(*6*) Report $(k_{pE})_{max}$, (k_{pE}), $(t_{1/2E})_{min}$, and $(t_{1/2E})$ for the summer and winter solstices using the appropriate L_λ values from § 796.3700(c)(3) Tables 3 to 6, closest to the latitude of the chemical manufacturing site.

(*7*) For chemicals that ionize or protonate, report the data for steps 1 through 6 for the experiments at the required pHs under paragraph (b)(1)(vi) of this section.

(3) *Hypothetical Illustrative Example: Determination of the Reaction Quantum Yield by the Low Optical Density Test Chemical and Actinometer Method and Sunlight Photolysis.* (i)(A) Consider a chemical plant just south of Peoria, Illinois on the Illinois River which produces an organic chemical A which is not an acid or a base. The waste effluent passes through a primary and secondary treatment plant and the waste, which still contains some chemical A, is then discharged into the river. The plant is located at 40.7 degrees north latitude. Information is needed on photolysis rates and half-lives of chemical A in aqueous media in the summer and winter seasons.

(B) The company research laboratory is located in Peoria, Illinois and the required photolysis data was needed in January. Since the temperature outdoors was well below freezing during this month, the outdoor sunlight photolysis experiments, described in § 796.3700 could not be carried out. Thus, it was necessary to carry out photolysis experiments in the laboratory. The research laboratory was equipped to carry out photolysis experiments with an Ace-type PMGRR and thus Procedure One was used.

(ii) Laboratory Experiments, Data, and Calculations: (A) *Phase 1 Experiments.* (*1*) Chemical A had a saturated water solubility of 3.9×10^{-3} M at 25 °C. In the Phase 1 procedure, the UV-visible absorption spectrum was obtained for chemical A at a concentration of 1.00×10^{-5} M in a 10.0 cm quartz cell in duplicate. Using the wavelength interval range from § 796.3700(c)(3) Table 1, the average absorbance of duplicate runs at λ center was obtained and the results are summarized in the following Table 1. (It must be emphasized that ϵ_{λ_c} has to be averaged over the wavelength intervals that correspond to the same intervals for the L_λ values centered at λ. This has already been taken care of in this section since the wavelength interval ranges listed in Table 1 coincide with the same wavelength intervals for L_λ centered at λ in § 796.3700(c)(3) Tables 3 to 6. In addition, the average absorbance was measured at 313.0 nm and at 366.0 nm and these results are summarized in the following Table 1:

TABLE 1—SUMMARY OF SPECTRAL AND PHOTOLYSIS DATA FOR CHEMICAL A

SPECTRAL DATA

λ center (nm)	A_{λ_c}	$\epsilon_{\lambda_c}(M^{-1}cm^{-1})$
297.5	0.5904	5904
300.0	0.5414	5414
302.5	0.4924	4924
305.0	0.4434	4434
307.5	0.3810	3810
310.0	0.3124	3124
312.5	0.2714	2714
315.0	0.2224	2224
317.5	0.2004	2004
320.0	0.1514	1514
323.1	0.1310	1310
330.1	0.1030	1030
340.0	0.0645	645
350.0	0.0473	473
360.0	0.0287	287
370.0	0.0170	170
380.0	0.0085	85
390.0	0.0030	30
400.0	0.0010	10
410.0	0.0000	0
313.0	0.2610	2610
366.0	0.0185	185

PHOTOLYSIS DATA

λ center (nm)	Summer		Winter	
	L_λ[1]	$\epsilon_{\lambda_c}L_\lambda(day^{-1})$	L_λ[1]	$\epsilon_{\lambda_c}L_\lambda(day^{-1})$
297.5	6.17(−5)	0.4	5.49(−7)	0.0
300.0	2.70(−4)	1.5	5.13(−6)	0.0
302.5	8.30(−4)	4.1	3.02(−5)	0.2
305.0	1.95(−3)	8.7	1.19(−4)	0.5
307.5	3.74(−3)	14.3	3.38(−4)	1.3
310.0	6.17(−3)	19.3	7.53(−4)	2.4
312.5	9.07(−3)	24.6	1.39(−3)	3.8
315.0	1.22(−2)	27.1	2.22(−3)	4.9
317.5	1.55(−2)	31.1	3.19(−3)	6.4
320.0	1.87(−2)	28.3	4.23(−3)	6.4
323.1	3.35(−2)	43.9	8.25(−3)	10.8
330.0	1.16(−1)	119.5	3.16(−2)	32.6
340.0	1.46(−1)	94.2	4.31(−2)	27.8
350.0	1.62(−1)	76.6	4.98(−2)	23.6
360.0	1.79(−1)	51.4	5.68(−2)	16.3
370.0	1.91(−1)	32.5	6.22(−2)	10.6
380.0	2.04(−1)	17.3	6.78(−2)	5.8
390.0	1.93(−1)	5.8	6.33(−2)	1.9
400.0	2.76(−1)	2.8	9.11(−2)	0.9
410.0	3.84(−1)	0.0	1.20(−1)	0.0
	$\Sigma\epsilon_{\lambda_c}L_\lambda = 603.4$		$\Sigma\epsilon_{\lambda_c}L_\lambda = 156.2$	

[1] The units of L_λ are in 10^{-3} einsteins cm^{-2} day^{-1}. The second number in the columns in parenthesis is the power of ten by which the first number is multiplied.

From the Table 1 data and Equation 25 under paragraph (c)(1)(i)(A)(*1*) of this section, the average molar absorptivity is

Equation 28

$$\epsilon_{\lambda_c} = 10^4 A_{\lambda_c}$$

From the average value of A_{λ_c} at λ center, the average molar absorptivity can be obtained from Equation 28 and these results are summarized in Table 1, Spectral Data. In addition, using A_{λ_c} at 313.0 and 366.0 nm in Equation 28, the molar absorptivity ϵ_{313_c} and ϵ_{366_c} can be obtained and these values are also given in Table 1, Spectral Data.

(*2*) Since the plant is located at 40.7 degrees north latitude, the closest L_λ values are at 40 degrees north latitude. These values are obtained from § 796.3700(c)(3) Table 5 and are summarized in Table 1, Photolysis Data under paragraph (c)(3)(ii)(A)(*1*) of this section for the summer and winter solstices. Using the data from Table 1, Photolysis Data under paragraph (c)(3)(ii)(A)(*1*) of this section and Equations 16 and 17 under paragraph (a)(3)(ii)(B)(*6*) of this section, the following results are obtained.

Summer solstice	Winter solstice
$(k_{pE})_{max} = \Sigma\epsilon_{\lambda_c}L_\lambda = 603$ $days^{-1}$ $(t_{1/2E})_{min} = 1.1 \times 10^{-3}$ days	$(k_{pE})_{max} = \Sigma\epsilon_{\lambda_c}L_\lambda = 156$ $days^{-1}$ $(t_{1/2E})_{min} = 4.4 \times 10^{-3}$ days.

Since the chemical transforms rapidly on the summer and winter solstices, it is necessary to determine the reaction quantum yield of chemical A in the laboratory using Procedure One and to obtain direct sunlight photolysis rates and half-lives in aqueous media during the summer and winter seasons.

(B) *Phase 2 Experiments*—(*1*) *Determination of the approximate rate constant of the test chemical.* (*i*) Chemical A was dissolved directly in pure water and a homogeneous solution was prepared. Analysis of duplicate samples indicated that the average concentration was 5.00×10^{-6} M. Using the UV spectral data obtained, the absorbance at 313 nm in a one cm absorption cell containing a solution at a concentration of 5.00×10^{-6} M was less than 0.02 [i.e., $A_{313_c} = 2610\ (5.00 \times 10^{-6})\ (1.00) = 0.0131$]. Hence, the test chemical solution in approximately 1 cm pathlength tubes was at low optical density.

(*ii*) A series of tubes (13×100 mm), with an effective pathlength of 1.12 cm, were filled with the aqueous solution of test chemical and sealed. Since $\epsilon_{313_c} > \epsilon_{366_c}$ in Table 1 under paragraph (c)(3)(ii)(A) (*1*) of this section photolysis experiments were carried out at 313 nm in a PMGRR using the procedure described in paragraph (b)(2)(ii)(A) of this section. Duplicate photolyzed and control samples were removed periodically and analyzed for the concentration of test chemical. At $t=39.4$ hours, the average concentration of photolyzed sample $(C_t)_c$ was 2.25×10^{-6}M and the average concentration of control sample was 4.99×10^{-6}M. Thus, approximately 55 percent of the test chemical transformed. Since no loss of chemical was observed in the control sample, no adventitious processes occurred and the loss of chemical was only due to photolysis. Using Equation 3 under paragraph (a)(3)(ii)(A)(*1*) of this section and the data in this paragraph:

Equation 29

$\ln(5.00\times10^{-6}/2.25\times10^{-6})=k_{pc}(39.4)$

$k_{pc}=0.0203$ hours^{-1}.

(*2*). *Actinometry experiments.* (*i*) Utilizing Equation 5 and the value of k_{pc} obtained in Equation 29, the half-life of the test chemical is then

Equation 30

$(t_{1/2})c=0.693/0.0203=34.1$ hours.

Since $(t_{1/2})c > 12$ hours, the PNAP/PYR actinometer is required for the photolysis experiments.

(*ii*) Using Equation 18 under paragraph (a)(3)(x)(A)(*1*)(*ii*) of this section and the approximate rate constant k_{pc} from equation 29 under paragraph (c)(3)(ii)(B)(*1*)(*ii*) of this section, the concentration of pyridine needed to make the rate constant of the actinometer approximately equal to the rate constant of the test chemical is given below:

Equation 31

$[PYR]=1.16\ k_{pc}=1.16(0.0203)$

$[PYR]=2.36\times10^{-2}M$

An actinometer solution was then prepared according to the procedure given in paragraph (b)(2)(ii)(B)(*1*) of this section and the concentration of pyridine was 2.36×10^{-2}M. The concentration of PNAP was measured in duplicate and the average concentration (C_o) was 0.900×10^{-5}M. Using UV spectral data, the absorbance at 313 nm in a one cm absorption cell containing an actinometer solution at a concentration of 0.900×10^{-5}M was less than 0.02 (i.e., $A_{313_a}=(2056)$ $(0.900\times10-5)$ $(1.00)=0.0185$). Hence, the actinometer solution in approximately 1 cm pathlength tubes was at low optical density.

(*iii*) A series of tubes, identical to those used in the test chemical photolysis experiments, were filled with the actinometer solution and sealed. The photolysis experiments were carried out in the same PMGRR at 313 nm using the procedure described in paragraph (b)(2)(ii)(B)(*2*) of this section. Duplicate photolyzed and control samples were periodically removed and analyzed for the concentration of PNAP. At 50.6 hours, the average concentration of photolyzed sample $(C_t)_a$ was 0.360×10^{-5} M and the average concentration of the control sample was 0.900×10^{-5}M. Thus, approximately 60 percent of PNAP transformed. Since no loss of chemical was observed in the control sample, no adventitious processes occurred and the loss of chemical was only due to photolysis. Using equation 2 under paragraph (a)(3)(ii)(A)(*1*) of this section and the data:

Equation 32

$\ln(0.900\times10^{-5}/0.360\times10^{-5})=k_{pa}\ (50.6)$

$k_{pa}=0.0181$ hours^{-1}.

Thus, at the molar concentration of pyridine given by equation 31, k_{pa} is approximately the same as k_{pc} (within ± 50 percent).

(C) *Phase 3 Experiments.* (*1*) A solution of test chemical was prepared according to the procedures described in paragraph (b)(2)(ii)(A)(*2*) of this section and the concentration $(C_o)_c$ was measured in duplicate and the average concentration was found to be 5.00×10^{-6}M. An actinometer solution (PNAP/PYR) was prepared according to the procedure described in paragraph (b)(2)(ii)(B)(*1*) of this section with a pryridine concentration of 2.36×10^{-2}M under Equation 31 in paragraph (c)(3)(B)(*2*)(*ii*) of this section. The average concentration of PNAP $(C_o)_a$ from duplicate samples was measured and found to be 9.00×10^{-6}M. These solutions were placed in identical tubes (13×100 mm), sealed, and photolyzed at 313 nm in the PMGRR according to the procedure given in paragraph (b)(2)(iii) of this section. The average concentration of duplicate samples of test chemical, test chemical control, actinometer (PNAP), and actinometer control, obtained in this photolysis experiment, is summarized in the following Table 2:

TABLE 2—PHOTOLYSIS DATA FOR TEST CHEMICAL A AND (PNAP/PYR) ACTINOMETER

t(hours)	Average test chemical concentration × 10⁶		Average PNAP concentration × 10⁶	
	Photolyzed samples	Controls	Photolyzed samples	Controls
0	5.00	5.00	9.00	9.00
12	3.92	4.98	7.24	8.99
24	3.07	5.00	5.83	8.98
36	2.41	4.99	4.69	9.00
48	1.89	4.96	3.78	8.98
60	1.48	4.99	3.04	8.99
72	1.16	5.00	2.44	9.00

(*2*)(*i*) Since no significant loss of PNAP or test chemical was observed in the control samples, no adventitious processes occurred and the loss of test chemical and PNAP was only due to photolysis. Utilizing the data in under paragraph (c)(3)(ii)(C)(*1*) Table 2, of this section $\ln(C_o/C_t)$ for the test chemical and actinometer solution can be calculated and the results are summarized in the following Table 3:

TABLE 3—PHOTOLYSIS DATA FOR TEST CHEMICAL AND ACTINOMETER (PNAP/PYR)

t (hours)	Test chemical		Actinometer (PNAP)	
	$(C_t)_c\times10^6$(M)	$\ln(C_o/C_t)_c$	$(C_t)_a\times10^6$(M)	$\ln(C_o/C_t)_a$
0	5.00	0.000	9.00	0.000
12	3.92	0.243	7.24	0.218
24	3.07	0.488	5.83	0.434
36	2.41	0.730	4.69	0.652
48	1.89	0.973	3.78	0.868
60	1.48	1.22	3.04	1.09
72	1.16	1.46	2.44	1.31

(*ii*) The ratio of the rate constants (k_{pc}/k_{pa}) is defined by equation 6 under paragraph (a)(3)(ii)(A)(*2*) of this section.

$\ln(C_o/C_t)_c=(k_{pc}/k_{pa})\ln(C_o/C_t)_a$.

Utilizing all the data in paragraph (c)(3)(ii)(C)(*2*)(*i*) Table 3 of this section including the time point $t=0$ and linear regression analysis, the slope is found to be 1.12 with a correlation coefficient of 1.000.

(*iii*) The quantum yield of the PNAP/PYR actinometer is given by equation 7 under paragraph (a)(3)(ii)(A)(*3*) of this section.

$\phi_a=0.0169\ [PYR]$.

Since the pyridine concentration in the actinometer was 2.36×10^{-2}M under Equation 31 in paragraph (c)(3)(B)(*2*)(*ii*) of this section, the quantum yield of the actinometer (ϕ_a) is 3.99×10^{-4}.

(*3*) The reaction quantum yield of the test chemical is given by Equation 1 under paragraph (a)(3)(ii)(A)(*1*) of this section.

$\phi_c=(k_{pc}/k_{pa})\ (\epsilon_{\lambda_a}/\epsilon_{\lambda_c})\ \phi a$.

The pertinent data are summarized as follows:

$\phi_a=3.99\times10^{-4}$, $(k_{pc}/k_{pa})=1.12$, $\epsilon_{\lambda_c}=2610$, and $\epsilon_{\lambda_a}=2056$. Substituting these data in Equation 1 under paragraph (a)(3)(ii)(A)(*1*) of this section yields

Equation 33

$\phi_c=(1.12)\ (2056/2610)\ (3.99\times10^{-4})$

$\phi_c=3.52\times10^{-4}$

(*4*) The rate constants for direct photolysis of test chemical in aqueous media and the half-life for water bodies and clear sky conditions for the winter and summer seasons can be calculated as follows: The values of $\Sigma\epsilon_{\lambda_c}L_\lambda$ have been calculated for the summer and winter solstices under paragraph (c)(3)(ii)(A)(*1*) Table 1, Photolysis Data of this section. For summer $\Sigma\epsilon_{\lambda_c}L_\lambda = 603$ days $^{-1}$; for winter $\Sigma\epsilon_{\lambda_c}L_\lambda = 156$ days $^{-1}$. The reaction quantum yield for chemical A is 3.52×10^{-4} Equation 33 under paragraph (c)(3)(B)(*3*) of this section. Using these data in Equation 16 under paragraph (a)(3)(B)(*6*) of this section yields

Equation 34 (summer)

$$k_{pE} = 3.52\times10^{-4}(603) = 0.213 \text{ days}^{-1}$$

Equation 35 (winter)

$$k_{pE} = 3.52\times10^{-4}(156) = 0.0549 \text{ days}^{-1}.$$

These values can be substituted into equation 18 under paragraph (b)(1)(x)(A)(*1*)(*ii*) of this section to obtain the half-lives for these two seasons.

Equation 36 (summer)

$$(t_{1/2E})_c = 0.693/0.213 = 3.3 \text{ days}$$

Equation 37 (winter)

$$(t_{1/2E})_c = 0.693/0.0549 = 13 \text{ days}$$

These results are valid for clear-sky conditions and at shallow depths in the Illinois River.

(4) *Glossary of important symbols.*

λ—Wavelength λ.

A_λ—Absorbance at wavelength λ.

$F_{s\lambda}$—Fraction of light absorbed by the system at wavelength λ.

$F_{c\lambda}$—Fraction of light absorbed by a chemical (c) at wavelength λ.

$\epsilon\lambda c$—Molar absorptivity of a chemical (c) at wavelength λ.

$\epsilon\lambda a$—Molar absorptivity of an actinometer (a) at wavelength λ.

$\alpha\lambda$—Absorption (or attenuation) coefficient of water at wavelength λ.

l—The light pathlength; the distance traveled by a beam of light passing through the system.

ϕ_a–Reaction quantum yield of an aqueous solution of actinometer (a).

ϕ_c–Reaction quantum yield of an aqueous solution of chemical (c).

ϕ_E—Sunlight reaction quantum yield of a chemical (c) in a water body in the environment.

$-d[C]/dt$—Direct photolysis rate of chemical (c).

k_{pE}–Direct photolysis sunlight rate constant in a water body in the environment.

$(k_{pE})_{max}$—Maximum direct photolysis rate constant in a water body in the environment.

k_{pc}—Direct photolysis rate constant of constant of chemical (c) in water measured in the laboratory.

k_{pa}—Direct photolysis rate constant of actinometer (a) in water measured in the laboratory.

$k_{a\lambda}$–Specific light absorption of a photoreactive chemical at low concentration and at wavelength λ.

k_a—Specific light absorption rate constant integrated over all wavelengths absorbed by the chemical.

$(t_{1/2})c$—Half-life of a chemical (c) in water.

$(t_{1/2})a$—Half-life of an actinometer (a) in water.

$t_{1/2E}$—Half-life of a chemical in a water body in sunlight in the environment.

$(t_{1/2E})_{min}$—The minimum sunlight half-life of a chemical in a water body in sunlight in the environment.

$I_{o\lambda}$–The number of photons (or einsteins) of light of wavelength λ in the system per cm^2 per second.

I_λ–The number of einsteins of light of wavelength λ in the system per liter per hour (or second).

L_λ—Solar irradiance in a water body at shallow depths in the units milli einsteins per cm^2 per second.

PYR—Pyridine.

[PYR]—Molar concentration of pyridine.

PNAP—p-Nitroacetophenone.

PNA—p-Nitroanisole.

o-NB—o-Nitrobenzaldehyde

PNAP/PYR—p-Nitroacetophenone-pyridine actinometer.

PNA/PYR—p-Nitroanisole-pyridine actinometer.

POB—Photochemical optical bench.

PMGRR—Photochemical "Merry-Go-Round" Reactor.

A_r—Aperture in the PMGRR.

(d) *References.* For additional background information on this test guideline the following references should be consulted:

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§ 796.3800 Gas phase absorption spectra and photolysis.

(a) *Introduction*—(1) *Background and purpose.* Numerous chemicals enter the atmosphere from a variety of sources. For example, chemicals enter the atmosphere as a result of the burning of coal, from the combustion of gasoline in cars and diesel fuel in trucks, and from the release of volatile organic chemicals during manufacture, processing, use, and disposal. Pesticides, applied from airplanes, enter the atmosphere directly and volatilize from soils and water bodies. Chemical pollutants present in the atmosphere can undergo photochemical transformation in the environment by direct photolysis in sunlight. Quantitative data in the form of rate constants and half-lives are needed to determine the importance of direct photolysis of pollutants in the atmosphere. This test method describes a first-tier screening level test method to estimate the maximum direct photolysis rate constant and minimum half-life of chemicals in the atmosphere in sunlight as a function of latitude and season of the year in the United States.

(2) *Definitions and units.* (i) "Radiant energy," or radiation, is defined as the energy traveling as a wave unaccompanied by transfer of matter. Examples include x-rays, visible light, ultraviolet light, radio waves, etc.

(ii) "Absorbance (A_λ)" is defined as the logarithm to the base 10 of the ratio of the initial intensity (I_o) of a beam of radiant energy to the intensity (I) of the same beam after passage through a sample at a fixed wavelength λ. Thus, $A_\lambda = \log_{10}(I_o/I)$.

(iii) The "Beer-Lambert law" states that the absorbance of a chemical in the gas phase, at a fixed wavelength, is proportional to the thickness of the absorbing material (*l*), or the light pathlength, and the concentration of the absorbing species (C).

(iv) "Cross section (σ_λ')" is defined as the proportionality constant in the Beer-Lambert law. Thus, $A_\lambda = \sigma_\lambda' Cl$, where A_λ is the absorbance, C is the concentration in molecules per cm^3 and *l* is the pathlength in cm. The units of the cross section σ_λ' are cm^2 $molecule^{-1}$. Numerical values of the cross section depend upon the nature of the absorbing species.

(v) A "first-order reaction" is defined as a reaction in which the rate of disappearance of a chemical is directly proportional to the concentration of the chemical and is not a function of the concentration of any other chemical present in the reaction mixture.

(vi) The "half-life ($t_{1/2}$)" of a chemical is defined as the time required for the concentration of the chemical being photolyzed to be reduced to one-half its initial value.

(vii) The "sunlight direct photolysis rate constant (k_{pE})" is the first-order rate constant in the units of day^{-1} and is a measure of the rate of disappearance of a chemical in the gas phase in sunlight.

(viii) The "actinic solar irradiance in the atmosphere (J_λ)" is related to the sunlight intensity in the atmosphere and is proportional to the average light flux (in the units of photons cm^{-2} day^{-1}) that is available to cause photoreaction in the wavelength interval Δλ, centered at λ, over a 24-hour day at a specific latitude and season date. It is the irradiance which would be measured by a weakly absorbing spherical actinometer exposed to direct solar radiation and sky radiation from all directions.

(ix) "The Grotthus-Draper law," the first law of photochemistry, states that only light which is absorbed can be effective in producing a chemical transformation.

(x) The "Stark-Einstein law," the second law of photochemistry, states that only one molecule is activated to an excited state per photon or quantum of light absorbed.

(xi) The "reaction quantum yield (ϕ_λ)" for an excited state process is defined as the fraction of absorbed light that results in photoreaction at a fixed wavelength λ. It is the ratio of a number of molecules that photoreact to the number of quanta of light absorbed or the ratio of the number of moles that photoreact to the number of einsteins of light absorbed at a fixed wavelength λ.

(xii) "Direct photolysis" is defined as the direct absorption of light by a chemical followed by a reaction which transforms the parent chemical into one or more products.

(3) *Principle of the test method.* (i) For weak absorbance of a chemical in the atmosphere, the first-order direct photolysis rate constant, k_{pe}, is given by the equation

Equation 1

$$k_{pE} = 2.30\Sigma\phi_\lambda\sigma'_\lambda J_\lambda.$$

Where ϕ_λ is the reaction quantum yield; ϕ'_λ is the cross section in the units cm^2 $molecule^{-1}$ averaged over a wavelength interval Δλ, centered at λ; J_λ is the actinic solar irradiance in the units photons cm^{-2} day^{-1} averaged over the wavelength interval Δλ, centered at λ; and the summation is taken over the range Δλ=290 to 800 nm. J_λ is the solar actinic irradiance in the atmosphere under clear sky conditions and is a function of latitude and season of the year.

(ii) Since this photolysis process is first-order, the half-life ($t_{1/2}$) of a chemical is then given by

Equation 2

$$t_{1/2} = 0.693/k_{pe}$$

(iii) A simple first-tier screening test has been developed using Equation 1. As an approximation, it is assumed that the reaction quantum yield ϕ_λ is equal to one, the maximum value. As a result, the upper limit for the direct photolysis sunlight rate constant in the gas phase is obtained and Equation 1 becomes

Equation 3

$$(k_{pE})_{max} = 2.30\Sigma\sigma'_\lambda J_\lambda$$

Using Equation 3 in Equation 2, the lower limit for the half-life is then given by

Equation 4

$$(t_{1/2})_{min} = 0.693/(k_{pE})_{max}.$$

The cross section can be determined experimentally by the procedures outlined in paragraph (b) of this section and the values of J_λ are given in Tables 1 to 4 under paragraph (c)(3) of this section as a function of latitude and season of the year in the United States. These data can then be used in Equation 3 to calculate $[k_{pE}]_{max}$. Finally, $(k_{pE})_{max}$ can be substituted in equation 4 to calculate $[t_{1/2}]_{min}$.

(4) *Applicability and specificity.* (i) This test method is applicable to all chemicals which have UV-visible absorptions in the range 290 to 800 nm. Some chemicals only have absorptions below 290 nm and consequently cannot undergo direct photolysis in sunlight (e.g., chemicals such as alkanes, alkenes, alkynes, dienes, and fluoroalkanes).

(ii) This test method is only applicable to pure chemicals and not to the technical grade.

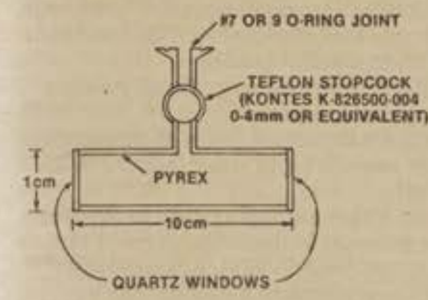
(iii) The first-tier screening test can be employed to estimate $(k_{pEX})_{max}$ and $(t_{1/2X})_{min}$. If these data indicate that gas phase photolysis is an important process relative to other gas phase transformation processes (e.g., oxidation with hydroxyl radicals or ozone), then it is recommended that an upper-tier photolysis test be carried out to determine the reaction quantum yield and thus obtain more precise environmentally relevant rate constants and half-lives in sunlight. The data obtained from this first-tier test method can be used to determine $(k_{pE})_{max}$ for a test chemical as a function of latitude and season of the year in the United States under clear sky conditions. These rate constants are in a form suitable for preliminary mathematical modeling for environmental fate of a test chemical.

(b) *Test procedures.* The procedures outlined in this test method are based on the method proposed by Mill et al. (1982) under paragraph (d)(1) of this section and developed by Pitts et al. (1981) under paragraph (d)(2) of this section. It is also recommended that § 796.— *Absorption in Aqueous Solution: Ultraviolet/Visible Spectra,* be consulted for additional guidance.

(1) *Test conditions*—(i) *Ultraviolet-Visible Spectrophotometer.* Although single-beam spectrophotometers may be used, recording double beam spectrophotometers are highly recommended. It is extremely important that the spectrophotometer be able to scan over the wavelength region 270 to 800 nm and have an absorbance sensitivity, at a signal/noise ratio of one, of approximately 0.001. It is important that the spectrophotometer be able to attain a 90 percent separation of two monochromatic spectral features approximately 4 nm apart, peak to peak (i.e., the resolution should be at least 4 nm). It is also desirable to have a spectrophotometer that can accommodate absorption cells of length >10 cm. A Cary 219 UV-Visible Spectrophotometer, or an equivalent model, is highly recommended.

(ii) *Vapor and Liquid Absorption Cells.* (A) Long pathlength cells are preferable; however, many commercial spectrophotometers will only accept absorption cells of 10 cm or less. A suitable vapor cell is depicted in the following Figure 1.

Figure 1—Gas absorption cell

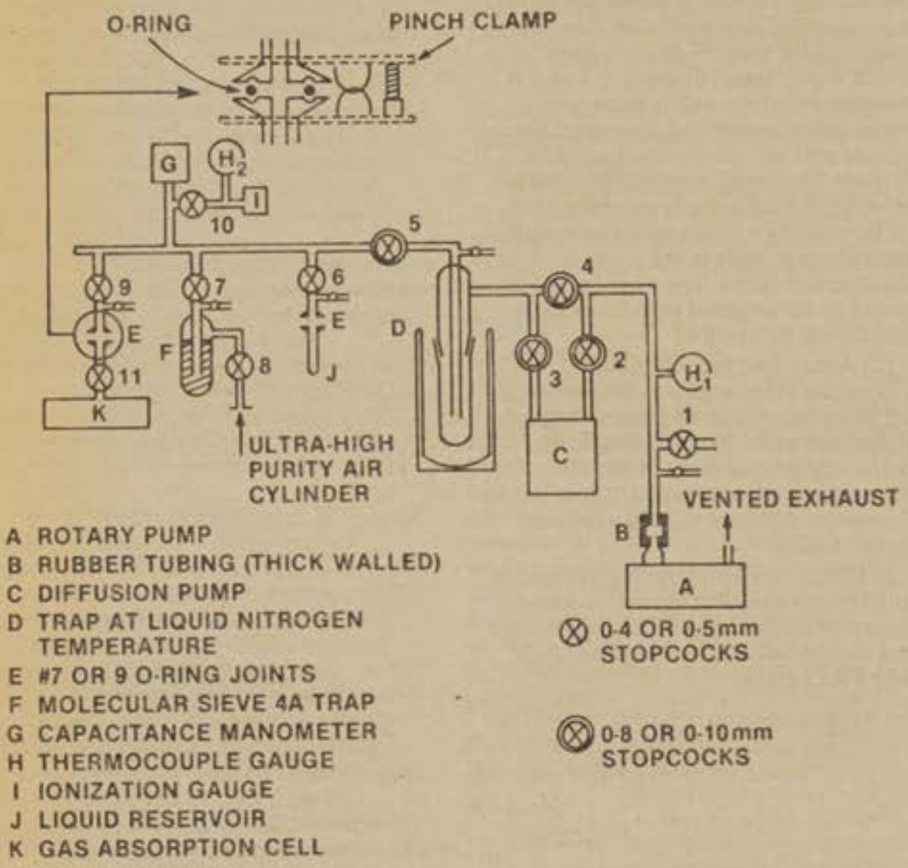


(B) A suitable vapor cell can be constructed as follows. The vapor cell should be constructed of Pyrex, 1 cm O.D. and 10 cm in length, and be fitted with plane parallel quartz windows at each end. The quartz windows can be conveniently attached to the Pyrex cell with vaccum tight epoxy resin (e.g., Torr-Seal, Varian Associates) only applied to the outside surface. A Teflon stopcock (or a Pyrex "o" ring stopcock) should be connected to the cell and contain an "o"-ring joint. The "o"-ring joint (e.g., #7 or #9, Kontes or Ace Glass) must match the one on the vacuum rack. Viton "o"-rings are highly recommended and should be frequently inspected for signs of deterioration which would result in vacuum leaks. A matched reference cell is extremely useful but not essential. However, the sample and reference cells should be very similar. Small spectral differences between the cells can be compensated for by running a blank with the sample and reference cells in the spectrophotometer. The use of stopcock grease is not required with these cells and should be avoided.

(C) A matched pair of liquid absorption cells is very desirable but is not essential. A pair of quartz ultraviolet absorption cells, 10 cm in length, and containing ground glass or Teflon stoppers are recommended. These liquid absorption cells are readily available commercially.

(iii) *Vacuum Gas Handling System.* A suitable gas handling system is shown diagramatically in the following Figure 2 and should be constructed completely with Pyrex glass.

Figure 2—Schematic of gas handling vacuum rack



The components of the gas handling system are discussed below. The use of stopcock grease is not required and should be avoided.

(A) *Vacuum Pumping System.* (*1*) In order to achieve a good vacuum, i.e., pressures $<10^{-5}$ torr (1.3×10^{-6} kPa), two pumps are required. The forepump (A) must be capable of achieving a pressure <0.05 torr (0.0065 kPa). A rotary pump (e.g., a Welch Model 1402 Duo-Seal or an equivalent model) is recommended. The forepump can be attached to the vacuum system by means of heavy-walled rubber vacuum tubing (B), or any flexible vacuum tubing. The exhaust from this pump should be vented into a hood.

(*2*) The second pump, a high vacuum model, should be a multistage oil diffusion pump (C) [e.g., a Consolidated Vacuum Corp. VMF-10 or VMF-20 or an equivalent model]. The pump fluid should be a silicone oil with a room temperature vapor pressure of $<10^{-6}$ torr (1.3×10^{-7} kPa) [e.g., Dow-Corning D.C. 702 or 703, or an equivalent grade].

(*3*) It is extremely important that the pumping system contain a trap (D) cooled with liquid nitrogen. The cone and socket joint on this trap can be conveniently sealed with Apiezon W wax, or an equivalent grade. This wax requires only gentle heating to apply and makes an effective vacuum seal. It is possible that a few test chemicals could dissolve Apiezon W wax. In this case, an inert silicone grease may be used to seal the trap.

(B) *Vacuum Rack.* The recommended vacuum rack assembly is depicted in Figure 2 under paragraph (b)(1)(iii) of this section. All stopcocks should be of Teflon with Viton "o"-rings [Kontes K–826500 or K–826510 series or equivalent grades (or Pyrex "o" ring stopcocks)]. The "o"-ring joints (E) [#7 or #9] must be compatible with those on the gas absorption cell (K) or on the liquid reservoir (J). These "o"-ring joints should be clamped by pinch clamps with a screw lock device (e.g., Thomas #18A, or an equivalent grade).

(C) *Pressure Gauges.* Three pressure gauges are required:

(*1*) An ionization gauge to measure high vacuum [$<10^{-3}$ torr (1.3×10^{-4} kPa)].

(*2*) A thermocouple gauge to monitor the pressure in the range 10^{-3} to 1 torr (1.3×10^{-4} to 0.13 kPa). A convenient pressure monitoring system which contains ionization and thermocouple gauges is a Consolidated Vacuum Corp. Model GIC–300A or an equivalent model.

(*3*) A pressure gauge to monitor the pressure of the test chemical and diluent in the range 0.01 to 760 torr (0.0013 to 101.3 kPa); for example, an MKS Baratron 310 BHS–1000 with the associated 170–6C electronics unit and a digital readout or an equivalent model. While this vacuum gauge exhibits a slow zero drift, it can be readily rezeroed using the ionization gauge, i.e., when the ionization gauge reads approximately 10^{-3} torr (0.00013 kPa) or less.

(2) *Operation of the Gas Handling System.* Since there are a wide variety of procedures available for operating a gas handling system, the method used is left to the discretion of the tester. For those testers who do not have experience in handling a vacuum system, the detailed procedure described in paragraph (c)(5) of this section is highly recommended.

(3) *Preparation of samples*—(i) *Preparation of the Gas Phase Test Chemical Sample: Preliminary Steps.* (A) If the test chemical is a gas at room temperature, then attach the gas container to the "o"-ring at the point where the liquid reservoir (J) is placed. Close stopcocks 2 and 3 and open 4. Pump until the pressure is less than 10^{-2} torr (1.3×10^{-3} kPa) as read on thermocouple gauge (H_2). Then open stopcocks 2 and 3 and close 4 and pump until the pressure is less than 10^{-5} torr (1.3×10^{-6} kPa) as read on the ionization gauge (I).

(B) If the test chemical is a liquid at room temperature, add a few cm^3 of liquid to a reservoir tube (J), sealed at one end and containing an "o"-ring at the other end, and connect the tube via the "o"-ring to stopcock 6. Freeze the sample with a Dewar containing liquid nitrogen, close stopcocks 2 and 3 and open 4 and 6. Degas the test chemical by allowing it to warm up to the liquid state, briefly degas, and refreeze the liquid. Repeat this process three or more times until the evolution of gas bubbles ceases upon thawing. Freeze the liquid, open stopcocks 2 and 3 and close 4. Pump until the pressure is less than 10^{-5} torr (1.3×10^{-6} kPa) as indicated by the ionization gauge (I). Close stopcock 6.

(ii) *Introduction of the test chemical into the Gas Absorption Cell.* (A) For introduction of the test chemical into the gas absorption cell, close stopcocks 5, 7 and 10, with 9 and 11 open. If the test chemical is a gas, then stopcock 6 should be opened and the gas container valve is gradually opened to admit the gas into the gas handling manifold and gas absorption cell until the desired pressure is attained, as read on the capacitance manometer (G). Close the gas container valve and stopcock 6 and allow approximately 5 minutes before the final pressure at (G) is read. If the pressure has not stabilized in approximately 5 minutes, allow the cell to condition for several hours before the final pressure at (G) is read.

(B) For a liquid chemical in the reservoir (J), which has been degassed and is at liquid nitrogen temperature, the liquid nitrogen Dewar should be removed and stopcock 6 opened. The cold liquid in the reservoir (J) is allowed to warm up until the required pressure is attained, as read by the capacitance manometer (G). Close stopcock 6 and cool the reservoir again with liquid nitrogen and allow approximately 5 minutes before the final pressure at (G) is read. If the pressure has not stabilized in approximately 5 minutes, allow the cell to condition for several hours before the final pressure at (C) is read.

(C) With stopcocks 6, 8 and 11 closed and 5, 7, 9, and 10 open, the gas handling manifold is evacuated as described previously to a pressure less than 10^{-5} torr (1.3×10^{-6} kPa). Stopcocks 5 and 10 are then closed and ultra-high purity air from a cylinder is admitted into the gas handling manifold via stopcock 8 and through the trap (F) containing Molecular Sieve 4A. When the manifold is at one atmosphere pressure, as measured by pressure gauge (C), stopcock 11 is briefly opened to pressure the gas absorption cell to one atmosphere, and then closed. Stopcocks 8 and 9 are closed and the gas handling system is evacuated as described previously. The gas absorption cell can then be removed from (E) and covered to avoid photolysis.

(D) Based on the pressure P of the test chemical, as measured by gauge (G), the concentration of the gas sample is

Equation 5

$$C\ (\text{molecules cm}^{-3}) = 9.657\times10^{18}\ P(\text{torr})/T(\text{K})$$

Equation 5a

$$C\ (\text{molecules cm}^{-3}) = 1.287\times10^{18}\ P(\text{kPa})/T(\text{K}).$$

where T is the room temperature in °K, which should be routinely monitored with a thermometer.

(E) The recommended pressure of the test chemical should be in the range 1–5 torr (0.13–0.65 kPa) where the Beer-Lambert law is obeyed. A final check on whether the test chemical obeys the Beer-Lambert law can be accomplished by demonstrating the constancy of the cross section at three partial pressures differing by a factor of 10.

(iii) *Preparation of solution phase test chemical sample.* (A) If the properties of the test chemical (i.e., small cross sections, low vapor pressure) are such that the maximum absorbance obtainable is one-tenth of the most sensitive spectrophotometer scale or less (i.e., ≤ 0.001 absorbance), a solution-phase study should be undertaken. The most sensitive scale may be limited by inherent spectrophotometer noise. For example, a given spectrophotometer's most sensitive scale is 0.00 to 0.10 absorbance units. Therefore, a test chemical for which the product of its maximum cross section and its concentration is less than 0.001 (in a 10 cm cell) could not be analyzed in the vapor phase with this particular spectrophotometer.

(B) The following spectroscopic grade chemicals are recommended to prepare solutions: chloroform, *n*-hexane, acetonitrile, and cyclohexane. Solutions of up to 10 percent by volume of test chemical can be prepared in one of these solvents in the standard manner.

(C) The concentration of the test chemical is given by the equations

Equation 6

$$C\ (\text{molecules cm}^{-3}) = 6.022\times10^{23}\ \text{mass (gms)}/\text{FW}\ (V_d)$$

Equation 6a

$$C\ (\text{molecules cm}^{-3}) = 6.022\times10^{23}\ V_s\rho/\text{FW}\ (V_d).$$

where V_s is the volume of test chemical delivered into a volume V_d of solvent in cm^3, FW is the formula weight of the test chemical in gms, and ρ is the density of the test chemical in gms cm^{-3} at the room temperature the solution was prepared.

(4) *Procedure for obtaining the spectrum*

As a general guide to obtaining uv-visible absorption spectra, the procedures outlined in § 796.1050 *Absorption in Aqueous Solution: Ultraviolet/Visible Spectra*, are highly recommended. Since the method presented in this procedure was developed by Pitts et al. (1981), it is highly recommended that this report be consulted for further details.

(i) *Determination of the cell pathlength.* The method for determining the cell pathlength of gas or liquid cells is left to the discretion of the tester. However, the method listed in § 796.1050, using one of their reference compounds, is highly recommended.

(ii) *Gas Phase Spectrum.* Measure the absorbance of the test chemical in duplicate relative to a matched cell filled with ultra-high purity air from the same cylinder similarly passed through trap (F) containing the molecular sieve. The absorbance should be measured at wavelengths $\lambda > 280$ nm using minimum slit widths. Record, in duplicate, the baseline when both the same reference cells are filled with high purity air dried through the molecular sieve and at the same settings as used for the test chemical sample. These data will be used to calculate the cross section, σ'_λ, at the appropriate wavelength intervals, centered at wavelength λ, listed in Tables 1 through 4, under paragraph (c)(3) of this section.

(iii) *Solution Phase Spectrum.* (A) Measure the absorbance of the test chemical in duplicate relative to a matched cell containing the solvent. The absorbance should be measured for wavelengths $\lambda \geq 280$ nm using the minimum slit widths. Record, in duplicate, the baseline when both the sample and reference cells are filled with the solvents. These data will be used to calculate the cross sections, σ'_λ, for the appropriate wavelength intervals, centered at λ, listed in Tables 1 through 4 under paragraph (c)(3) of this section.

(B) The concentration of the test chemical should be in the range where the Beer-Lambert law is obeyed. A check on whether the test chemical obeys this law can be accomplished by demonstrating the constancy of the cross section at 3 concentrations differing by a factor of 10.

(c) *Data and reporting*—(1) *Treatment of results*—(i) *Determination of the cross section from the Gas Phase Spectrum.* (A) The cross section, σ_λ, can be determined from the gas phase absorption spectrum and the Beer-Lambert law in the form

Equation 7

$$\sigma_\lambda' = A_\lambda/Cl$$

where A_λ is the absorbance at wavelength λ, centered in the wavelength interval $\Delta\lambda$, C is the concentration of test chemical in molecules cm^{-3}, and *l* is the cell pathlength in cm. The cross section of the test chemical should be determined for the wavelength intervals listed in Tables 1 through 4 under paragraph (c)(3) of this section.

(B) There are at least three nondestructive methods of determining the absorbance over a specified wavelength interval: Estimation, square counting, and planimetry. For many spectra, estimating an average absorbance over a small wavelength interval is sufficient to yield accurate results. However, for spectra containing rapidly changing absorptions and complex fine structure, square counting or planimetry should be used. These two methods require the integration of a definite region (in $A_\lambda \times$ nm) followed by division by the width of the region in nm to obtain absorbance. The method using a compensating polar planimeter is the most accurate and is highly recommended. The absorbance should be obtained from the average of three tracings.

(ii) *Determination of the gross section from the Solution Phase Spectrum.* The cross section, σ'_λ, can be determined from the solution phase spectrum using equation 7 for the wavelength intervals listed in Tables 1 through 4 under paragraph (c)(3) of this section. For solution spectra, estimating an average absorbance over the wavelength intervals is sufficient to yield accurate results.

(iii) *Estimation of the maximum direct photolysis rate constant and minimum half-life in the gas phase.* (A) Using the cross sections obtained from the spectra and the values of J_λ from Tables 1–4 under paragraph (c)(3) of this section, the maximum direct photolysis rate constant $(k_{pE})_{max}$ can be calculated at a specific latitude and season for the year using equation 3. The minimum half-life, $(t_{1/2E})_{min}$, can be calculated using this $(k_{pE})_{max}$ in equation 4.

(B) An example is presented in under paragraph (c)(4) of this section, to illustrate how the test data obtained in this section can be used.

(2) *Test data report.* (i) Submit the original chart, or photocopy, containing a plot of absorbance vs. wavelength plus the baseline. Spectra should include a readable wavelength scale, preferably marked at 10 nm intervals. Each spectrum should be clearly marked.

(ii) Gas Phase Spectra. (A) Report the pressure of the test chemical in torr (or kPa), the concentration in molecules cm^{-3}, and the pathlength of the sample cell in cm. Describe the method used to determine the pathlength and report the experimental data.

(B) Report the wavelength λ, the wavelength interval for each 10 nm over the region of absorption, the value of the absorbance (A_λ) for each replicate, the mean absorbance, and the mean cross section in cm^2 $molecule^{-1}$.

(C) Report the estimated maximum direct photolysis rate constant in $days^{-1}$ and the corresponding minimum half-life in days at 20°, 30°, 40°, and 50° north latitude for the summer and winter solstices.

(iii) Solution Phase Spectra. (A) Report the concentration of the test chemical in molecules cm^{-3}, the type of cell used (quartz or borosilicate), and the pathlength in cm. Describe the method used to determine the pathlength and report the experimental results.

(B) Report the identity of the solvent.

(C) Report the wavelength λ, the wavelength interval over the region of absorption, the value of the absorbance (A_λ) of each replicate, the mean absorbance, and the mean cross section (σ'_λ) in cm^2 $molecule^{-1}$.

(D) Report the estimated maximum direct photolysis rate constant in $days^{-1}$ and the corresponding minimum half-life in days at 20°, 30°, 40°, and 50° north latitude for the summer and winter solstices.

(iv) Report the name, structure, and purity of the test chemical.

(v) Submit a recent spectrum on appropriate reference chemicals for photometric and wavelength accuracy.

(vi) Report the name and model of the spectrophotometer used.

(vii) Report the various control settings employed with the spectrophotometer. These might include scan speed, slit width, given, etc.

(viii) Report anything unusual about the test; e.g., if the Beer-Lambert law is not obeyed at a pressure of 1 to 5 torr (0.13 to 0.65 kPa), report the pressure at which the deviation was overcome and the experimental data. If the Beer-Lambert law is not obeyed in solution at high concentrations, report the concentration at which the deviation was overcome and the experimental data.

(ix) Report any other relevant information.

(3) *Tables of solar irradiance.*

TABLE 1—J_λ VALUES AT 20 °N. LATITUDE

Wavelength center ^{a}	Summer solstice ^{a}	Equinox ^{a}	Winter solstice ^{a}	Wavelength center ^{b}	Fall or winter average ^{a}	Spring or summer average ^{a}	Wave-length center ^{b}
290	0.0000811	0.00000131	0.000000108	290	0.000000696	0.0000625	290.
300	0.0810	0.0611	0.0212	300	0.0359	0.0769	300.
310	1.10	0.9148	0.499	310	0.663	1.05	310.
320	2.74	2.35	1.52	320	1.855	2.62	320.
330	4.82	4.20	2.90	330	3.42	4.63	330.
340	5.27	4.61	3.28	340	3.82	5.06	340.
350	5.94	5.22	3.77	350	4.36	5.71	350.
360	6.22	5.47	4.01	360	4.61	5.98	360.
370	7.76	6.84	5.06	370	5.79	7.46	370.
380	7.60	6.71	5.02	380	5.71	7.31	380.
390	7.77	6.88	5.19	390	5.88	7.48	390.
400	10.6	9.44	7.17	400	8.10	10.2	400.
410	13.5	12.0	9.17	410	10.3	13.0	410.
420	14.1	12.5	9.65	420	10.8	13.6	420.
430	14.3	12.7	9.85	430	11.1	13.7	430.
440	15.8	14.1	11.0	440	12.2	15.2	440.
450	18.2	16.2	12.7	450	14.1	17.5	450.
460	19.7	17.5	13.7	460	15.3	18.9	460.
470	20.2	18.1	14.2	470	15.8	19.5	470.
480	20.5	18.3	14.4	480	16.0	19.8	480.
490	20.6	18.4	14.5	490	16.1	19.8	490.
500	20.9	18.7	14.8	500	16.4	20.2	500.
510	21.1	18.8	14.9	510	16.4	20.3	510.
520	21.1	18.9	14.9	520	16.5	20.3	520.
530	21.3	19.0	15.1	530	16.7	20.5	530.

TABLE 1—J_λ VALUES AT 20 °N. LATITUDE—Continued

Wavelength center [b]	Summer solstice [a]	Equinox [a]	Winter solstice [a]	Wavelength center [b]	Fall or winter average [a]	Spring or summer average [a]	Wavelength center [b]
540	21.2	19.0	15.0	540	16.6	20.4	540
550	21.1	18.8	14.9	550	16.5	20.3	550
560	21.3	19.0	15.1	560	16.7	20.5	560
570	21.6	19.3	15.3	570	16.9	20.8	570
580	22.1	19.7	15.7	580	17.3	21.2	580
590	22.3	19.9	15.8	590	17.5	21.5	590
600	22.5	20.0	15.9	600	17.6	21.6	600
610	22.6	20.2	16.0	610	17.7	21.8	610
620	22.6	20.1	16.1	620	17.7	21.8	620
630	22.6	20.1	16.2	630	17.7	21.7	630
640	23.1	20.6	16.6	640	18.2	22.3	640
650	23.6	21.1	16.9	650	18.6	22.8	650
660	24.0	21.4	17.2	660	18.9	23.1	660
670	24.3	21.7	17.4	670	19.2	23.4	670
680	24.3	21.7	17.5	680	19.2	23.4	680
690	24.3	21.7	17.5	690	19.2	23.4	690
700	24.2	21.7	17.5	700	19.2	23.0	700
710	24.1	21.5	17.4	710	19.1	23.2	710
720	23.9	21.4	17.3	720	19.0	23.0	720
730	23.8	21.3	17.2	730	18.9	22.9	730
740	23.6	21.1	17.2	740	18.8	22.8	740
750	23.5	21.0	17.1	750	18.7	22.6	750
760	23.3	20.9	17.0	760	18.6	22.5	760
770	23.2	20.8	16.9	770	18.5	22.3	770
780	23.1	20.6	16.8	780	18.4	22.2	780
790	22.9	20.5	16.7	790	18.3	22.1	790
800	22.8	20.4	16.7	800	18.2	21.9	800

[a] J_λ values are in units of 10^{19} photons cm^{-2} day^{-1}.

[b] Wavelength intervals are uniformly 10 nm wide, extending from 5 nm lower than the center wavelength to 5 nm higher. Thus, the first interval centered on 290 extends from 265-295 nm.

TABLE 2—J_λ VALUES AT 30° N. LATITUDE

Wavelength center [b]	Summer solstice [a]	Equinox [a]	Wintar solstice [a]	Wavelength center [b]	Fall or winter avg. [a]	Spring or summer avg. [a]	Wavelength center [b]
290	0.0000768	0.00000203	0.000000213	290	0.000000457	0.0000352	290
300	0.0831	0.0457	0.00835	300	0.0208	0.0704	300
310	1.14	0.787	0.300	310	0.480	1.02	310
320	2.84	2.13	1.06	320	1.47	2.60	320
330	5.02	3.88	2.13	330	2.81	4.62	330
340	5.49	4.30	2.48	340	3.19	5.08	
350	6.28	4.88	2.89	350	3.68	5.74	350
360	6.49	5.15	3.10	360	3.91	6.02	360
370	8.09	6.45	3.95	370	4.94	7.51	370
380	7.93	6.25	3.95	380	4.91	7.37	380
390	8.12	6.53	4.12	390	5.08	7.55	390
400	11.1	8.97	5.73	400	7.02	10.4	400
410	14.1	11.4	7.37	410	8.99	13.2	410
420	14.7	12.—	7.81	420	9.46	13.7	420
430	14.9	12.2	8.00	430	9.66	13.9	430
440	16.5	13.5	8.94	440	10.8	15.4	440
450	19.0	15.5	10.4	450	12.4	17.8	450
460	20.6	16.8	11.3	460	13.5	19.2	460
470	21.2	17.3	11.7	470	13.9	19.8	470
480	21.5	17.6	11.9	480	14.2	20.1	480
490	21.5	17.7	12.0	490	14.3	20.1	490
500	21.9	18.0	12.2	500	14.5	20.5	500
510	22.1	18.1	12.3	510	14.6	20.6	510
520	22.1	18.1	12.4	520	14.7	20.7	520
530	22.3	18.3	12.5	530	14.8	20.9	530
540	22.1	18.2	12.4	540	14.7	20.6	540
550	22.1	18.1	12.4	550	14.7	20.6	550
560	22.6	18.3	12.5	560	14.8	20.8	560
570	22.6	18.6	12.7	570	15.1	21.1	570
580	23.1	19.0	13.0	580	15.4	21.6	580
590	23.3	19.2	13.2	590	15.6	21.8	590
600	23.5	19.3	13.3	600	15.7	22.0	600
610	23.7	19.5	13.4	610	15.8	22.1	610
620	23.6	19.3	13.6	620	15.9	22.1	620
630	23.6	19.2	13.7	630	16.0	22.1	630
640	24.2	19.8	14.0	640	16.3	22.6	640
650	24.7	20.4	14.2	650	16.7	23.1	650
660	25.1	20.7	14.4	660	16.9	23.5	660
670	25.4	21.0	14.7	670	17.2	23.8	670
680	25.4	21.0	14.7	680	17.2	23.8	680
690	25.4	21.0	14.8	690	17.3	23.8	690
700	25.3	21.0	14.8	700	17.3	23.7	700
710	25.2	20.8	14.7	710	17.2	23.6	710
720	25.0	20.7	14.6	720	17.1	23.4	720
730	24.9	20.6	14.6	730	17.0	23.3	730
740	24.7	20.5	14.5	740	16.9	23.2	740
750	24.6	20.4	14.5	750	16.8	23.0	750
760	24.4	20.3	14.4	760	16.8	22.9	760
770	24.3	20.1	14.3	770	16.7	22.7	770
780	24.1	20.0	14.3	780	16.6	22.6	780
790	24.0	19.9	14.2	790	16.5	22.5	790
800	23.8	19.8	14.2	800	16.4	22.3	800

[a] J_λ values are in units of 10^{15} photons cm^{-2} day^{-1}.
[b] Wavelength intervals are uniformly 10 nm wide, extending from 5 nm lower than the center wavelength to 5 nm higher. Thus, the first interval centered on 290 extends from 285-295 nm.

TABLE 3—J_λ VALUES AT 40° N. Latitude

Wavelength center [b]	Summer solstice [a]	Equinox [a]	Winter solstice [a]	Wavelength center [b]	Fall or winter avg. [a]	Spring or summer avg. [a]	Wavelength center [b]
290	0.0000136	0.000000121	0.000000000615	290	0.0000000814	0.00000349	290.
300	0.0769	0.0293	0.00145	300	0.00939	0.0587	300.
310	1.12	0.618	0.132	310	0.298	0.940	310.
320	2.87	1.81	0.591	320	1.04	2.49	320.
330	5.11	3.41	1.31	330	2.90	4.49	330.
340	5.62	3.83	1.58	340	2.43	4.77	340.
350	6.35	4.39	1.88	350	2.84	5.64	350.
360	6.61	4.65	2.05	360	3.05	5.93	360.
370	8.32	5.86	2.64	370	3.88	7.43	370.
380	8.17	5.80	2.67	380	3.88	7.30	380.
390	8.37	5.99	2.82	390	4.05	7.50	390.
400	11.5	8.26	3.97	400	5.64	10.3	400.
410	14.6	10.5	5.15	410	7.26	13.1	410.
420	15.2	11.1	5.51	420	7.69	13.9	420.
430	15.5	11.3	6.69	430	7.89	15.4	430.
440	17.1	12.5	6.41	440	8.82	17.8	440.
450	19.7	14.5	7.47	450	10.2	19.2	450.
460	21.3	15.7	8.15	460	11.1	19.8	460.
470	22.0	16.2	8.51	470	11.5	20.1	470.
480	22.3	16.5	8.74	480	11.8	20.2	480.
490	22.3	16.6	8.83	490	11.9	20.6	490.
500	22.7	16.9	8.99	500	12.1	20.7	500.
510	22.9	17.0	9.07	510	12.2	20.8	510.
520	22.9	17.0	9.14	520	12.3	21.0	520.
530	23.2	17.2	9.24	530	12.4	21.0	530.
540	23.0	17.1	9.18	540	12.3	20.8	540.
550	22.9	17.0	9.15	550	12.3	20.7	550.
560	23.1	17.2	9.23	560	12.4	20.9	560.
570	23.5	17.4	9.38	570	12.6	21.2	570.
580	24.0	17.8	9.62	580	12.9	21.9	580.
590	24.2	18.0	9.79	590	13.1	21.9	590.
600	24.4	18.2	9.85	600	13.2	22.1	600.
610	24.6	18.3	9.93	610	13.2	22.2	610.
620	24.5	18.3	10.2	620	13.4	22.2	620.
630	24.5	18.3	10.2	630	13.5	22.1	630.
640	25.1	18.8	10.5	640	13.8	22.7	640.
650	25.7	19.2	10.7	650	14.1	23.3	650.
660	26.1	19.5	10.9	660	14.3	23.6	660.
670	26.4	19.8	11.1	670	14.5	24.0	670.
680	26.3	19.9	11.1	680	14.6	24.0	680.
690	26.4	19.9	11.2	690	14.6	24.0	690.
700	26.4	19.9	11.3	700	14.7	24.0	700.
710	26.2	19.8	11.2	710	14.6	23.9	710.
720	26.1	19.7	11.2	720	14.6	23.7	720.
730	25.9	19.6	11.2	730	14.5	23.5	730.
740	25.8	19.5	11.2	740	14.5	23.4	740.
750	25.6	19.4	11.2	750	14.4	23.3	750.
760	25.5	19.3	11.2	760	14.4	23.1	760.
770	25.3	19.2	11.3	770	14.3	23.0	770.
780	25.2	19.1	11.3	780	14.3	22.4	780.
790	25.0	19.0	11.2	790	14.2	22.7	790.
800	24.8	1.89	11.2	800	14.1	22.6	800.

[a] J_λ values are in units of 10^{15} photons cm^{-2} day^{-1}.
[b] Wavelength intervals are uniformly 10nm wide, extending from 5 nm lower than the center wavelength to 5 nm higher. Thus, the first interval centered on 290 extends from 285-295 nm.

TABLE 4—J_λ VALUES AT 50° N. Latitude

Wavelength center [b]	Summer solstice [a]	Equinox [a]	Winter solstice [a]	Wavelength center [b]	Fall or winter avg. [a]	Spring or summer avg. [a]	Wavelength center [b]
290	0.00000185	0.000000200	0.0000000112	290	0.0000000391	0.00000152	290.
300	0.0635	0.0140	0.0000681	300	0.00296	0.0433	300.
310	1.05	0.423	0.321	310	0.147	0.810	310.
320	2.81	1.41	0.214	320	0.610	2.28	320.
330	5.10	2.78	0.555	330	1.33	4.23	330.
340	5.64	3.19	0.711	340	1.59	4.73	340.
350	6.41	3.70	0.864	350	1.88	5.40	360.
360	6.75	3.96	0.953	360	2.04	5.71	350.
370	8.46	5.03	1.25	370	2.63	7.18	370.
380	8.32	5.01	1.28	380	2.66	7.09	380.
390	8.56	5.21	1.37	390	2.80	7.31	390.
400	11.8	7.22	1.95	400	3.93	10.1	400.
410	15.0	9.27	2.57	410	5.09	12.8	410.
420	15.7	9.79	2.79	420	5.45	13.5	420.
430	15.9	10.0	2.92	430	5.62	13.7	430.
440	17.6	11.2	3.33	440	6.33	15.2	440.
450	20.3	12.9	3.92	450	7.37	17.6	450.
460	22.0	14.0	4.31	460	8.05	19.0	460.
470	22.7	14.5	4.54	470	8.40	19.7	470.
480	23.1	14.8	4.70	480	8.52	20.0	480.
490	22.1	15.0	4.78	490	8.72	20.1	490.
500	23.5	15.2	4.88	500	8.87	20.4	500.
510	23.7	15.3	4.94	510	9.00	20.6	510.
520	23.8	15.4	4.98	520	9.03	20.6	520.
530	24.0	15.6	5.05	530	9.12	20.8	530.

TABLE 4—J_λ VALUES AT 50° N. Latitude—Continued

Wavelength center [b]	Summer solstice [a]	Equinox [a]	Winter solstice [a]	Wavelength center [b]	Fall or winter avg. [a]	Spring or summer avg. [a]	Wavelength center [b]
540	23.8	15.5	5.02	540	9.07	20.7	540.
550	23.7	15.4	5.01	550	9.05	20.6	550.
560	24.0	15.5	5.04	560	9.11	20.8	560.
570	24.3	15.8	5.11	570	9.26	21.1	570.
580	24.8	16.1	5.27	580	9.50	21.6	580.
590	25.1	16.4	5.38	590	9.66	21.8	590.
600	25.3	16.5	5.42	600	9.73	22.0	600.
610	25.5	16.6	5.47	610	9.80	22.1	610.
620	25.4	16.8	5.61	620	9.96	22.1	620.
630	25.3	17.0	5.77	630	10.1	22.1	630.
640	26.0	17.3	5.93	640	10.4	22.7	640.
650	26.7	17.6	6.10	650	10.6	23.3	650.
660	27.1	17.8	6.24	660	10.8	23.6	660.
670	27.5	18.1	6.39	670	11.0	24.0	670.
680	27.5	18.2	6.47	680	11.0	24.0	680.
690	27.5	18.2	6.56	690	11.1	24.0	690.
700	27.5	18.2	6.64	700	11.2	24.0	700.
710	27.3	18.1	6.67	710	11.2	29.9	710.
720	27.2	18.1	6.72	720	11.2	23.8	720.
730	27.0	18.0	6.75	730	11.2	23.6	730.
740	26.9	17.9	6.78	740	11.2	23.5	740.
750	26.7	17.8	6.82	750	11.1	23.4	750.
760	26.6	17.8	6.82	760	11.1	23.3	760.
770	26.4	17.7	6.82	770	11.1	23.1	770.
780	26.3	17.6	6.82	780	11.1	23.0	780.
790	2.61	17.5	6.80	790	11.0	22.9	790.
800	26.0	17.4	6.80	800	11.0	22.8	800.

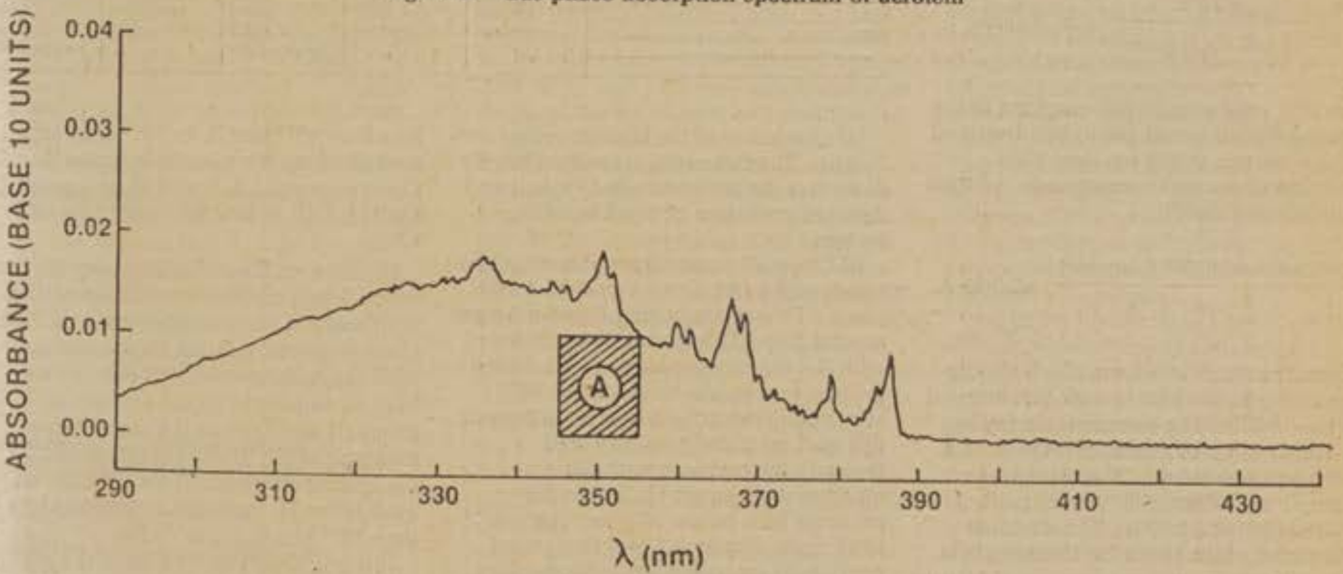
[a] J_λ values are in units of 10^{14} photons cm^{-2} day^{-1}.

[b] Wavelength intervals are uniformly 10 nm wide, extending from 5 nm lower than the center wavelength to 5 nm higher. Thus, the first interval centered on 290 extends from 285–295 nm.

(4) *Example of application of methodology.* (i) Consider a chemical plant located in Freeport, Texas, which produces acrolein [$CH_2=CHCHO$] continuously every day of the year. Despite the fact that all acrolein wastes, including vented vapors, are treated in a waste-treatment plant, some acrolein escapes into the atmosphere. The chemical plant is located at 29° north latitude. Estimate the maximum sunlight direct photolysis rate constant and the corresponding minimum half-life in the atmosphere in the vicinity of the plant for the winter and summer season solstices under clear sky conditions.

(ii) The vapor phase spectrum of acrolein was obtained by the procedure outlined in this test method and is depicted in the following Figure 3:

Figure 3.—Gas phase absorption spectrum of acrolein



The spectral data were taken from the work of Pitts et al. (1981) under paragraph (d)(2) of this section.

The pathlength of the sample gas absorption cell was measured according to the recommended procedure and was found to be 9.98 cm. The gas absorption cell contained 6.52×10^{16} molecules cm^{-3} of acrolein. A compensating polar planimeter was used to integrate each 10 nm interval throughout the region of absorption from 285 nm to 425 nm in both the sample and blank spectra. Based on triplicate measurements, one square, corresponding to 0.001 absorbance units (A), was found to be 0.148 vernier units (v.u.). The mean absorbance (A_λ) was obtained from these spectra and the mean cross section (σ'_λ) was obtained using Equation 7 under paragraph (c)(1)(i)(A) of this section for each wavelength interval, centered at λ. All the results are summarized in the following Table 5:

TABLE 5—ABSORBANCE AND CROSS SECTION FOR ACROLEIN VAPOR [1]

Wavelength λ (nm)	Wavelength interval (nm)	Mean absorbance [Aλ]	Mean cross section σ'_λ] (cm² molecule⁻¹)
290	285–295	0.0037	5.69×10^{-21}
300	295–305	0.0068	1.01×10^{-20}
310	305–315	0.0104	1.60×10^{-20}
320	315–325	0.0137	2.11×10^{-20}
330	325–335	0.0156	2.40×10^{-20}
340	335–345	0.0156	2.40×10^{-20}
350	345–355	0.0151	2.32×10^{-20}
360	355–365	0.0096	1.48×10^{-20}
370	365–375	0.0073	1.12×10^{-20}
380	375–385	0.0031	4.76×10^{-21}
390	385–395	0.0016	2.46×10^{-21}
400	395–405	0.0004	6.15×10^{-22}
410	405–415	0.0003	4.61×10^{-22}
420	415–425	0.0000	0.00

[1] 6.52×10^{16} molecules cm⁻³ in a 9.98 cm gas absorption cell.

(iii) A sample calculation is given for the wavelength λ=305 nm centered over the wavelength interval 345 to 355 nm. For convenience, the area A, corresponding to 100 squares was blocked off in this absorption area and was not integrated with the planimeter. The average vernier reading of the remaining absorption area was 7.2 v.u. Hence,

$$\frac{7.2\text{ v.u}}{0.148\text{ v.u./square}}=49\text{ squares,}$$

and the total area in the spectrum in the wavelength interval 345 to 355, centered at λ=350 nm, is 149 squares. This number of squares corresponds to 0.0149 absorbance units:

$$\frac{(149\text{ squares})\ (0.001\text{ A/square})}{10}=0.0149\text{ A.}$$

From the blank spectrum, the baseline absorbance (A_λ blank) over this interval was −0.0001. The sample trace lay at −0.0001 absorbance units relative to a zero point at 450 nm. The observed sample absorbance is then equal to 0.0150 (0.0149+0.0001). The absolute corrected absorbance for the sample is given by

$$A^{corr}{}_{\lambda sample}=A^{obs}{}_{\lambda sample}-A_{\lambda\ blank}$$

$$A^{corr}{}_{\lambda sample}=0.0150-(-0.001)=0.0151\text{ A.}$$

(iv) Using Equation 7 under paragraph (c)(1)(i)(A) of this section and the values for the corrected sample absorbance, l, and C, the mean cross section for the wavelength λ=350 nm, centered over the wavelength interval 345–355 nm, is

$$\sigma'_\lambda=\frac{0.0151}{6.52\times10^{16}\text{ molecules cm}^{-3}\ (9.98\text{ cm})}$$

$$\sigma'_\lambda=2.3\times10^{-20}\text{ cm}^2\text{ molecule}^{-1}.$$

(v) Since the plant is located at 29° north latitude, the closest J_λ values are at 30° north latitude. These values are obtained from Table 2 under paragraph (c)(3) of this section and are summarized in Table 6 for the summer and winter season solstices. Using the data in Tables 5 and 6 under paragraph (c)(4)(ii) of this section, the products $\sigma_\lambda J_\lambda$ are calculated for each wavelength interval, centered at λ, and the results are summarized in the following Table 6 for each of the solstices:

Summer	Winter
$(k_{pE})_{max}=16.9$ days^{-1} $(t_{1/2})_{min}=0.041$ days	$(k_{pE})_{max}=7.60$ days^{-1} $(t_{1/2})_{max}=0.091$ days.

The terms $\Sigma\sigma_\lambda J_\lambda$ are also summarized for each solstice at the bottom of Table 6. Using these data in Equations 3 and 4 yields:

Summer	Winter
$(k_{pE})_{max}=16.9$ days^{-1} $(t_{1/2})_{min}=0.041$ days	$(k_{pE})_{max}=7.60$ days^{-1} $(t_{1/2})_{min}=0.091$ days.

Thus, acrolein transforms rapidly under clear sky conditions in the vicinity of the plant at Freeport, Texas on the summer and winter season solstices.

TABLE 6—CALCULATION OF $(K_{pE})_{max}$ FOR ACROLEIN VAPOR; RATE AT 30 °N ON WINTER AND SUMMER SOLSTICES

Wavelength λ (nm)	Wavelength interval (nm)	Summer solstice		Winter solstice	
		J_λ photons (cm⁻² day⁻¹)	$\sigma'_\lambda J_\lambda$ (day⁻¹)	J_λ photons (cm⁻² day⁻¹)	$\sigma'_\lambda J_\lambda$ (day⁻¹)
290	285–295	1.0×10^{16}	0.000	2.1×10^{13}	0.000
300	295–305	8.31×10^{17}	0.008	8.35×10^{16}	0.001
310	305–315	1.14×10^{19}	0.182	3.00×10^{18}	0.048
320	315–325	2.84×10^{19}	0.599	1.06×10^{19}	0.224
330	325–335	5.02×10^{19}	1.205	2.13×10^{19}	0.511
340	335–345	5.49×10^{19}	1.318	2.48×10^{19}	0.595
350	345–355	6.28×10^{19}	1.457	2.89×10^{19}	0.671
360	355–365	6.49×10^{19}	0.961	3.10×10^{19}	0.459
370	365–375	8.09×10^{19}	0.906	3.95×10^{19}	0.442
380	375–385	7.93×10^{19}	0.378	3.95×10^{19}	0.188
390	385–395	8.12×10^{19}	0.200	4.12×10^{19}	0.101
400	395–405	1.11×10^{20}	0.068	5.73×10^{19}	0.035
410	405–415	1.41×10^{20}	0.065	7.37×10^{19}	0.034
420	415–425	1.47×10^{20}	0.000	7.81×10^{19}	0.000
			$\Sigma\sigma'_\lambda J_\lambda=7.34_1$		$\Sigma\sigma'_\lambda J_\lambda=3.30_4$

(5) *Operation of the Gas Handling System.* The following procedure briefly describes the recommended typical and detailed operation of a gas handling system.

(i) Close all stopcocks and turn on the rotary pump (A). Open stopcock 4 and place a Dewar containing liquid nitrogen around trap (D). Measure the pressure with the thermocouple gauge H_1. When the pressure is less than 0.1 torr (0.013 kPa) open stopcocks 5 and 10, pump out this portion of the manifold, and measure the pressure with the thermocouple gauge H_2. When the pressure falls below 10^{-2} torr (1.3×10^{-3} kPa), open stopcock 7 and evacuate F containing activated Linde Molecular Sieve 4A or an equivalent grade. Heat F to approximately 150 °C for 1 to 2 hours under vacuum until the pressure falls to less than 10^{-2} torr (1.3×10^{-3} kPa) as measured on thermocouple gauge H_2. Open stopcocks 6, 9, and 11 and pump until H_2 falls below 10^{-2} torr (1.3×10^{-3} kPa).

(ii) Turn on the diffusion pump (C) and when this pump has reached operating temperature, open stopcocks 2 and 3 and close stopcock 4. Pump on the manifold until the pressure is $<10^{-5}$ torr (1.3×10^{-6} kPa) as measured by the ionization gauge (I) and zero on the capacitance manometer (G). It should be noted that the ionization gauge (I) should only be used when H_2 indicates a pressure less than 10^{-2} torr (1.3×10^{-3} kPa).

(iii) It is good practice, after the gas phase spectrum has been obtained, to evacuate the gas absorption cell (K) and the trap (F) prior to shutting down the gas handling system. The gas handling system can be shut down by the following procedure: (A) closing stopcocks 5 to 11; (B) switching off the diffusion pump; (C) closing stopcocks 2 and 3 and opening 4, after the diffusion pump is cool; (D) removing the Dewar from trap (D) and allowing it to warm up; (E) then closing stopcock 4 and switching off the rotary pump; and (F) opening stopcock 1 to admit air to the rotary pump, thus preventing suck-back of the rotary pump oil. With this procedure, the vacuum manifold, the trap (D), and the diffusion pump are left under vacuum. The method of cleaning the liquid reservoir (J) is left to the discretion of the tester. However, as a final step it should be cleaned with reagent grade methanol or dichloromethane as solvent and dried. It is then ready for use. In operating a vacuum system with the diffusion pump working, do not expose the diffusion pump to pressures $\geq$0.1 torr of air (1.3×10^{-2} kPa) to avoid the degradation of the pump oil.

(d) *References.* For additional background information on this test guideline, the following references should be consulted: (1) Mill, T., Mabey, W.R., Bomberger, D.C., Chou, T-W., Hendry, D.G., and Smith, J.H. *Laboratory Protocols for Evaluating the Fate of Organic Chemicals in Air and Water.* Chapter 5. EPA 600/3-82-022 (1982).

(2) Pitts, J.N., Jr., Winer, A.M., Fitz, D.R., Knudsen, A.K., and Atkinson, R. *Experimental Protocol for Determining Absorption Cross Sections of Organic Chemicals,* EPA Report No. 600/3-81-051 (1981).

(3) U.S. Environmental Protection Agency. Mill, T., Davenport, J.E., Winterle, J.S., Maby, W.R., Drossman, H., Tse, D., and Lin, A. "Section 5. Photolysis in Air," by J.E., Davenport, *Toxic Substances Process Generation and Protocol Development.* Work Assignment 12, Draft final report. (Athens, Georgia, and Washington, D.C., 1984).

PART 797—ENVIRONMENTAL EFFECTS TESTING GUIDELINES

Subpart A—[Reserved]

Subpart B—Aquatic Guidelines

Sec.
797.1050 Algal acute toxicity test.
797.1060 Freshwater algal acute toxicity test.
797.1075 Freshwater and marine algae acute toxicity test.
797.1160 Lemna acute toxicity test.
797.1300 Daphnid acute toxicity test.
797.1330 Daphnid chronic toxicity test.
797.1350 Daphnid chronic toxicity test.
797.1400 Fish acute toxicity test.
797.1440 Fish acute toxicity test.
797.1520 Fish bioconcentration test.
797.1560 Fish bioconcentration test.
797.1600 Fish early life stage toxicity test.
797.1800 Oyster acute toxicity test.
797.1830 Oyster bioconcentration test.
797.1930 Mysid shrimp acute toxicity test.
797.1950 Mysid shrimp chronic toxicity test.
797.1970 Penaeid shrimp acute toxicity test.

Subpart C—Terrestrial Guidelines

797.2050 Avian dietary toxicity test.
797.2130 Bobwhite reproduction test.
797.2150 Mallard reproduction test.
797.2175 Avian acute oral toxicity test.
797.2750 Seed germination/root elongation toxicity test.
797.2800 Early seedling growth toxicity test.
797.2850 Plant uptake and translocation test.

Authority: 15 U.S.C. 2603.

Subpart A—[Reserved]

Subpart B—Aquatic Guidelines

§ 797.1050 Algal acute toxicity test.

(a) *Purpose.* The guideline in this section is intended for use in developing data on the acute toxicity of chemical substances and mixtures ("chemicals") subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94-469, 90 Stat. 2003, 15 U.S.C. 2601 et seq.). This guideline prescribes test procedures and conditions using freshwater and marine algae to develop data on the phytotoxicity of chemicals. The United States Environmental Protection Agency (U.S. EPA) will use data from these tests in assessing the hazard of a chemical to the environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA) and the definitions in Part 792—Good Laboratory Practice Standards of this chapter apply to this test guideline. The following definitions also apply to this guideline:

(1) "Algicidal" means having the property of killing algae.

(2) "Algistatic" means having the property of inhibiting algal growth.

(3) "ECx" means the experimentally derived chemical concentration that is calculated to effect X percent of the test criterion.

(4) "Growth" means a relative measure of the viability of an algal population based on the number and/or weight of algal cells per volume of nutrient medium or test solution in a specified period of time.

(5) "Static system" means a test container in which the test solution is not renewed during the period of the test.

(c) *Test procedures*—(1) *Summary of the test.* (i) In preparation for the test, fill test containers with appropriate volumes of nutrient medium and/or test solution. Start the test by introducing algae into the test and control containers in the growth chambers. Environmental conditions within the growth chambers are established at predetermined limits.

(ii) At the end of 96 hours enumerate the algal cells in all containers to determine inhibition or stimulation of growth in test containers compared to controls. Use data to define the concentration-response curve, and calculate the EC_{10}, EC_{50}, and EC_{90} values.

(2) [Reserved].

(3) *Range-finding test.* (i) A range-finding test should be conducted to determine:

(A) If definitive testing is necessary.

(B) Test chemical concentrations for the definitive test.

(ii) Algae are exposed to a widely spaced (e.g., log interval) chemical concentration series. The lowest value in the series, exclusive of controls, should be at the chemical's detection limit. The upper value, for water soluble compounds, should be the saturation concentration. No replicates are required; and nominal concentrations of the chemical are acceptable unless definitive testing is not required.

(iii) The test is performed once for each of the recommended algal species or selected alternates. Test chambers should contain equal volumes of test solution and approximately 1×10^4 *Selenastrum* cells/ml or 7.7×10^4 *Skeletonema* cells/ml of test solution. The algae should be exposed to each concentration of test chemical for up to 96 hours. The exposure period may be shortened if data suitable for the purposes of the range-finding test can be obtained in less time.

(iv) Definitive testing is not necessary if the highest chemical concentration tested (water saturation concentration or 1000 mg/l) results in less than a 50 percent reduction in growth or if the lowest concentration tested (analytical detection limit) results in greater than a 50 percent reduction in growth.

(4) *Definitive test.* (i) The purpose of the definitive test is to determine the concentration response curves, the EC_{10}'s, EC_{50}'s, and EC_{90}'s for algal growth for each species tested, with a minimum amount of testing beyond the range-finding test.

(ii) Algae should be exposed to five or more concentrations of the test chemical in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 2, 4, 6, 8, 16, 32, and 64 mg/l). Algae should be placed in a minimum of three replicate test containers for each concentration of test chemical and control. More than three replicates may be required to provide sufficient quantities of test solution for determination of test substance concentration at the end of the test. Each test chamber should contain equal volumes of test solution and approximately 1×10^4 *Selenastrum* cells ml^{-1} or 7.7×10^4 *Skeletonema* cells/ml of test solution. The chemical concentrations should result in greater than 90 percent of algal growth being inhibited or stimulated at the lowest concentrations of test substance compared to controls.

(iii) Every test should include a control consisting of the same nutrient medium, conditions, procedures, and algae from the same culture, except that none of the test substance is added. If a carrier is present in any of the test chambers, a separate carrier control is required.

(iv) The test begins when algae from 7 to 10-day-old stock cultures are placed in the test chambers containing test solutions having the appropriate concentrations of the test substance. Algal growth in controls should reach the logarithmic growth phase by 96 hours (at which time the number of algal cells should be approximately 1.5×10^6/ml for *Skeletonema* or 3.5×10^6/ml for *Selenastrum*). If growth in controls does not reach this logarithmic phase within this 96-hour period, the test is invalidated and should be repeated. At the end of 96 hours the algal growth response (number or weight of algal cells/ml) in all test containers and controls should be determined by an indirect (spectrophotometry, electronic cell counters, dry weight, etc.) or a direct (actual microscopic cell count) method. Indirect methods should be calibrated by a direct microscopic count. The percentage inhibition or stimulation of growth for each concentration, EC_{10}, EC_{50}, EC_{90} and the concentration-response curves are determined from these counts.

(v) At the end of the definitive test, the following additional analyses of algal growth response should be performed:

(A) Determine whether the altered growth response between controls and test algae was due to a change in relative cell numbers, cell sizes or both. Also note any unusual cell shapes, color differences, flocculations, adherence of algae to test containers, or aggregation of algal cells.

(B) In test concentrations where growth is maximally inhibited, algistatic effects may be differentiated from algicidal effects by the following two methods:

(*1*) Add 0.5 ml of a 0.1 percent solution (weight/volume) of Evans blue stain to a 1 milliliter aliquot of algae from a control container and to a 1 milliliter aliquot of algae from the test container having the lowest concentration of test chemical which completely inhibited algal growth (if algal growth was not completely inhibited, select an aliquot of algae for staining from the test container having the highest concentration of test chemical which inhibited algal growth). Wait 10 to 30 minutes, examine microscopically, and determine the percent of the cells which stain blue (indicating cell mortality). A staining control should be performed concurrently using heat-killed or formaldehyde-preserved algal cells; 100 percent of these cells should stain blue.

(*2*) Remove 0.5 ml aliquots of test solution containing growth-inhibited algae from each replicate test container having the concentration of test substance evaluated in paragraph (c)(4)(v)(B)(*1*) of this section. Combine these aliquots into a new test container and add a sufficient volume of fresh nutrient medium to dilute the test chemical to a concentration which does not affect growth. Incubate this subculture under the environmental conditions used in the definitive test for a period of up to 9 days, and observe for algal growth to determine if the algistatic effect noted after the 96-hour test is reversible. This subculture test may be discontinued as soon as growth occurs.

(5) [Reserved]

(6) *Analytical measurements*—(i) *Chemical*. (A) Glass distilled or deionized water should be used in the preparation of the nutrient medium. The pH of the test solution should be measured in the control and test containers at the beginning and at the end of the definitive test. The concentration of test chemical in the test containers should be determined at the beginning and end of the definitive test by standard analytical methods which have been validated prior to the test. An analytical method is unacceptable if likely degradation products of the chemical, such as hydrolysis and oxidation products, give positive or negative interference.

(B) At the end of the test and after aliquots have been removed for algal growth-response determinations, microscopic examination, mortal staining, or subculturing, the replicate test containers for each chemical concentration may be pooled into one sample. An aliquot of the pooled sample may then be taken and the concentration of test chemical determined. In addition, the concentration of test chemical associated with the algae alone should be determined. Separate and concentrate the algal cells from the test solution by centrifuging or filtering the remaining pooled sample and measure the test substance concentration in the algal-cell concentrate.

(ii) *Numerical*. Algal growth response (as percent of inhibition or stimulation in the test solutions compared to the controls) is calculated at the end of the test. Mean and standard deviation should be calculated and plotted for each treatment and control. Appropriate statistical analyses should provide a goodness-of-fit determination for the concentration response curves. The concentration response curves are plotted using the mean measured test solution concentrations obtained at the end of the test.

(d) *Test conditions*—(1) *Test species*. Species of algae recommended as test organisms for this test are the freshwater green alga, *Selenastrum capricornutum*, and the marine diatom, *Skeletonema costatum*. Algae to be used in acute toxicity tests may be initially obtained from commercial sources and subsequently cultured using sterile technique. Toxicity testing should not be performed until algal cultures are shown to be actively growing (i.e., capable of logarithmic growth within the test period) in at least 2 subcultures lasting 7 days each prior to the start of the definitive test. All algae used for a particular test should be from the same source and the same stock culture. Test algae should not have been used in a previous test, either in a treatment or a control.

(2) *Facilities*—(i) *General*. (A) Facilities needed to perform this test include: a growth chamber or a controlled environment room that can hold the test containers and will maintain the air temperature, lighting intensity and photoperiod specified in this test guideline; apparatus for culturing and enumerating algae; a source of distilled and/or deionized water; and apparatus for carrying out analyses of the test chemical.

(B) Disposal facilities should be adequate to accommodate spent glassware, algae and test solutions at the end of the test and any bench covering, lab clothing, or other contaminated materials.

(ii) *Test containers*. Erlenmeyer flasks should be used for test containers. The flasks may be of any volume between 125 and 500 ml as long as the same size is used throughout a test and the test solution volume does not exceed 50 percent of the flask volume.

(iii) *Cleaning and sterilization.* New test containers may contain substances which inhibit growth of algae. They should therefore be cleaned thoroughly and used several times to culture algae before being used in toxicity testing. All glassware used in algal culturing or testing should be cleaned and sterilized prior to use according to standard good laboratory practices.

(iv) *Conditioning.* Test containers should be conditioned by a rinse with the appropriate test solutions prior to the start of the test. Decant and add fresh test solutions after an appropriate conditioning period for the test chemical.

(v) *Nutrient medium.* (A) Formulation and sterilization of nutrient medium used for algal culture and preparation of test solutions should conform to those currently recommended by the U.S. EPA for freshwater and marine algal bioassays. No chelating agents should be included in the nutrient medium used for test solution preparation. Nutrient medium should be freshly prepared for algal testing, and may be dispensed in appropriate volumes in test containers and sterilized by autoclaving or filtration. The pH of the nutrient medium should be 7.5 for *Selenastrum* and 8.1 for *Skeletonema* at the start of the test and may be adjusted prior to test chemical addition with 0.1N NaOH or HCl.

(B) Dilution water used for preparation of nutrient medium and test solutions should be filtered, deionized or glass distilled. Saltwater for marine algal nutrient medium and test solutions should be prepared by adding a commercial, synthetic, sea salt formulation or a modified synthetic seawater formulation to distilled/deionized water to a concentration of 30 parts per thousand.

(vi) *Carriers.* Nutrient medium should be used in making stock solutions of the test chemical. If a carrier other than nutrient medium is absolutely necessary to dissolve the chemical, the volume used should not exceed the minimum volume necessary to dissolve or suspend the chemical in the test solution.

(3) *Test parameters.* (i) The test temperature should be maintained at 24°±1 °C for *Selenastrum* and 20°± °C for *Skeletonema.* Temperature should be recorded hourly during the test.

(ii) Test chambers containing *Selenastrum* should be illuminated continuously and those containing *Skeletonema* should be provided a 14-hour light and 10-hour dark photoperiod with a 30 minute transition period under fluorescent lamps providing 300 ±25 uEin/m^2 sec (approximately 400 ft-c) measured adjacent to the test chambers at the level of test solution.

(iii) Stock algal cultures should be shaken twice daily by hand. Test containers should be placed on a rotary shaking apparatus and oscillated at approximately 100 cycles/min for *Selenastrum* and at approximataely 60 cycles/min for *Skeletonema* during the test. The rate of oscillation should be determined at least once daily during testing.

(iv) The pH of nutrient medium in which algae are subcultured should be 7.5 for *Selenastrum* and 8.1 for *Skeletonema*, and is not adjusted after the addition of the algae. The pH of all test solutions and controls should be measured at the beginning and end of the test.

(v) Light intensity should be monitored at least daily during the test at the level of the test solution.

(e) *Reporting.* The sponsor should submit to the EPA all data developed by the test that are suggestive or predictive of acute phytotoxicity. In addition to the general reporting requirements prescribed in Part 792—Good Laboratory Practice Standards of this chapter, the following should be reported:

(1) Detailed information about the test organisms, including the scientific name, method of verification, and source.

(2) A description of the test chambers and containers, the volumes of solution in the containers, the way the test was begun (e.g., conditioning, test substance additions, etc.), the number of replicates, the temperature, the lighting, and method of incubation, oscillation rates, and type of apparatus.

(3) The concentration of the test chemical in the control and in each treatment at the end of the test and the pH of the solutions.

(4) The number of algal cells in each treatment and control and the method used to derive these values at the beginning and end of the test; the percentage of inhibition or stimulation of growth relative to controls; and other adverse effect in the control and in each treatment.

(5) The 96-hour EC_{10}, EC_{50}, and EC_{90} values and their 95-percent confidence limits, the methods used to derive these values, the data used to define the shape of the concentration-response curve and the goodness-of-fit determination.

(6) Methods and data records of all chemical analyses of water quality and test substance concentrations, including method validations and reagent blanks.

(7) The results of any optional analyses such as: microscopic appearance of algae, size or color changes, percent mortality of cells and the fate of subcultured cells, the concentration of test substance associated with algae and test solution supernate or filtrate.

(8) If the range-finding test showed that the highest concentration of the chemical tested (not less than 1000 mg/l or saturation concentration) had no effect on the algae, report the results and concentration and a statement that the chemical is of minimum phytotoxic concern.

(9) If the range-finding test showed greater than a 50 percent inhibition of algal growth at a test concentration below the analytical detection limit, report the results, concentration, and a statement that the chemical is phytotoxic below the analytical detection limit.

§ 797.1060 Freshwater algae acute toxicity test.

(a) *Purpose.* (1) A unicellular green alga is used as a model system initially to estimate the concentration of a chemical which could affect the primary production of plants. Because regeneration times for unicellular algal species are measured in hours, this relatively short test can assess effects over several generations. Results allow the assessment of effects on initial organisms from short term exposures and give an indication of the effect on algal populations.

(2) Many different protocols for algal tests are available. This growth test is easy to perform and gives reproducible results with the recommended species. This Test Guideline can be adapted for other algal species. If such an adaptation is used, a description of the method should be provided with the test report.

(b) *Definitions.* The following definitions apply to this guideline:

(1) "EC-X" means the experimentally derived chemical concentration that is calculated to effect X percent of the test criterion.

(2) "Growth rate" means an increase in biomass or cell numbers of algae per unit time.

(3) "Inhibition" means any decrease in the growth rate of the test algae compared to the control algae.

(4) "Limited water-soluble substances" means chemicals which are soluble in water at less than 1,000 mg/l.

(5) "Readily water-soluble substances" means chemicals which are soluble in water at a concentration equal to or greater than 1,000 mg/l.

(c) *Test procedures*—(1) *Summary of the test.* (A) The procedures for the preparation of the algal suspension, the stock solution of the test chemical, and the test media, are dependent on the solubility of the chemical and modifications in the testing procedure may be necessary due to the chemical's solubility in water. For chemicals with low solubility under test conditions, it may not be possible to quantitatively determine the EC_{50}.

(B) For purposes of the test, algae are grown in Erlenmeyer flasks in an environmentally controlled growth chamber. The test is started when 50 ml of algal suspension (1×10^4 or 2×10^4 cells/ml) and 50 ml of the appropriate test chemical dilutions are placed in the flasks. Algal growth is measured at 24 hour intervals for at least 96 hours. A Coulter Counter, counting chamber, or other appropriate instruments may be used to determine cell density. The data are used to define the concentration-response curve and the time-growth curve, and to calculate the EC_{50} and the no-effect level for (EC_0) for the chemical.

(2) [Reserved]

(3) *Range-finding test.* A range-finding test should be conducted to establish test chemical concentrations for the definitive test.

(4) *Definitive test.* (i) A definitive test is used to determine time-growth and concentration-response curves, as well as the EC_{50} and no effect level (EC_0) of the test chemical. The testing method will vary slightly depending upon whether the chemical is readily water-soluble, of limited solubility, or volatile.

(ii) Criteria for a valid definitive test. (A) Algae in the control flasks should exhibit log phase growth within 48 hours of test initiation and should produce a standing crop of at least 10^5 cells/ml in 96 hours.

(B) At 96 hours, one test concentration should show no significant decrease in growth rate and one concentration should show greater than a 50 percent decrease in growth rate relative to the control.

(C) The pH of each test solution should be measured before use and, if necessary, adjusted to 7.5 ± 0.2 using HCl or NaOH.

(D) The test chemical concentration should be determined before and after the test when practical.

(E) No more than 20 percent of the test chemical should be lost by volatilization. If more is lost (or is likely to be), the test should be conducted in closed flasks with a resulting lower standing crop.

(iii) *Test procedures dependent on solubility.*—(A) *Readily water-soluble chemicals.* When readily water-soluble chemicals are tested, the following procedures are recommended:

(*1*) A stock solution of the readily water-soluble chemical should be prepared with micropore-filtered (0.45 μm pore size) medium. The concentration of the stock solution should be twice as high as the highest concentration to be used in the test. From this stock solution at least five dilutions should be made. The dilutions should be in a geometric series with a ratio of 10 or n/10 (where n=number of dilutions). A minimum of five concentrations should be used such that the highest concentration results in at least 50 percent growth inhibition and the lowest concentration shows no significant difference ($p=0.05$) from the control.

(*2*) The algal suspension for testing readily-soluble chemicals should have a density of approximately 2×10^4 cells/ml of medium. The cell density should be quantified prior to beginning the test.

(*3*) The test should be carried out in triplicate (i.e., 3 replicate flasks per concentration). The test begins by transferring 50 ml of algal suspension to 250 ml Erlenmeyer flasks. Then, in sequence of increasing concentrations, a 50 ml volume of each prepared dilution is added to the appropriate flask. The control flasks receive 50 ml of medium. The flasks are then gently shaken and placed in the test chambers. The algal concentration in samples from each flask is determined at intervals of at least 24, 48, 72, and 96 hours after the start of the test and the number of cells or biomass (dry weight) per ml is calculated for each sample.

(*4*) A fluorimeter or spectrometer can be used to calculate cell number or biomass, but will not provide precise measurements at the start of the test and at 24 hours. If a Coulter Counter, or spectrophotometer, is used to enumerate algae at the beginning of the test, 100 ml of medium should be used to determine the background.

(B) *Limited water-soluble chemicals.* When chemicals with limited water-solubility are tested, the following modifications of the above procedure are recommended:

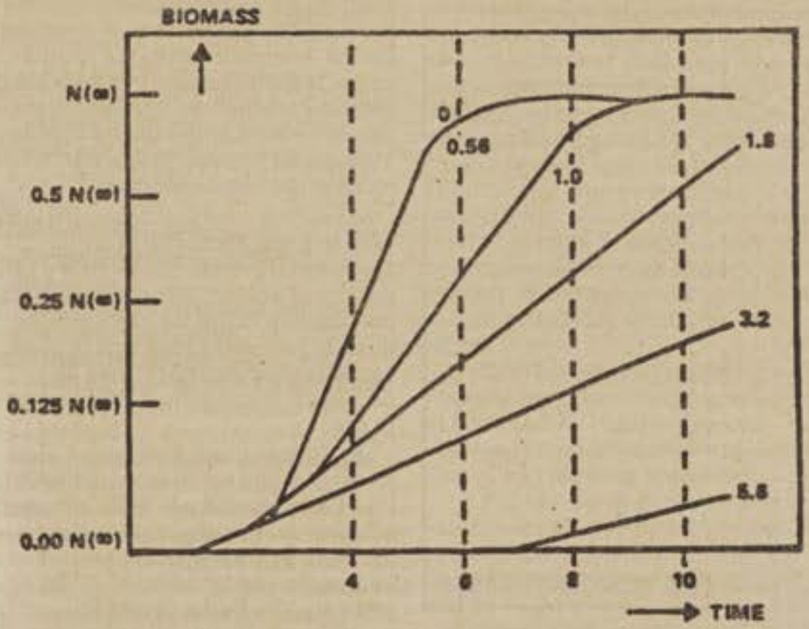
(*1*) A stock solution for chemicals with limited water-solubility should be prepared with a suitable organic solvent. This stock solution should be 10^4 times as concentrated as the highest concentration to be tested and the amount of solvent necessary to dissolve the chemical should not exceed 0.1 ml/l at the highest chemical concentration used.

(*2*) An algal suspension for testing chemicals with limited water-solubility should have a density of approximately 10^4 cells/ml. One hundred (100) ml of this algal suspension (10^4 cells/ml) are placed in each flask and 10 μl of the various dilutions of test chemical and solvent are added. Ten (10) μl of solvent are added to the solvent control flasks. Otherwise the test should be conducted as described for readily-soluble chemicals.

(iv) *Test procedures dependent on volatility.* When volatile chemicals are tested, the following modification of the procedures in paragraph (c)(4)(iii) (A) and (B) of this section is recommended. The modification prescribes the use of 250 ml conical flasks with ground glass stoppers instead of 250 ml Erlenmeyer flasks prescribed in paragraph (c)(4)(iii)(A)(*3*) of this section. These flasks are filled as described above with the algal suspension and test chemical solution. For each measurement interval the complete contents of the flasks should be used. Therefore, it is necessary to use a sufficient number of flasks to allow for this destructive sampling with time.

(5) *Test results.* (i) The results of the measurements should be tabulated and plotted on semi-logarithmic paper to yield growth curves as illustrated in the following Figure 1:

Figure 1—Theoretical example of a result of a toxicity test with algae



The growth curve is plotted on a simple logarithmic scale. The figures give the concentrations of the test compound in weight per volume. The broken lines show the measurements. N(∞) is the maximum cell density that can be achieved.

(ii) The reported chemical effects on algae should include both the EC$_{50}$ value and the no-effect level (EC$_0$). A number of methods have been described for determining the EC$_{50}$ and EC$_0$. Two examples are provided.

(A) *EC$_5$0 and EC$_0$ determined from the specific maximum growth rate (μ).* The mean value of the three replicate measurements and the 95 percent confidence limits should be calculated for the 24, 48, 72, and 96 hour sampling times, and plotted as in Figure 1. Two values are selected from the log-linear portion of the curve and the specific growth rate (μ) is calculated according to the following formula:

$$\mu = \frac{\log_{10} \frac{N_1}{N_2}}{0.434(t_2 - t_1)}$$

where:

N_1 = the lower cell number chosen in the log phase at t_1

N_2 = the higher cell number chosen in the log phase at t_2

t = time in hours

u = maximum specific growth rate

0.434 = coefficient to convert $\log_{10}$ data to $\log_e$

The percent reduction of the growth rate compared to the control can be calculated:

$$\text{"\% inhibition"} = \frac{1 - u(\text{tox})}{u(\text{b})} \times 100$$

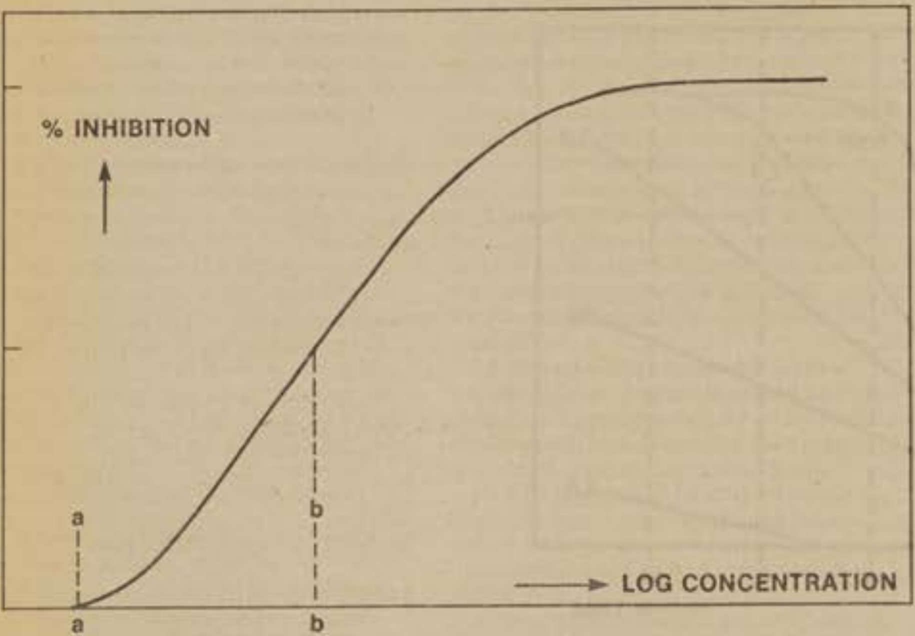
where:

u(tox) = the growth rate in the presence of the chemical

u(b) = the growth rate in the control

The calculated percentages ("% inhibition") are plotted against the log concentration as shown in the following Figure 2:

Figure 2—Theoretical example of the relation between the logarithm of the concentration of the chemical and the percentage inhibition



The highest concentration showing no difference from the control (*1*) and the EC_{50} (*2*) can then be read from this graph.

(B) *EC_{50} determined from the mean relative growth rate.* (*1*) The mean relative growth rate (RGR) during log phase growth for each culture can be calculated as follows:

$$RGR = \frac{\mathrm{Log}_{10} n_2 - \mathrm{Log}_{10} n_1}{t_2 - t_1}$$

where:

n_1 = number of cells/ml at t_1
n_2 = number of cells/ml at t_2
t = time (hours).

(*2*) The RGR of the three control replicates can be calculated and its 95 percent confidence limits determined. Treatment RGR values that are greater than the control upper 95 percent confidence limit indicate algal stimulation and should be ignored. Those above the control RGR but within the 95 percent confidence interval should be assumed to equal the control mean. Treatment RGR values that are less than the lower 95 percent confidence limit of the control should be used in the following calculations: The low treatment RGR values should be expressed as a percentage of the control RGR. The percentage values including the highest concentration of test compound at which growth is 100 percent that of control (zero inhibition and the EC_0) and the lowest concentration with a value of 0 percent (100 percent inhibition) should be included in an appropriate statistical analysis to determine the EC_{50} value and its 95 percent confidence limits.

(*3*) Interpretation of results. Algal populations rapidly regenerate themselves upon removal of stress; consequently, concentrations that produce effects need careful interpretation.

(6) [Reserved]

(d) *Test conditions*—(1) *Test species*—(i) *Selection.* (A) It is recommended that the algae used be a fast-growing species that is convenient for culturing and testing. The following freshwater species are considered suitable:

(*1*) *Selenastrum capricornutum.*

(*2*) *Scenedesmus quadricauda.*

(*3*) *Chlorella vulgaris.*

(B) Axenic cultures are recommended and are highly desirable when testing biodegradable compounds. However, pure monocultures of algae are required.

(ii) *Stock culture.* (A) The stock cultures are algal cultures that are regularly transferred to fresh medium to act as initial test material. Cultures that are not used regularly should be streaked out on sloped agar tubes. The tube cultures should be transferred to fresh medium at least once every 2 months.

(B) The stock cultures should be grown in Erlenmeyer flasks containing the appropriate medium (volume about 100 ml). When the algae are incubated at 20 °C with continuous illumination, a weekly transfer is recommended. An amount of "old" culture is transferred with sterile pipettes into a flask of fresh medium, for an approximate 100-fold dilution. The growth rate of a species can be determined from the growth curve. If this is known, it is possible to estimate at what density the culture should be transferred to new medium. This should be done before the culture reaches the senescent phase.

(iii) *Selection of test algae.* (A) Algae used in a test should be in an exponential growth phase, with a cell density of at least 10^4 cells/ml. If it is not possible to use the stock culture directly for testing (due to lack of or excessive cell growth) it may be necessary to pre-culture the algae prior to use.

(B) To preculture the algae, 2 liters of sterile nutrient solution are added to a 3 liter Erlenmeyer flask. Sufficient algal suspension from the stock culture is added to give an algal concentration of approximately 10^4 cells/ml (± 25 percent). The flasks should be continuously illuminated with fluorescent light, and the temperature maintained at 20 °C. Cultures should be shaken by hand at least once every day; this is particularly important for non-motile species. The cell concentration in the culture should be determined daily so that the desired concentration may be obtained for testing.

(C) Other methods of culturing may also be used. Some algae can be grown rapidly in shake cultures. Bubbling with air containing additional carbon dioxide may also accelerate growth. Furthermore, under the culturing conditions described above, the pre-culture, for the algal species recommended in this test, should be optimal for inoculation of test flasks after 4 to 5 days. If the algal cultures contain deformed or otherwise abnormal cells (e.g., clumped, chlorotic), they should not be used for the test.

(2) *Facilities*—(i) *Apparatus.* (A) In order to avoid contamination with bacteria and other algae, all stock culture maintenance operations should be carried out under sterile conditions. Possible contamination should be checked by suitable methods.

(B) Testing and culturing of algae should be done in an environmentally controlled cabinet or chamber capable of maintaining a temperature of 20 °C ± 2 °C and continuous illumination of approximately 300 to 400 uE/m² sec.

(C) In addition to normal laboratory apparatus and equipment for algal testing, a counting apparatus (e.g., Coulter Counter, counting chamber, fluorometer, spectrophotometer, colorimeter) to determine cell numbers is also necessary.

(D) The following apparatus and equipment are necessary for algal culturing:

(*1*) Incubators or climate rooms capable of maintaining temperature and light at the recommended levels.

(*2*) Filtering apparatus, accompaning membrane filters, (0.45 μm) and 5 liter flasks.

(*3*) Inoculation needle.

(*4*) Sterile graduated pipettes.

(*5*) pH-meter.

(*6*) Culture tubes (150 × 18 mm) with sponge and/or metal caps.

(*7*) Erlenmeyer flasks, 200 ml, with ground glass stoppers, and a 3 liter flask.

(ii) *Dilution water.* Redistilled water should be used for preparation of stock, chemical, and nutrient media solutions.

(3) *Test parameters*—(i) *Carriers.* If solvents, are used, they should not themselves be toxic at the concentrations used and should not affect algal growth. In no case should the concentration of an organic solvent exceed 0.1 ml/1 in the highest concentration.

(ii) *Lighting.* Algae should be kept under continuous, uniform illumination of approximately 300 to 400 uE/m^2 sec. The light source should be fluorescent lights.

(iii) *Loading.* For readily water-soluble and volatile chemicals, an algal suspension containing approximately 2×10^4 cells/ml should be used. For chemicals with limited water solubility the algal density should be approximately 10^4 cells/ml.

(iv) *Temperature.* The temperature for culturing and testing algae should be 20 °C ± 2 °C.

(v) *Nutrient media.* The recommended media for adequate growth of algae is shown in the following Table 1:

TABLE I—MEDIUM FOR FRESHWATER ALGAE

	Amount
Nutrient salts:	
$NaNO_3$	0.5 g.
NH_4NO_3	0.33 g.
$K_2HPO_4.3H_2O$	0.052 g.
$CaCl_2.2H_2O$	0.035 g.
$Na_2CO_3.10H_2O$	0.054 g.
Fe_3-citrate	0.006 g.
Citric acid monohydrate	0.006 g.
Trace element solution (see below).	1.0 ml.
Redistilled water made up to	1.0liter.
pH	7.7 ±0.3.
Trace Element Solution:	
H_3BO_3	2.90 g.
$MnCl_2.4H_2O$	1.81 g.
$ZnCl_2$	0.11 g.
$CuSO_4.5H_2O$	0.08 g.
$(NH_4)_6Mo_7.4H_2O$	0.018 g.
Redistilled water made up to	1.0 liter.

All nutrient solutions can be prepared as concentrated stock solutions using the salts shown in Table 1 under this paragraph and stored in the dark and cold. These solutions should be sterilized by filtration or by autoclaving. The medium is prepared by adding the correct amount of stock solutions, or the nutrients salts directly, to sterile distilled water, to give the final concentrations listed. For solid medium, 1.5 to 2 percent agar can be added. Other media may be necessary if species other than those above are used.

(e) *Reporting.* In addition to a description of the type of test and method, the report submitted to EPA should include the following information.

(1) For the chemical tested: Manufacturer, empirical formula, batch number and its degree of purity, chemical characterization (e.g., trade name), and physical properties.

(2) For the test organisms: Origin of innoculum, laboratory culture and strain number, and method of cultivation (including whether cultures aerated and/or shaken).

(3) For the test conditions: Date of beginning and end of the test and its duration, temperature, light intensity and light quality in the growth chamber, type of test flask (and if closed or open), initial pH of test solution, what carrier is used and how much, concentrations of test chemical, and the counting method.

(4) For the results: A tabulation of cell number or biomass per ml for each flask at each sampling period, the plotted time-growth curves for each concentration and the concentration-effect curve, the EC_{50} value and the highest concentration showing no statistical growth inhibition (EC_0) and the statistical methods used to calculate them, and other observed effects, e.g. algicidal vs. algistatic effects, clumping or chlorosis of cells.

(5) For the laboratory performing the test: The name of the person responsible for carrying out the test (study director) as well as the name of the person carrying out the test, the name and address of the testing laboratory, and the date and signature of the person responsible for the test.

§ 797.1075 Freshwater and marine algae acute toxicity test.

(a) *Purpose.* (1) A unicellular alga is used as a model system initially to estimate the concentration of a chemical which could affect survival and growth of freshwater or marine algae. Because generation times for unicellular algal species are measured in hours, this relatively short test can assess effects over several generations. Results allow the assessment of effects on initial organisms from short term exposures and give an indication of the effect on algal populations.

(2) Many different protocols for algal tests are available. This growth test is easy to perform and gives reproducible results with the recommended species. This test guideline can be adapted for other algal species. If such an adaptation is used, a description of the method should be provided with the test report.

(b) *Definitions.* The following definitions apply to this guideline:

(1) "EC–X" means the experimentally derived chemical concentration that is calculated to effect X percent of the test criterion.

(2) "Growth rate" means the rate at which the algal population grows, estimated by the increase in cell numbers or biomass over a specified period of time.

(3) "Inhibition" means any decrease in the growth rate of the population of test algae compared to the controls.

(4) "Limited water-soluble substances" means chemicals which are soluble in water at less than 1,000 mg/l.

(5) "Readily water-soluble substances" means chemicals which are soluble in water at a concentration equal to or greater than 1,000 mg/l.

(c) *Test procedures*—(1) *Summary of the test.*

(A) The procedures for the preparation of the algal suspension, the stock solution of the test chemical, and the test media, are dependent on the solubility of the chemical. Modifications in the testing procedure may be necessary due to the chemical's solubility in water. For chemicals with low solubility under test conditions, it may not be possible to quantitatively determine the EC_{50}.

(B) For purposes of the test, algae are grown in Erlenmeyer flasks in an environmentally controlled growth chamber. The test is started when an algal suspension in nutrient solution (10^4 or 2×10^4 cells/ml) and the appropriate test chemical dilutions are placed in the flasks. Algal growth is measured at 24-hour intervals for at least 96 hours. A Coulter Counter, cell counting chamber, or other appropriate instruments may be used to determine population density. The data are used to define the concentration-response curve and the time-growth curve, and to calculate the EC_{50} and EC_0 (no-effect level) for the chemical.

(2) [Reserved]

(3) *Range-finding test.* A range-finding test should be conducted to establish test chemical concentrations for the definitive test.

(4) *Definitive test.* (i) The definitive test is used to determine time-growth and concentration-response curves, as well as the EC_{50} and EC_0 of the test chemical. The testing method will vary slightly depending upon whether the chemical is readily water-soluble, of limited solubility, and/or volatile.

(ii) Criteria for a valid definitive test. (A) Algae in the control flasks should exhibit log phase growth within 48 hours of test initiation and should produce a population density of at least 10^5 cells/ml in 96 hours.

(B) At 96 hours, one test concentration should show no significant decrease in growth rate and one concentration should show greater than a 50 percent decrease in growth rate relative to the control.

(C) The pH of each test solution should be measured before use and, if necessary, adjusted to 7.5 ± 0.2 for freshwater and 8.0 ± 0.1 for marine species using HCl or NaOH.

(D) The concentration of the test chemical in the medium should be determined before and after the test.

(E) No more than 20 percent of the test chemical should be lost by volatilization. If more is lost (or is anticipated to be), the test should be conducted in closed flasks. Carbon dioxide limitations resulting from the closed flasks typically result in population densities lower than the standard in (A) above.

(iii) Test procedures dependent on solubility—(A) *Readily water-soluble chemicals.* When readily water-soluble chemicals are tested, the following procedures are recommended:

(*1*) Just prior to testing, a stock solution of the readily water-soluble chemical should be prepared with micropore-filtered (0.45 um pore size) medium. The concentration of the stock solution should be twice as high as the highest concentration to be used in the test. From this stock solution at least five dilutions should be made. The dilutions should be in a geometric series with a ratio of 10 or n/10 (where n = number of dilutions). The highest concentration should result in at least 50 percent growth inhibition while the lowest concentration should result in no significant difference ($\alpha = 0.05$) from the growth in the controls.

(*2*) The algal suspension for testing readily-soluble chemicals should have a density of approximately 2×10^4 cells/ml of nutrient solution. The cell density should be quantified prior to beginning the test.

(*3*) The test should be carried out in triplicate (i.e., three replicate flasks per concentration). The test begins by transferring 50 ml of algal suspension to 250 ml Erlenmeyer flasks. Then, in sequence of increasing concentrations, a 50 ml volume of each prepared dilution is added to the appropriate flask. The control flasks receive 50 ml of nutrient solution. The flasks are then gently shaken and placed in the growth chambers. The flasks should be shaken by hand at least once every day; this is particularly important for nonmotile species. The algal concentration in samples from each flask is determined at intervals of at least 24, 48, 72, and 96 hours after the start of the test and the number of cells or biomass (dry weight per ml) is calculated for each sample.

(*4*) If a fluorometer or spectrometer is used to calculate cell number or biomass, the measurements will be inaccurate during the first 24 hours of the test period. Therefore, a calibration curve of meter readings of cell numbers versus test concentrations should be plotted. Calculations should be limited to the straight portion of the calibration curve.

(B) *Limited water-soluble chemicals.* When chemicals with limited water-solubility are tested, the following modifications of the above procedure are recommended:

(*1*) Just prior to testing, stock solutions of test chemicals should be prepared with a suitable organic solvent. Each stock solution should be 10^4 times more concentrated than the highest concentration to be tested. The amount of solvent necessary to dissolve the chemical should not exceed 0.1 ml/l of stock solution.

(*2*) The algal suspension should have a density of approximately 10^4 cells/ml. Otherwise the test should be conducted as described for readily-soluble chemicals.

(*3*) Each test flask should contain 100 ml of algal suspension and a 10 μl aliquote of the diluted chemical stock. A series of dilutions is made of the chemical stock so that when 10 μl of each dilution is combined with 100 ml of algal suspension the desired test concentrations are obtained. Two controls should be included, one possessing the solvent (10 μl of 0.1 ml/l) and no test chemical, and the second without either.

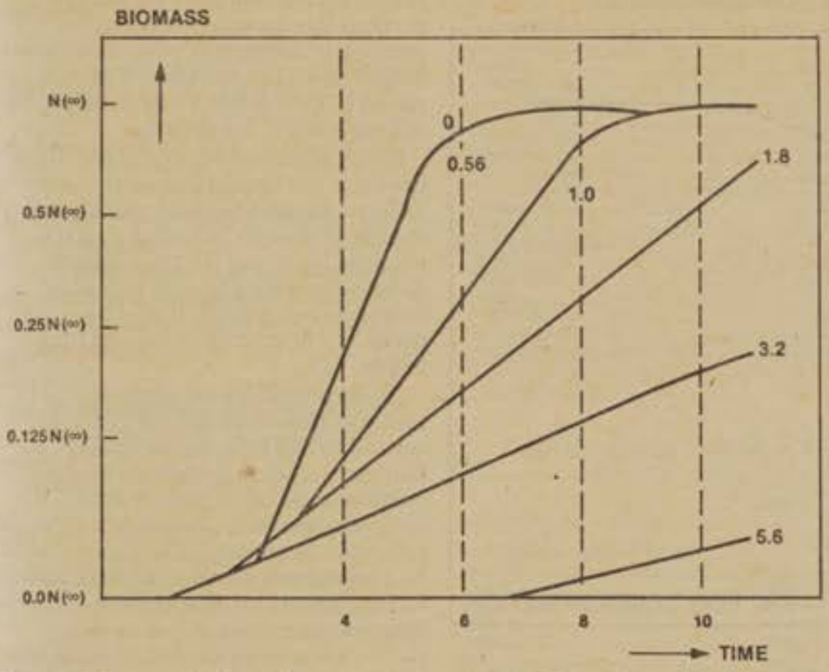
(iv) Test procedures dependent on volatility. When volatile chemicals are tested, the following modifications of the procedures in paragraph (c)(4)(iii) (A) and (B) of this section are recommended. The test is performed as described above (depending on the chemical's solubility in water) except that 250 ml conical flasks with ground glass stoppers should be used. These flasks are filled, as described above, with the algal suspension and test chemical solution. For each measurement interval the complete contents of the flasks should be used. Therefore, it is necessary to use a sufficient number of flasks to allow for this destructive sampling with time.

(5) *Test results.* (i) The results of the measurements should be tabulated. Growth curves resulting from the experiment should be drawn on semi-logarithmic paper.

(ii) The reported chemical effects on algae should include both the EC_{50} and EC_0. A number of methods have been described for determining the EC_{50} and EC_0. Two examples are provided.

(A) *EC_{50} and EC_0 determined from the specific maximum growth rate (u).* The mean value of the three replicate measurements and the 95 percent confidence limits should be calculated for the 24, 48, 72, and 96 hour sampling times, and plotted as in the following Figure 1:

Figure 1—Theoretical example of a result of a toxicity test with algae



The growth curve is plotted on a simple logarithmic scale. The figures give the concentrations of the test compound in weight per volume. The broken lines show the measurements. N(∞) is the maximum cell density that can be achieved.

Two values are selected from the log-linear portion of the curve and the specific growth rate (u) is calculated according to the following formula:

$$u = \frac{\ln (N_2/N_1)}{(t_2 - t_1)}$$

where:

N_1 = the lower cell number chosen in the log phase at t_1

N_2 = the higher cell number chosen in the log phase at t_2

t = time in hours

u = maximum specific growth rate

The percent reduction of the growth rate compared to the control can be calculated:

"% inhibition" = 1 − (u(tox)/(u(b))) × 100

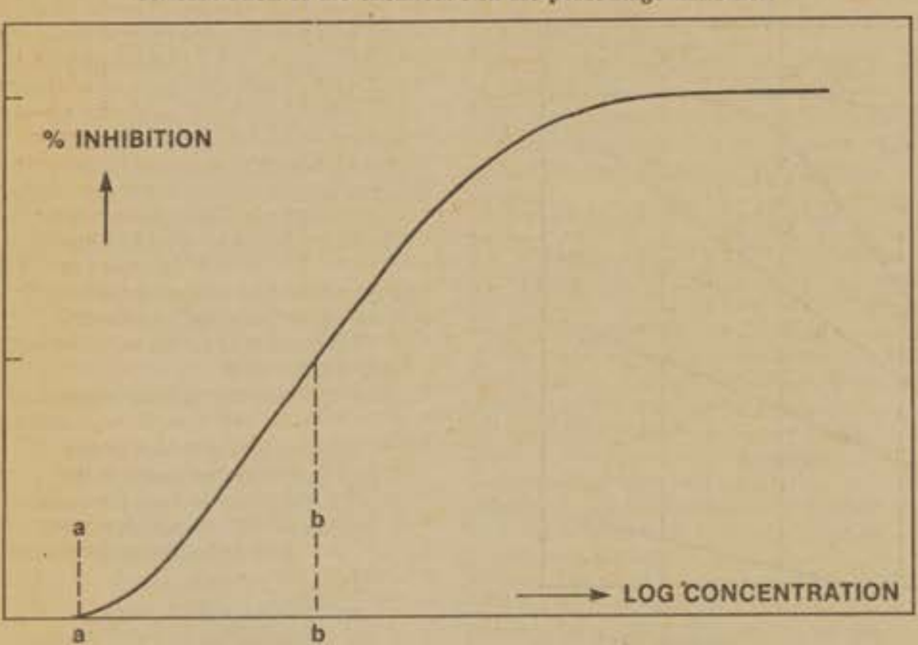
where:

u(tox) = the growth rate in the presence of the chemical

u(b) = the growth rate in the control

The calculated percentages ("% inhibition") are plotted against the log concentration as shown in the following Figure 2. The highest concentration showing no difference from the control (*1*) and the EC_{50} (*2*) can then be read from this graph.

Figure 2—Theoretical example of the relation between the logarithm of the concentration of the chemical and the percentage inhibition



(B) *EC_{50} determined from the mean relative growth rate.* (*1*) The mean relative growth rate (RGR) during log phase growth for each culture can be calculated as follows:

$RGR = (\log_{10} n_2 - \log_{10} n_1)/(t_2 - t_1)$

where:

n_1 = number of cells/ml at t_1
n_2 = number of cells/ml at t_2
t = time (hours)

(*2*) The mean relative growth rates (RGR) of the controls and treatment along with the 95 percent confidence intervals (95 percent CI) are calculated. Treatment RGR values that are greater than the control upper 95 percent CI will be ignored. Those above the mean control RGR but within the 95 percent CI should be assumed to equal the control mean. The treatment RGR values are then expressed as a percentage of the control mean RGR. The percentage values including the highest concentration of test compound at which growth is 100 percent that of control (if values for all higher concentrations are less than 100 percent) and the lowest concentration with a value of 0 percent (if values for higher concentrations are less than or equal to 0 percent) are included in a probit analysis to determine the EC_{50} value with its 95 percent confidence limits.

(*3*) Interpretation of results. Algal populations subjected to chemical stress may quickly establish normal growth rates when the stress condition is removed; consequently, concentrations that produce effects need careful interpretation.

(*d*) *Test conditions*—(1) *Test Species*—(i) *Selection.* (A) It is recommended that the algae used be fast-growing species that are convenient for culturing and testing. The following species are considered suitable:

(*1*) *Freshwater Algae*—

(*i*) *Selenastrum capricornutum.*

(*ii*) *Scenedesmus quadricauda.*

(*iii*) *Chlorella vulgaris.*

(*2*) *Marine Algae*—

(*i*) *Skeletonema costatum.*

(*ii*) *Thallassiosira pseudonana.*

(*iii*) *Isochrysis galbana.*

(B) Monocultures of algae are required, and axenic cultures are recommended.

(ii) *Stock culture.* (A) The stock cultures are algal cultures that are regularly transferred to fresh medium to act as initial test material. Cultures that are not used regularly should be streaked out on sloped agar slants. The tube cultures should be transferred to fresh medium at least once every 2 months. Stock cultures taken from agar slants should be subcultured in liquid medium once or twice before use.

(B) The stock cultures should be grown in Erlenmeyer flasks containing the appropriate medium (volume about 100 ml). When the algae are grown at 20 °C under continuous cool-white fluorescent light, a weekly transfer is recommended. An aliquot removed from a mature culture transferred with a sterile pipette into a flask of fresh medium, for an approximate 100-fold dilution. The growth rate of a species can be determined from the growth curve. If this growth rate is known, it is possible to estimate at what density the culture should be transferred to new medium. This should be done before the culture is out of log growth.

(iii) *Selection of test algae.* (A) Algae used in a test should be in an exponential growth phase, with a cell density of at least 10^4 cells/ml. If it is not possible to use the stock culture directly for testing (due to lack of or excessive cell growth) it may be necessary to preculture the algae prior to use.

(B) To preculture the algae, 200 ml of sterile nutrient solution are added to several 500 ml Erlenmeyer flasks. Sufficient algal suspension from stock cultures is added to give an algal concentration of approximately 10^4 cells/ml ± 25 percent). The flasks should be continuously illuminated with cool-white fluorescent light, and the temperature maintained at 20 °C. Cultures should be shaken by hand at least once every day; this is particularly important for nonmotile species. The cell concentration in the culture should be determined daily so that the desired concentration may be obtained for testing.

(C) Other methods of culturing may also be used. Some algae can be grown rapidly in shake cultures. Bubbling with filtered air containing additional carbon dioxide may also accelerate growth. Furthermore, under the culturing conditions described above, the preculture for the algal species recommended in this test, should be optimal for inoculation of test flasks after 4 to 5 days. If the algal cultures contain deformed or otherwise abnormal cells (e.g., clumped, chlorotic), they should not be used for the test.

(2) *Facilities*—(i) *Apparatus.* (A) In order to avoid contamination with bacteria and other algae, all stock culture maintenance operations should be carried out under aseptic conditions. Cultures should be periodically checked for contamination.

(B) Testing and culturing of algae should be done in an environmentally controlled cabinet or chamber capable of maintaining a temperature of 20 °C ± 2 °C and continuous illumination of approximately 150 to 200 $\mu E/m^2$ sec.

(C) In addition to normal laboratory apparatus and equipment for algal testing, a counting apparatus (e.g., Coulter Counter, cell counting chamber, fluorometer, spectrophotometer, or colorimeter) to determine cell numbers is also necessary.

(i) The following apparatus and equipment are necessary for algal culturing:

(*1*) Incubators or climate rooms capable of maintaining temperature and light at the recommended intensity.

(*2*) Filtering apparatus, accompanying membrane filters, (0.45 um) and flasks.

(*3*) Inoculation needle.

(*4*) Sterile graduated pipettes.

(*5*) pH-meter.

(*6*) Culture tubes (150×18 mm) with stainless steel closures.

(*7*) Erlenmeyer flasks, 250 ml, with ground glass stoppers, and a 3 liter flask.

(ii) *Dilution water.* Redistilled or deionized water should be used for preparation of stock, chemical, and nutrient solutions.

(3) *Test parameters*—(i) *Carriers.* If solvents, are used, they should not themselves be toxic at the concentrations used and should not affect algal growth. In no case should the concentration of an organic solvent exceed 0.1 ml/l in the highest concentration.

(ii) *Lighting.* Algae should be kept under continuous, uniform illumination of approximately 150 to 200 $\mu E/m^2$ sec. The light source should be cool-white fluorescent lights.

(iii) *Inoculation.* For readily water-soluble and volatile chemicals, an algal suspension containing approximately 2×10^4 cells/ml should be used. For chemicals with limited water solubility the algal density should be approximately 10^4 cells/ml. After dilution, both initial algal densities should be approximately 10^4 cells/ml.

(iv) *Temperature.* The temperature for culturing and testing algae should be 20 °C ± 2 °C.

(v) *Nutrient media.* The recommended media for adequate growth of algae are shown in the following Table 1 and Table 2. All nutrient solutions can be prepared as concentrated stock solutions using the salts shown in the following Tables 1 and 2 and stored in the dark and cold. These solutions should be sterilized by filtration or by autoclaving. The medium is prepared by adding the correct amount of stock solutions, or the nutrient salts directly, to sterile distilled or deionized water, to give the final concentrations listed. For solid medium, 1.5 to 2 percent agar (wt/vol) should be added to the medium prior to autoclaving. Other media may be necessary if species other than those above are used.

TABLE 1.—MEDIUM FOR FRESHWATER ALGAE

	Amount
Solution 1 (Nutrient salts):	
$NaNO_3$	0.5 g
NH_4NO_3	0.33 g
$K_2HPO_4.3H_2O$	0.052 g
$CaCl_2.2H_2O$	0.035 g
$Na_2CO_3.10H_2O$	0.054 g
Fe_3-citrate	0.006 g
Citric acid monohydrate	0.006 g
Trace element solution (sol. 2) (see below)	1.0 ml
Redistilled water made up to	1.0 liter
pH	7.7±0.3
Solution 2 (Trace Metals):	
H_3BO_3	2.90 g
$MnCl_2.4H_2O$	1.81 g
$ZnCl_2$	0.11 g
$CuSO_4.5H_2O$	0.08 g
$(NH_4)_6MO_7.4H_2O$	0.018 g
Redistilled water made up to	1.0 liter

TABLE 2.—MEDIUM FOR MARINE ALGAE

	mmol/1	g/kg solution
Solution 1 (Nutrient salts)		
NaCl	362.661	20.758
Na_2SO_4	24.993	3.477
KCl	8.038	0.587
KBr	0.7249	0.0845
H_3BO_3	0.3715	0.0225
NaF	0.0657	0.0027
$MgCl_2.6H_2O$	47.176	9.395
$CaCl_2.2H_2O$	9.139	1.316
$SrCl_2.6H_2O$	0.0082	0.0214
Solution 2		
$NaHCO_3$[1]	2.066	0.170
Solution 3 (Trace Metals)[3]	g/l	umol/1[2]
$NaNO_3$	4.667	549.09
$Na_2SiO_3 \cdot 9H_2O$[4]	3.000	105.60
Na_2 glycerol PO_4	0.667	21.79
Na_2EDTA[5]	0.553	14.86
H_3BO_3	0.380	61.46
$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	0.234	5.97
$FeCl_3 \cdot 6H_2O$	0.016	0.592
$MnSO_4 \cdot 4H_2O$	0.054	2.42
$ZnSO_4 \cdot 7H_2O$	0.0073	0.254
$CoSO_4 \cdot 7H_2O$	0.0016	0.0569
Solution 4 (Vitamins[6])		
Thiamine HCl	0.1	0.297
Vitamin B_{12}	0.002	0.00147
Biotin	0.001	0.00409

[1] Dry, autoclave the $NaHCO_3$ then add to autoclaved solution 1.

[2] Final concentration in solution.

[3] Should be autoclaved (50 minutes).

[4] Should be neutralized before adding to other solutions. To neutralize, 100 ml of $Na_2SiO_3 \cdot 9H_2O$(3.0 g/l) is added to 20 ml 1 *N* HCl and then added to solution.

[5] NA_2 EDTA, $FeCl_3 \cdot 6H_2O$, $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ has to be in solution before remaining salts (Mn, Zn, Co) are added.

[6] Filter, sterilize, store frozen.

(e) *Reporting.* In addition to a description of the type of test and method, the report submitted to EPA should include the following information.

(1) For the chemical tested: manufacturer, empirical formula, batch number and its degree of purity, chemical characterization, and physical properties.

(2) For the test organisms: origin of innoculum, laboratory culture and strain number, and method of cultivation (including whether and how cultures were aerated and/or shaken).

(3) For the test conditions: date of beginning and end of the test and its duration, temperature, light intensity and light quality in the growth chamber, type of test flask (and if closed), initial pH of test solution, what carrier was used and how much, concentrations of test chemical, and the counting method.

(4) For the results: A tabulation of cell number or biomass (dry weight per ml) for each flask at each sampling period; the plotted time-growth curves for each concentration and the concentration-effect curve; the EC_{50} and EC_0 values and the statistical methods used to calculate them; and other observed effects, e.g., stimulation of growth, algicidal vs. algistatic effects, clumping or chlorosis of cells.

(5) For the laboratory performing the test: the name of the person responsible for carrying out the test (e.g., study director) as well as the name of the person carrying out the test, the name and address of the testing laboratory, and the date and signature of the person responsible for the test.

§ 797.1160 Lemna acute toxicity test.

(a) *Purpose.* This guideline prescribes test procedures and conditions using the freshwater aquatic plant *Lemna gibba* G3 to develop data on the phytotoxicity of chemicals. The United States Environmental Protection Agency (USEPA) will use data from these tests in assessing the hazard of a chemical to the environment under the Toxic Substances Control Act (TSCA) (Pub. L. 94–469, 90 Stat. 2003, 15 U.S.C. 2601 et seq.).

(b) *Definitions.* The definitions in section 3 of TSCA and Part 792—Good Laboratory Practice Standards of this chapter are applicable to this guideline. The following definitions also apply:

(1) "Axenic" means a culture of *Lemna* fronds free from other organisms.

(2) "Colony" means an aggregate of mother and daughter fronds attached to each other.

(3) "EC–X" means the experimentally derived chemical concentration that is calculated to effect X percent of the test criterion.

(4) "Frond" means a single *Lemna* "leaf-like" structure.

(5) "Frond mortality" means dead fronds which may be identified by a total discoloration (yellow, white, black or clear) of the entire frond.

(6) "Static-replacement test" means a test method in which the test solution is periodically replaced at specific intervals during the test.

(c) *Test procedures*—(1) *Summary of the test.* (i) In preparation for the test, containers are filled with appropriate volumes of nutrient medium and/or the test solutions. The test is started by introducing *Lemna* fronds into each of the containers. Nutrient medium and test solutions should be replaced on days 3 and 6 or as needed to prevent nutrient limitation or depletion of the test chemical.

(ii) Colonies should be inspected for changes in frond number and appearance at the beginning of the exposure period (day 0), on days 3 and 6, and at the end of the exposure period (day 7). On day 7, the total number of living and/or dead fronds are counted. Any frond which is visible as a bud when viewed under a hand lens or dissecting microscope should be counted. Concentration response curves are plotted for total frond number, growth rate (as number of fronds per day) and mortality (percentage of dead fronds to total number of fronds). EC_{10}'s, EC_{50}'s, and EC_{90}'s are determined from the curves.

(2) [Reserved]

(3) *Range-finding test.* (i) A range-finding test should be conducted to establish (A) if definitive testing is necessary and (B) test solution concentrations for the definitive test.

(ii) The recommended procedure is to expose *Lemna* to a chemical concentration series (e.g., 0.1, 1.0, 10, 100 and 1000 mg/)l plus controls. Three, four-frond colonies should be selected and exposed to equal volumes of each chemical concentration for a period of seven days. The exposure period may be shortened if data suitable for the purpose of the range-finding test are obtained.

(iii) The lowest chemical concentration in a test series, exclusive of controls, should be the lowest concentration which can be analytically quantified. The highest concentration should be at least 1000 mg/l. Replicates are not needed and nominal concentrations of the chemical are acceptable for range-finding. If the calculated EC_{50} is greater than 1000 mg/l or is less than the analytical detection limit, definitive testing is not necessary. However, replicates and measured concentrations of the appropriate dose are needed to substantiate this result.

(4) *Definitive test.* (i) The purpose of the definitive test is to determine the EC_{10}'s, EC_{50}'s, and EC_{90}'s for *Lemna* growth based on total frond number, growth rate and/or frond mortality with a minimum amount of testing beyond the range-finding test.

(ii) At least five concentrations of chemical, exclusive of controls, should be used in the definitive test. The concentration range should be selected to define the concentration response curve between the EC_{10} and EC_{90}. For each concentration and control at least seven replicate containers should be used, each containing 150 ml of test solution and three randomly selected four-frond colonies. Fewer replicates, each containing a greater number of colonies, may be used; however, test containers and solution volumes will have to be adjusted accordingly. The range of chemical concentrations tested should result in the highest concentration affecting at least 90 percent of the fronds and the lowest concentration affecting no more than 10 percent of the fronds compared with the controls.

(iii) Every test should include controls consisting of the same nutrient medium, number of fronds, environmental conditions, and procedures as the test containers except that none of the chemical is added. If a solvent or carrier is used to dissolve or suspend the test chemical, additional controls containing the solvent or carrier should also be included in the test to determine any effect of the solvent or carrier on the plants.

(iv) The colonies should be transferred to freshly prepared test solutions on days 3 and 6. No more than 20 percent of the test substance should be lost by volatilization between replacements. The colonies may have to be transferred more frequently for highly volatile test substances in order to maintain 80 percent of the initial test substance concentration. Transfer should be done in a clean, draft-free area as quickly as possible to minimize contamination of the colonies.

(v) Observations of frond numbers and appearance should be made of the colonies on day 0, 3, 6, and 7. A dissecting microscope will facilitate observations.

(vi) Concentration response curves should be plotted. These curves can provide the basis for determining the EC_{10}'s, EC_{50}'s, and EC_{90}'s for total frond number, growth rate, and mortality.

(vii) Any change in frond development or appearance such as increase in number (a frond is counted regardless of size as long as it is visible adjacent to the parent frond), necrosis, chlorosis, etc., should be reported. Chlorophyll content of the colonies may be used as an indicator of chlorosis. Any additional observations such as sedimentation of test solution, sinking of fronds, or other abnormalities should also be recorded.

(viii) A randomized complete block design is recommended for the definitive test with blocks delineated within the test chamber. If, for any reason, blocking is not feasible, total randomization within chambers is suggested.

(5) [Reserved].

(6) *Analytical measurements*—(i) *Chemical.* Stock solutions should be prepared just prior to use and diluted with glass distilled or deionized water to obtain the test solutions. Standard analytical methods, if available, should be used to establish concentrations of these solutions and should be validated before beginning the test. An analytical method is not acceptable if likely degradation products of the chemical, such as hydrolysis and oxidation products, give positive or negative interference. The pH of the test solutions should also be measured prior to and after use.

(ii) *Numerical.* The number of fronds is counted at the end of the definitive test. Means and standard deviations are calculated and plotted for each treatment and control. Appropriate statistical analyses are used to provide a goodness-of-fit determination for the concentration response curves.

(d) *Test conditions*—(1) *Test Species.* The test species to be used in these tests is *Lemna gibba* G3. Axenic cultures may be obtained from laboratory cultures or commercial sources. A stock culture grown from a single isolated frond should be used to inoculate all the flasks used in a given test.

(2) *Acclimation.* Axenic stock cultures should be grown in the aquaria for 2 weeks (with necessary transfers) prior to being used in a test. Four-frond plants used in a test should be randomly selected from the culturing tank. Inocula should be taken from cultures which are less than 2 weeks old.

(3) *Facilities*—(i) *Apparatus.* (A) A controlled environment growth chamber or an enclosed area capable of maintaining the specified test parameters (see Section 4 below) is needed.

(B) Laboratory facilities for the mixing and diluting of nutrient medium and a source of distilled or deionized water are needed. An autoclave (for sterilizing glassware and media) and a sterile transfer hood (for maintenance of an axenic *Lemna* culture) are also necessary. Disposal facilities should be adequate to accommodate spent test solutions and plant materials as well as any bench covering, lab clothing, or other contaminated materials.

(ii) *Containers and support media.* Test containers may be glass beakers or Erlenmeyer flasks, large enough to hold 150 ml of test solution and the *Lemna* colonies without crowding for the duration of the test; 250 ml beakers are recommended. All containers should be of the same type and size. If fewer replicates are used, larger containers may be necessary to hold the additional colonies and test solution volume. The ratio of test solution to container volume should not exceed 3:5. For each test concentration and control the same number of replicates should be used.

(iii) *Cleaning and sterilization.* All glassware and equipment should be cleaned following good laboratory practice. The Nytex screen or inoculating loops used for transferring the *Lemna* should be disposed of after use or thoroughly cleaned and sterilized before reuse.

(iv) *Nutrient media.* Hoagland's nutrient medium is recommended for maintaining *Lemna* cultures and for use as the diluent in the preparation of various concentrations of test solutions. Deionized or distilled water should be used to make the nutrient medium. The medium should be freshly prepared prior to each transfer of *Lemna* cultures and for the preparation of new test solutions during the course of a test. The pH of the medium should be adjusted to between 4.8 to 5.2 by the addition of 0.1N NaOH for culture maintenance prior to addition of the test chemical. Nutrient media should not contain EDTA, other chelating agents, or organic metabolites such as sucrose.

(v) *Carriers.* Stock solutions of substances of low aqueous solubility may be prepared by use of organic solvents, emulsifiers or dispersants of low phytotoxicity to plants. When a solvent or carrier is used, a second set of controls should contain the same concentration of the solvent or carrier as that used in the highest concentration of the test substance. The concentration of the solvent or carrier should not exceed 0.1 ml/1.

(4) *Test parameters.* Environmental conditions should be maintained as specified below:

(i) Temperature at 25 ± 2 °C.

(ii) The pH of the nutrient medium between 4.8 and 5.2. Test solution pH may vary from the nutrient medium after the addition of the test chemical and/or carrier (if used). Any such changes should be recorded but not adjusted.

(iii) Continuous irradiation of 400 ± 50 uEinsteins/m^2 sec measured at the test solution or nutrient medium surface.

(e) *Reporting.* Reporting requirements of Part 792—Good Laboratory Practice Standards of this chapter apply to this guideline. The following data should also be reported.

(1) Source of *Lemna* and taxonomic verification.

(2) Description of test chambers, type of lights, size of beakers or flasks used, number of concentrations and replicates per concentration, number of colonies per replicate, solution volumes, physical parameters of growth chambers (e.g., humidity, temperature, and light intensity).

(3) The pH and concentration of the test chemical in the test solutions prior to use and discarding on day 3, 6 and 7.

(4) Number of fronds per test concentration and control at the end of the test, the percent inhibition and/or stimulation of growth rate, and percent frond mortality for each test concentration compared to controls.

(5) If the range-finding test showed that the highest concentration of the chemical tested (not less than 1000 mg/l had no effect on *Lemna*, report the results and measured concentrations and a statement that the chemical is not phytotoxic at concentrations less than 1,000 mg/l.

(6) If the range-finding test showed greater than a 50 percent effect with a test concentration below the analytical detection limit, report the results and a statement that the chemical is phytotoxic below the analytical detection limit.

(7) Means and standard deviations for frond number, growth rate, and percent frond mortality in each test concentration. In addition, concentration response curve(s) with 95 percent confidence limits delineated, goodness-of-fit determination, and EC_{10}'s, EC_{50}'s, and EC_{90}'s, identified.

(8) Methods and data records from chemical and numerical analyses including validation methods and quality assurance procedures.

§ 797.1300 Daphnid acute toxicity test.

(a) *Purpose.* This guideline is intended for use in developing data on the acute toxicity of chemical substances and mixtures ("chemicals") subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94–469, 90 Stat. 2003, 15 U.S.C. 2601 *et seq.*). This guideline prescribes an acute toxicity test in which daphnids (*Daphnia magna* or *D. pulex*) are exposed to a chemical in static and flow-through systems. The United States Environmental Protection Agency will use data from this test in assessing the hazard a chemical may present in the acquatic environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA) and Part 792—Good Laboratory Practice Standards of this chapter apply to this test guideline. In addition, the following definitions apply to this guideline:

(1) "Brood stock" means the animals which are cultured to produce test organisms through reproduction.

(2) "EC_{50}" means that experimentally derived concentration of test substance in dilution water that is calculated to affect 50 percent of a test population during continuous exposure over a specified period of time. In this guideline, the effect measured is immobilization.

(3) "Ephippium" means a resting egg which develops under the carapace in response to stress conditions in daphnids.

(4) "Flow-through" means a continuous or an intermittent passage of test solution or dilution water through a test chamber or culture tank with no recycling.

(5) "Immobilization" means the lack of movement by the test organisms except for minor activity of the appendages.

(6) "Loading" means the ratio of daphnid biomass (grams, wet weight) to the volume (liters) of test solution in a test chamber at a point in time, or passing through the test chamber during a specific interval.

(7) "Static system" means a test system in which the test solution and test organisms are placed in the test chamber and kept there for the duration of the test without renewal of the test solution.

(c) *Test procedures*—(1) *Summary of the test.* (i) Test chambers are filled with appropriate volumes of dilution water. In the flow-through test, the flow of dilution water through each chamber is adjusted to the rate desired. The test chemical is introduced into each treatment chamber. The addition of test chemical in the flow-through system is conducted at a rate which is sufficient to establish and maintain the desired concentration in the test chamber. The test is started within 30 minutes after the test chemical has been added and uniformly distributed in static test chambers or after the concentration of test chemical in each flow-through test chamber reaches the prescribed level and remains stable. At the initiation of the test, daphnids which have been cultured and acclimated in accordance with the test design are randomly placed into the test chambers. Daphnids in the test chambers are observed periodically during the test, the immobile daphnids removed, and the findings recorded.

(ii) Dissolved oxygen concentration, pH, temperature, the concentration of test chemical and other water quality parameters are measured at specified intervals in selected test chambers. Data are collected during the test to develop concentration-response curves and determine EC_{50} values for the test chemical.

(2) [Reserved]

(3) *Range-finding test.* (i) A range-finding test should be conducted to

establish test solution concentrations for the definitive test.

(ii) The daphnids should be exposed to a series of widely spaced concentrations of the test chemical (e.g., 1, 10, 100 mg/1, etc.), usually under static conditions.

(iii) A minimum of five daphnids should be exposed to each concentration of test chemical for a period of 48 hours. The exposure period may be shortened if data suitable for the purpose of the range-finding test can be obtained in less time. No replicates are required and nominal concentrations of the chemical are acceptable.

(4) *Definitive test.* (i) The purpose of the definitive test is to determine the concentration-response curves and the 24- and 48-hour EC_{50} values with the minimum amount of testing beyond the range-finding test.

(ii) A minimum of 20 daphnids per concentration should be exposed to five or more concentrations of the chemical chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 2, 4, 8, 16, 32, and 64 mg/l). An equal number of daphnids should be placed in two or more replicates. If solvents, solubilizing agents or emulsifiers have to be used, they should be commonly used carriers and should not possess a synergistic or antagonistic effect on the toxicity of the test chemical. The concentration of solvent should not exceed 0.1 ml/l. The concentration ranges should be selected to detemine the concentration-response curves and EC_{50} values at 24 and 48 hours. Concentration of test chemical in test solutions should be analyzed prior to use.

(iii) Every test should include controls consisting of the same dilution water, conditions, procedures and daphnids from the same population (culture container), except that none of the chemical is added.

(iv) The dissolved oxygen concentration, temperature and pH should be measured at the beginning of the test and at 24 and 48 hours in each chamber.

(v) The test duration is 48 hours. The test is unacceptable if more than 10 percent of the control organisms appear to be immobilized, stressed or diseased during the 48-hour test period. Each test chamber should be checked for immobilized daphnids at 3, 6, 12, 24, and 48 hours after the beginning of the test. Concentration-response curves and 24-hour and 48-hour EC_{50} values for immobilization should be determined along with their 95 percent confidence limits.

(vi) In addition to immobility, any abnormal behavior or appearance should also be reported.

(vii) Distribution of daphnids among test chambers should be randomized. In addition, test chambers within the testing area should be positioned in a random manner or in a way in which appropriate statistical analyses can be used to determine the variation due to placement.

(viii) The concentration of dissolved test chemical (that which passes through a 0.45 micron filter) in the chambers should be measured as often as is feasible during the test. In the static test the concentration of test chemical should be measured, at a minimum, at the beginning of the test and at the end of the test in each test chamber. In the flow-through test the concentration of test chemical should be measured at a minimum: (A) in each chamber at the beginning of the test and at 24 and 48 hours after the start of the test; (B) in at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system. Among replicate test chambers of a treatment concentration, the measured concentration of the test chemical should not vary more than 20 percent (+ or −).

(5) [Reserved]

(6) *Analytical measurements*—(i) *Test chemical.* Deionized water should be used in making stock solutions of the test chemical. Standard analytical methods should be used whenever available in performing the analyses. The analytical method used to measure the amount of test chemical in a sample should be validated before beginning the test by appropriate laboratory practices. An analytical method is not acceptable if likely degradation products of the test chemical, such as hydrolysis and oxidation products, give positive or negative interferences which cannot be systematically identified and corrected mathematically.

(ii) *Numerical.* The number of immobilized daphnids should be counted during each definitive test. Appropriate statistical analyses should provide a goodness-or-fit determination for the concentration-response curves. A 24- and 48-hour EC_{50} and corresponding 95 percent interval should be calculated.

(d) *Test conditions*—*(1) Test species*—(i) *Selection.* (A) The cladocerans, *Daphnia magna* or *D. pulex*, are the test species to be used in this test. Either species may be used for testing of a particular chemical. The species identity of the test organisms should be verified using appropriate systematic keys. First instar daphnids, <24 hours old, are to be used to start the test.

(B) Daphnids to be used in acute toxicity tests should be cultured at the test facility. Records should be kept regarding the source of the initial stock and culturing techniques. All organisms used for a particular test should have originated from the same source and be from the same population (culture container).

(C) Daphnids should not be used for a test (*1*) if cultures contain ephippia; (*2*) if adults in the cultures do not produce young before day 12; (*3*) if more than 20 percent of the culture stock die during the 2 days preceding the test; (*4*) if adults in the culture do not produce an average of at least 3 young per adult per day over the 7-day period prior to the test and (*5*) if daphnids have been used in any portion of a previous test, either in a treatment or in a control.

(ii) *Acclimation.* (A) Daphnids should be maintained in 100 percent dilution water at the test temperature for at least 48 hours prior to the start of the test. This is easily accomplished by culturing them in the dilution water at the test temperature. Daphnids should be fed prior to the test.

(B) During culturing and acclimation to the dilution water, daphnids should be maintained in facilities with background colors and light intensities similar to those of the testing area.

(iii) *Care and handling.* (A) Daphnids should be cultured in dilution water under similar environmental conditions to those used in the test. Organisms should be handled as little as possible. When handling is necessary it should be done as gently, carefully and quickly as possible. During culturing and acclimation, daphnids should be observed carefully for ephippia and other signs of stress, physical damage and mortality. Dead and abnormal individuals should be discarded. Organisms that touch dry surfaces or are dropped or injured in handling should be discarded.

(B) Smooth glass tubes (I.D. greater than 5 mm) equipped with rubber bulb should be used for transferring daphnids with minimal culture media carry-over. Care should be exercised to introduce the daphnids below the surface of any solution to avoid trapping air under the carapace.

(iv) *Feeding.* A variety of foods (e.g., unicellular green algae) have been demonstrated to be adequate for daphnid culture. Daphnids should not be fed during testing.

(2) *Facilities*—(i) *Apparatus.* (A) Facilities needed to perform this test include: (*1*) Containers for culturing and acclimating daphnids; (*2*) a mechanism for controlling and maintaining the water temperature during the culturing, acclimation, and test periods; (*3*)

apparatus for straining particulate matter, removing gas bubbles, or aerating the water as necessary; and (*4*) an apparatus for providing a 16-hour light and 8-hour dark photoperiod with a 15 to 30 minute transition period. In addition, the flow-through system should contain appropriate test chambers in which to expose daphnids to the test chemical and an appropriate test substance delivery system.

(B) Facilities should be well ventilated and free of fumes and disturbances that may affect the test organisms.

(C) Test chambers should be loosely covered to reduce the loss of test solution or dilution water due to evaporation and to minimize the entry of dust or other particulates into the solutions.

(ii) *Construction materials.* (A) Materials and equipment that contact test solutions should be chosen to minimize sorption of test chemicals from the dilution water and should not contain substances that can be leached into aqueous solution in quantities that can affect the test results.

(B) For static tests, daphnids can be conveniently exposed to the test chemical in 250 ml beakers or other suitable containers.

(C) For flow-through tests, daphnids can be exposed in glass or stainless steel containers with stainless steel or nylon screen bottoms. The containers should be suspended in the test chamber in such a manner to insure that the test solution flows regularly into and out of the container and that the daphnids are always submerged in at least 5 centimeters of test solution. Test chambers can be constructed using 250 ml beakers or other suitable containers equipped with screened overflow holes, standpipes or V-shaped notches.

(iii) *Dilution water.* (A) Surface or ground water, reconstituted water or dechlorinated tap water are acceptable as dilution water if daphnids will survive in it for the duration of the culturing, acclimation and testing periods without showing signs of stress. The quality of the dilution water should be constant and should meet the following specifications:

Substance	Maximum concentration
Particulate matter	20 mg/liter.
Total organic carbon or	2 mg/liter.
Chemical oxygen demand	5 mg/liter.
Un-ionized ammonia	1 µg/liter.
Residual chlorine	<3 µg/liter.
Total organophosphorus pesticides	50 ng/liter.
Total organochlorine pesticides plus polychlorinated biphenyls (PCBs) or	50 ng/liter.
Organic chlorine	25 ng/liter.

(B) The above water quality parameters under paragraph (d)(2)(iii)(A) of this section should be measured at least twice a year or whenever it is suspected that these characteristics may have changed significantly. If dechlorinated tap water is used, daily chlorine analysis should be performed.

(C) If the diluent water is from a ground or surface water source, conductivity and total organic carbon (TOC) or chemical oxygen demand (COD) should be measured. Reconstituted water can be made by adding specific amounts of reagent-grade chemicals to deionized or distilled water. Glass distilled or carbon-filtered deionized water with a conductivity less than 1 µohm/cm is acceptable as the diluent for making reconstituted water.

(iv) *Cleaning.* All test equipment and test chambers should be cleaned before each test using standard laboratory procedures.

(v) *Test substance delivery system.* In flow-through tests, proportional diluters, metering pump systems, or other suitable devices should be used to deliver test chemical to the test chambers. The system should be calibrated before each test. Calibration includes determining the flow rate through each chamber and the concentration of the test chemical in each chamber. The general operation of the test substance delivery system should be checked twice daily during a test. The 24-hour flow-through a test chamber should be equal to at least 5 times the volume of the test chamber. During a test, the flow rates should not vary more than 10 percent from any one test chamber to another or from one time to any other.

(3) *Test parameters.* Environmental parameters of the water contained in test chambers should be maintained as specified below:

(i) Temperature of 20 ± 1 °C.

(ii) Dissolved oxygen concentration between 60 and 105 percent saturation. Aeration, if needed to achieve this level, should be done before the addition of the test chemical. All treatment and control chambers should be given the same aeration treatment.

(iii) The number of daphnids placed in a test chamber should not affect test results. Loading should not exceed 40 daphnids per liter test solution in the static system. In the flow-through test, loading limits will vary depending on the flow rate of dilution water. Loading should not cause the dissolved oxygen concentration to fall below the recommended levels.

(iv) Photoperiod of 16 hour light and 8-hours darkness, with a 15 to 30 minute transition period.

(e) *Reporting.* The sponsor should submit to the U.S. EPA all data developed by the test that are suggestive or predictive of acute toxicity and all concomitant gross toxicological manifestations. In addition to the reporting requirements prescribed in Part 792—Good Laboratory Practice Standards of this chapter, the reporting of test data should include the following:

(1) The name of the test, sponsor, testing laboratory, study director, principal investigator and dates of testing.

(2) A detailed description of the test chemical including its source, lot number, composition (identity and concentration or major ingredients and major impurities), known physical and chemical properties and any carriers or other additives used and their concentrations.

(3) The source of the dilution water, its chemical characteristics (e.g., conductivity, hardness, pH, etc.) and a description of any pretreatment.

(4) Detailed information about the daphnids used as brood stock, including the scientific name and method of verification, age, source, treatments, feeding history, acclimation procedures, and culture method. The age (in hours) of the daphnids used in the test is also reported.

(5) A description of the test chambers, the volume of solution in the chambers, the way the test was begun (e.g., conditioning, test chemical additions), the number of test organisms per test chamber, the number of replicates per treatment, the lighting, the method of test chemical introduction or the test substance delivery system and the flow rate (in flow-through test) expressed as volume additions per 24 hours.

(6) The concentration of the test chemical in each test chamber at times designated for static and flow-through tests.

(7) The number and percentage of organisms that were immobilized or showed any adverse effects in each test chamber at each observation period.

(8) Utilizing the average measured test chemical concentration, concentration-response curves should be fitted to immobilization data at 24 and 48 hours. A statistical test of goodness-of-fit should be performed and the results reported.

(9) The 24- and 48-hour EC_{50} values and their respective 95 percent confidence limits using the mean measured test chemical concentration and the methods used to calculate both

the EC_{50} values and their confidence limits.

(10) All chemical analyses of water quality and test chemical concentrations, including methods, method validations and reagent blanks.

(11) The data records of the culture, acclimation and test temperatures.

(12) Any deviation from this test guideline and anything unusual about the test, e.g., diluter failure, temperature fluctuations, etc.

§ 797.1330 Daphnid chronic toxicity test.

(a) *Purpose.* This guideline is intended for use in developing data on the chronic toxicity of chemical substances and mixtures ("chemicals") subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94–469, 90 Stat. 2003, 15 U.S.C. 2601 *et seq.*). This guideline prescribes a chronic toxicity test in which daphnids are exposed to a chemical in a renewal or a flow-through system. The United States Environmental Protection Agency will use data from this test in assessing the hazard a chemical may present to the aquatic environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA), and the definitions in Part 792 Good Laboratory Practice Standards of this chapter apply to this test guideline. In addition, the following definitions apply to this guideline:

(1) "Brood stock" means the animals which are cultured to produce test organisms through reproduction.

(2) "Chronic toxicity test" means a method used to determine the concentration of a substance in water that produces an adverse effect on a test organism over an extended period of time. In this test guideline, mortality and reproduction (and optionally, growth) are the criteria of toxicity.

(3) "EC_{50}" means that experimentally derived concentration of test substance in dilution water that is calculated to affect 50 percent of a test population during continuous exposure over a specified period of time. In this guideline, the effect measured is immobilization.

(4) "Ephippium" means a resting egg which develops under the carapace in response to stress conditions in daphnids.

(5) "Flow-through" means a continuous or intermittent passage of test solution or dilution water through a test chamber or culture tank with no recycling.

(6) "Immobilization" means the lack of movement by daphnids except for minor activity of the appendages.

(7) "Loading" means the ratio of daphnid biomass (grams, wet weight) to the volume (liters) of test solution in a test chamber at a point in time or passing through the test chamber during a specific interval.

(8) "MATC (Maximum Acceptable Toxicant Concentration)" means the maximum concentration at which a chemical can be present and not be toxic to the test organism.

(9) "Renewal system" means the technique in which test organisms are periodically transferred to fresh test solution of the same composition.

(c) *Test procedures*—(1) *Summary of the test.* (i) Test chambers are filled with appropriate volumes of dilution water. In the flow-through test the flow of dilution water through each chamber is then adjusted to the rate desired. The test substance is introduced into each test chamber. The addition of test substance in the flow-through system is done at a rate which is sufficient to establish and maintain the desired concentration of test substance in the test chamber.

(ii) The test is started within 30 minutes after the test substance has been added and uniformly distributed in the test chambers in the renewal test or after the concentration of test substance in each test chamber of the flow-through test system reaches the prescribed level and remains stable. At the initiation of the test, daphnids which have been cultured or acclimated in accordance with the test design, are randomly placed into the test chambers. Daphnids in the test chambers are observed periodically during the test, immobile adults and offspring produced are counted and removed, and the findings are recorded. Dissolved oxygen concentration, pH, temperature, the concentration of test substance, and other water quality parameters are measured at specified intervals in selected test chambers. Data are collected during the test to determine any significant differences ($p \leqslant 0.05$) in immobilization and reproduction as compared to the control.

(2) [Reserved]

(3) *Range-finding test.* (i) A range-finding test should be conducted to establish test solution concentrations for the definitive test.

(ii) The daphnids should be exposed to a series of widely spaced concentrations of the test substance (e.g., 1, 10, 100 mg/l), usually under static conditions.

(iii) A minimum of five daphnids should be exposed to each concentration of test substance for a period of time which allows estimation of appropriate chronic test concentrations. No replicates are required and nominal concentrations of the chemical are acceptable.

(4) *Definitive test.* (i) The purpose of the definitive test is to determine concentration-response curves, EC_{50} values and effects of a chemical on immobilization and reproduction during chronic exposure.

(ii) A minimum of 20 daphnids per concentration should be exposed to five or more concentrations of the chemical chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 2, 4, 8, 16, 32, 64 mg/l). An equal number of daphnids should be placed in two or more replicates. The concentration ranges should be selected to determine the concentration-response curves, EC_{50} values and MATC. Solutions should be analyzed for chemical concentration prior to use and at designated times during the test.

(iii) Every test should include controls consisting of the same dilution water, conditions, procedures and daphnids from the same population (culture container), except that none of the chemical is added.

(iv) The test duration is 21 days. The test is unacceptable if:

(A) More than 20 percent of the control organisms appear to be immobilized, stressed or diseased during the test.

(B) Each control daphnid living the full 21 days produces an average of less than 60 young.

(C) Any ephippia are produced by control animals.

(v) The number of immobilized daphnids in each chamber should be recorded on days 7, 14, and 21 of the test. After offspring are produced, they should be removed from the test chambers every 2 or 3 days. Counts of the cumulative number of offspring per adult (number of young divided by the number of adults in each chamber) and the cumulative number of immobilized offspring per adult should be recorded on days 14 and 21 of the test. Concentration-response curves, EC_{50} values and associated 95 percent confidence limits for adult immobilization should be determined for days 7, 14; and 21. A MATC should be determined for the most sensitive test criteria measured (number of adult animals immobilized, number of young per adult and number of immobilized young per adult).

(vi) In addition to immobility, any abnormal behavior or appearance should also be reported.

(vii) Distribution of daphnids among the test chambers should be randomized. In addition, test chambers within the testing area should be positioned in a random manner or in a way in which appropriate statistical analyses can be used to determine the variation due to placement.

(5) [Reserved]

(6) *Analytical measurements*—(i) *Test chemical.* Deionized water should be used in making stock solutions of the test substance. Standard analytical methods should be used whenever available in performing the analyses. The analytical method used to measure the amount of test substance in a sample should be validated before beginning the test by appropriate laboratory practices. An analytical method is not acceptable if likely degradation products of the test substance, such as hydrolysis and oxidation products, give positive or negative interferences which cannot be systematically identified and corrected mathematically.

(ii) *Numerical.* The number of immobilized adults, total offspring per adult and immobilized offspring per adult should be counted during each definitive test. Appropriate statistical analyses should provide a goodness-of-fit determination for the adult immobilization concentration-response curves calculated on days 7, 14, and 21. A 7-, 14-, and 21-day EC_{50}, based on adult immobilization and corresponding 95 percent confidence intervals, should be calculated. Appropriate statistical tests (e.g., analysis of variance, mean separation test) should be used to test for significant chemical effects on chronic test criteria (cumulative number of immobilized adults, cumulative number of offspring per adult and cumulative number of immobilized offspring per adult) on days 7, 14, and 21. An MATC should be calculated using these chronic test criteria.

(d) *Test conditions*—(1) *Test species*—(i) *Selection.* (A) The cladocerans, *Daphnia magna* or *D. pulex*, are the species to be used in this test. Either species can be utilized for testing of a particular chemical. The species identity of the test organisms should be verified using appropriate systematic keys.

(B) First instar daphnids, <24 hours old, are to be used to start the test.

(ii) *Acquisition.* (A) Daphnids to be used in chronic toxicity tests should be cultured at the test facility. Records should be kept regarding the source of the initial stock and culturing techniques. All organisms used for a particular test should have originated from the same population (culture container).

(B) Daphnids should not be used for a test if:

(*1*) Cultures contain ephippia.

(*2*) Adults in the cultures do not produce young before day 12.

(*3*) More than 20 percent of the culture stock die in the 2 days preceding the test.

(*4*) Adults in the culture do not produce an average of at least 3 young per adult per day over the 7-day period prior to the test.

(*5*) Daphnids have been used in any portion of a previous test either in a treatment or in a control.

(iii) *Feeding.* (A) During the test the daphnids should be fed the same diet and with the same frequency as that used for culturing and acclimation. All treatments and control(s) should receive, as near as reasonably possible, the same ration of food on a per-animal basis.

(B) The food concentration depends on the type used. Food concentrations should be sufficient to support normal growth and development and to allow for asexual (parthenogenic) reproduction. For automatic feeding devices, a suggested rate is 5 to 7 mg food (either solids or algal cells, dry weight) per liter dilution water or test solution. For manual once-a-day feeding, a suggested rate is 15 mg food (dry weight) per liter dilution water or test solution.

(iv) *Loading.* The number of test organisms placed in a test chamber should not affect test results. Loading should not exceed 40 daphnids per liter in the renewal system. In the flow-through test, loading limits will vary depending on the flow rate of the dilution water. Loading should not cause the dissolved oxygen concentration to fall below the recommended level.

(v) *Care and handling of test organisms.* (A) Daphnids should be cultured in dilution water under similar environmental conditions to those used in the test. A variety of foods have been demonstrated to be adequate for daphnid culture. They include algae, yeasts and a variety of mixtures.

(B) Organisms should be handled as little as possible. When handling is necessary it should be done as gently, carefully and quickly as possible. During culturing and acclimation, daphnids should be observed carefully for ephippia and other signs of stress, physical damage and mortality. Dead and abnormal individuals should be discarded. Organisms that touch dry surfaces or are dropped or injured during handling should be discarded.

(C) Smooth glass tubes (I.D. greater than 5mm) equipped with a rubber bulb can be used for transferring daphnids with minimal culture media carry-over.

(D) Care should be exercised to introduce the daphnids below the surface of any solution so as not to trap air under the carapace.

(vi) *Acclimation.* (A) Daphnids should be maintained in 100 percent dilution water at the test temperature for at least 48 hours prior to the start of the test. This is easily accomplished by culturing them in the dilution water at the test temperature. Daphnids should be fed the same food during the test as is used for culturing and acclimation.

(B) During culturing and acclimation to the dilution water, daphnids should be maintained in facilities with background colors and light intensities similar to those of the testing area.

(2) *Facilities*—(i) *General.* (A) Facilities needed to perform this test include:

(*1*) Containers for culturing and acclimating daphnids.

(*2*) A mechanism for controlling and maintaining the water temperature during the culturing, acclimation and test periods.

(*3*) Apparatus for straining particulate matter, removing gas bubbles, or aerating the water when water supplies contain particulate matter, gas bubbles, or insufficient dissolved oxygen, respectively.

(*4*) An apparatus for providing a 16-hour light and 8-hour dark photoperiod with a 15- to 30-minute transition period.

(*5*) An apparatus to introduce food if continuous or intermittent feeding is used.

(*6*) In addition, the flow-through test should contain appropriate test chambers in which to expose daphnids to the test substance and an appropriate test substance delivery system.

(B) Facilities should be well ventilated and free of fumes and other disturbances that may affect the test organisms.

(ii) *Test chambers.* (A) Materials and equipment that contact test solutions should be chosen to minimize sorption of test chemicals from the dilution water and should not contain substances that can be leached into aqueous solution in quantities that can affect test results.

(B) For renewal tests, daphnids can be conveniently exposed to the test solution in 250 ml beakers or other suitable containers.

(C) For flow-through tests daphnids can be exposed in glass or stainless steel containers with stainless steel or nylon screen bottoms. Such containers should be suspended in the test chamber in such a manner to ensure that the test solution flows regularly into and out of the container and that the daphnids are always submerged in at least 5 centimeters of test solution. Test chambers can be constructed using 250 ml beakers or other suitable containers equipped with screened overflow holes, standpipes or V-shaped notches.

(D) Test chambers should be loosely covered to reduce the loss of test solution or dilution water due to evaporation and to minimize the entry of dust or other particulates into the solutions.

(iii) *Test substance delivery system.* (A) In the flow-through test, proportional diluters, metering pump systems or other suitable systems should be used to deliver the test substance to the test chambers.

(B) The test substance delivery system used should be calibrated before and after each test. Calibration includes determining the flow rate through each chamber and the concentration of the test substance in each chamber. The general operation of the test substance delivery system should be checked twice daily during a test. The 24-hour flow rate through a test chamber should be equal to at least five times the volume of the test chamber. During a test, the flow rates should not vary more than 10 percent from any one test chamber to another or from one time to any other. For the renewal test, test substance dilution water should be completely replaced at least once every three days.

(iv) *Dilution water.* (A) Surface or ground water, reconstituted water, or dechlorinated tap water are acceptable as dilution water if daphnids will survive in it for the duration of the culturing, acclimation, and testing periods without showing signs of stress. The quality of the dilution water should be constant and should meet the following specificiations:

Substance	Maximum concentration
Particulate matter	20 mg/l.
Total organic carbon or	2 mg/l.
Chemical oxygen demand	5 mg/l.
Un-ionized ammonia	20 µg/l.
Residual chlorine	<3 µg/l.
Total organophosphorus pesticides	50 ng/l.
Total organochlorine pesticides plus polychlorinated biphenyls (PCBs).	50 ng/l.
or organic chlorine	25 ng/l.

(B) The water quality characteristics listed above should be measured at least twice a year or when it is suspected that these characteristics may have changed significantly. If dechlorinated tap water is used, daily chlorine analysis should be performed.

(C) If the diluent water is from a ground or surface water source, conductivity and total organic carbon (TOC) or chemical oxygen demand (COD) should be measured. Reconstituted water can be made by adding specific amounts of reagent-grade chemicals to deionized or distilled water. Glass distilled or carbon filtered deionized water with a conductivity of less than 1 microohm/cm is acceptable as the diluent for making reconstituted water.

(D) If the test substance is not soluble in water an appropriate carrier should be used.

(v) *Cleaning of test system.* All test equipment and test chambers should be cleaned before each test following standard laboratory procedures. Cleaning of test chambers may be necessary during the testing period.

(3) *Test parameters.* (i) Environmental conditions of the water contained in test chambers should be maintained as specified in this paragraph:

(A) Temperature of 20 ± 1 °C.

(B) Dissolved oxygen concentration between 60 and 105 percent saturation. Aeration, if needed to achieve this level, should be done before the addition of the test substance. All treatment and control chambers should be given the same aeration treatment.

(C) Photoperiod of 16-hours light and 8-hours darkness, with a 15 to 30 minute transition period.

(ii) Additional measurements include:

(A) The concentration of dissolved test substance (that which passes through a 0.45 micron filter) in the chambers should be measured during the test.

(B) At a minimum, the concentration of test substance should be measured as follows:

(*1*) In each chamber before the test.

(*2*) In each chamber on days 7, 14, and 21 of the test.

(*3*) In at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system. Among replicate test chambers of a treatment concentration, the measured concentration of the test substance should not vary more than 20 percent.

(C) The dissolved oxygen concentration, temperature and pH should be measured at the beginning of the test and on days 7, 14, and 21 in each chamber.

(e) *Reporting.* The sponsor should submit to the U.S. Environmental Protection Agency all data developed by the test that are suggestive or predictive of chronic toxicity and all associated toxicologic manifestations. In addition to the reporting requirements prescribed in the Part 792—Good Laboratory Practice Standards of this chapter the reporting of test data should include the following:

(1) The name of the test, sponsor, testing laboratory, study director, principal investigator, and dates of testing.

(2) A detailed description of the test substance including its source, lot number, composition (identity and concentration of major ingredients and major impurities), known physical and chemical properties, and any carriers or other additives used and their concentrations.

(3) The source of the dilution water, its chemical characteristics (e.g., conductivity, hardness, pH), and a description of any pretreatment.

(4) Detailed information about the daphnids used as brood stock, including the scientific name and method of verification, age, source, treatments, feeding history, acclimation procedures, and culture methods. The age (in hours) of the daphnids used in the test should be reported.

(5) A description of the test chambers, the volume of solution in the chambers, the way the test was begun (e.g., conditioning, test substance additions), the number of test organisms per test chamber, the number of replicates per treatment, the lighting, the renewal process and schedule for the renewal chronic test, the test substance delivery system and flow rate expressed as volume additions per 24 hours for the flow-through chronic test, and the method of feeding (manual or continuous) and type of food.

(6) The concentration of the test substance in test chambers at times designated for renewal and flow-through tests.

(7) The number and percentage of organisms that show any adverse effect in each test chamber at each observation period.

(8) The cumulative adult and offspring immobilization values and the progeny produced at designated observation times, the time (days) to first brood and the number of offspring per adult in the control replicates and in each treatment replicate.

(9) All chemical analyses of water quality and test substance concentrations, including methods, method validations and reagent blanks.

(10) The data records of the culture, acclimation, and test temperatures.

(11) Any deviation from this test guideline, and anything unusual about the test, (e.g., dilution failure, temperature fluctuations).

(12) The MATC to be reported is calculated as the geometric mean between the lowest measured test substance concentration that had a significant ($p \leq 0.05$) effect and the highest measured test substance concentration that had no significant

(p□0.05) effect on day 7, 14, or 21 of the test. The most sensitive of the test criteria (number of adult animals immobilized, the number of young per female and the number of immobilized young per female) is used to calculate the MATC. The criterion selected for MATC computation is the one which exhibits an effect (a statistically significant difference between treatment and control groups; $p \leq 0.05$) at the lowest test substance concentration for the shortest period of exposure. Appropriate statistical tests (analysis of variance, mean separation test) should be used to test for significant test substance effects. The statistical tests employed and the results of these tests should be reported.

(13) Concentration-response curves utilizing the average measured test substance concentration should be fitted to cumulative adult immobilization data at 7, 14, and 21 days. A statistical test of goodness-of-fit should be performed and the results reported.

(14) An EC_{50} value based on adult immobilization with corresponding 95 percent confidence limits when sufficient data are present for days 7, 14, and 21. These calculations should be made using the average measured concentration of the test substance.

§ 797.1350 Daphnid chronic toxicity test.

(a) *Purpose.* The proposed *Daphnia* chronic toxicity test standard is designed to assess the effects of test substances on the survival and reproduction of Daphnia as a representative freshwater invertebrate. The duration of the test permits the daphnids to be exposed to a chemical from shortly after birth until well into adulthood. The organisms are exposed long enough to allow the adults to produce several broods of progeny. Initiating exposure shortly after birth allows an assessment of the possible effects of the test chemical on such processes as reproduction, maturation, fecundity and growth.

(b) *Definitions.* The following definitions apply to this standard:

(1) "Acute lethal toxicity" is the lethal effect produced on an organism within a short period of time of exposure to a chemical.

(2) "Confidence limits" are the limits within which, at some specified level of probability, the true value of a result lies.

(3) "LC_{50}" is the median lethal concentration, i.e., that concentration of a chemical in air or water killing 50 percent of a test batch of organisms within a particular period of exposure (which shall be stated).

(4) "Reference substance" is a chemical used to access the constancy of response of a given species of test organisms to that chemical, usually by use of the acute LC_{50}. (It is assumed that any change in sensitivity to the reference substance will indicate the existence of some similar change in degree of sensitivity to other chemicals whose toxicity is to be determined.)

(5) "Static test" is a toxicity test with aquatic organisms in which no flow of test solution occurs. Solutions may remain unchanged throughout the duration of the test.

(6) "Renewal test" is a test without continuous flow of solution, but with occasional renewal of test solutions after prolonged periods, e.g., 24 hours.

(7) "Flow-through test" is a toxicity test in which water is renewed continuously in the test chambers, the test chemical being transported with the water used to renew the test medium.

(8) "Time-response curve" is the curve relating cumulative percentage response of a test batch of organisms, exposed to a single dose or single concentration of a chemical, to a period of exposure.

(9) "Toxicity curve" is the curve produced from toxicity tests when LC_{50} values are plotted against duration of exposure. (This term is also used in aquatic toxicology, but in a less precise sense, to describe the curve produced when the median period of survival is plotted against test concentrations).

(10) "Units" all concentrations are given in weight per volume (e.g., in mg/liter).

(c) *Test procedures*—(1) *Summary of the test.* (i) Test chambers are filled with appropriate volumes of dilution water. In the flow-through test the flow of dilution water through each chamber is then adjusted to the rate desired. The test substance is introduced into each test chamber. The addition of test substance in the flow-through system is done at a rate which is sufficient to establish and maintain the desired concentration of test substance in the test chamber.

(ii) For the renewal test, the test is started within 30 minutes after the test substance has been added and uniformly distributed in the test chambers. In the flow-through test the test begins after the concentration of test substance in each test chamber of the flow-through test system reaches the prescribed level and remains stable. At the initiation of the test, daphnids which have been cultured or acclimated in accordance with the test design, are randomly placed into the test chambers. Daphnids in the test chambers are observed periodically during the test, immobile adults and offspring produced are counted and removed, and the findings are recorded. Dissolved oxygen concentration, pH, temperature, the concentration of test substance, and other water quality parameters are measured at specified intervals in selected test chambers. Data are collected during the test to determine any significant difference ($p \leq 0.05$) in immobilization and reproduction as compared to the control.

(2) [Reserved]

(3) *Range-finding test*—(i) *General.* A range-finding test should be conducted to establish test solution concentrations for the definitive test.

(ii) *Introductory information for range-finding test.* (A) *Prerequisites:*

(*1*) Water solubility.

(*2*) Vapor pressure.

(B) *Guidance information:*

(*1*) Structural formula.

(*2*) Purity of the substance.

(*3*) Methods of analysis for the quantification of the substance in water.

(*4*) Chemical stability in water and light.

(*5*) *n*-octanol/water partition coefficient.

(*6*) Results of a test on biodegradability.

(C) *Qualifying statement.* For chemicals with low solubility under test conditions, it may not be possible to quantitatively determine the EC_{50}.

(iii) *Methods for range-finding test.* (A) *Definitions and units:*

(*1*) *24 hour EC_{50}.* The concentration (based upon nominal concentration) calculated to have immobilized 50 percent of the daphnids by 24 hours exposure. (If another definition is used, this shall be reported, together with its reference.)

(*2*) *Immobilization.* Those animals not able to swim for 15 seconds after gentle agitation of the test container are considered to be immobile. (If another definition is used, this shall be reported, together with its reference.)

(B) *Reference substances.* In the course of the acute immobilization phase a reference substance may occasionally be tested for EC_{50} with the test compound as a means of assuring that the laboratory test conditions are adequate and have not changed significantly. An example of such a useful reference substance is $K_2CR_2O_7$.

(C) *Conditions for the validity of the range-finding test:*

(*1*) The mortality in the controls should not exceed 10 percent at the end of the test.

(*2*) The oxygen concentration at the end of the test shall be >70 percent of the air saturation value at the temperature used.

(*3*) Test Daphnia should not have been trapped at the surface of the water, at least in the control.

(*4*) If conducted, the results with the reference compound should be within the normal range for the laboratory conducting the test.

(*5*) If the EC$_{50}$ is not calculable due to an inadequate number of intermediate response levels, it is acceptable to merely report the highest concentration causing complete immobility, provided that the concentration factor between doses was <1.8.

(D) *Performance of the range-finding test:*

(*1*) Equipment which will come into contact with the test solutions should be glass. This glassware should be cleaned with solvents known to remove previously tested chemicals.

(*2*) Any water, either reconstituted or natural water, can be used, provided that it will sustain growth and reproduction of *Daphnia* without signs of stress.

(*3*) At least 20 animals should be used at each test concentration, preferably divided into four batches of five animals each.

(*4*) At least 2 ml of test solution should be provided for each animal.

(*5*) The test temperature should be between 18 and 22 °C, and for each test it should be constant within ±0.5 °C.

(*6*) A light-dark cycle is optional.

(*7*) The concentrations should be formulated in a geometric series, preferably without using any solvents. If solvents, solubilizing agents, emulsifiers, etc., have to be used, they should be commonly used adjuvants and not be toxic in themselves at the levels used. Neither should they have a synergistic or antagonistic effect on the toxicity of the substance tested. In no case should the concentration of an organic solvent exceed 0.1 ml/l.

(*8*) The test solution should be prepared before introduction of the daphnids.

(*9*) The test solutions should not be aerated.

(*10*) The daphnids shall not be fed during the test.

(*11*) The highest concentration to be tested should not exceed 1.0 g/l.

(*12*) Concentrations sufficient to lead to zero and 100 percent immobilization and the 24 hour EC$_{50}$ should be tested together with a control.

(*13*) The pH and the oxygen concentration of the blank and all the test concentrations should be measured at the beginning and the end of the test. The pH of the test solutions should not be modified.

(*14*) Volatile compounds should be tested in completely filled, closed containers, large enough to prevent lack of oxygen.

(4) *Definitive test.* (i) *General.* The results of the range-finding test are used to determine, with judgment, the concentration levels to be used in the definitive test. It is suggested that this reproduction test be carried out using a geometrical concentration series of at least 5 concentrations with an interval of at least 10, starting at approximately the 24 hour EC$_{50}$ concentration and ending at $\frac{1}{100}$ of the 24 hour EC$_{50}$. If necessary, lower concentrations are to be tested.

(ii) *Introductory information for the definitive test.* (A) *Prerequisities:*

(*1*) Water solubility.

(*2*) Vapor pressure.

(*3*) Chemical stability in water and light.

(*4*) Results of a test on biodegradability.

(*5*) 24 hour EC$_{50}$ or the highest concentration producing no immobility and the lowest concentration causing complete immobility.

(B) *Guidance information:*

(*1*) Structural formula.

(*2*) Purity of the substance.

(*3*) *n*-octanol/water partition coefficient.

(C) *Recommendations.* (*1*) Instead of a 2 week test in which 3 batches of young should be born per female, a test of 3 or 4 weeks may be preferred in order to obtain a more thorough judgment of the influence of the test substance on mortality and reproduction. In this period approximately six to nine batches of young should be born per female.

(*2*) It is recommended that a statistical test (such as an analysis of variance) be used to determine whether the test replications can be analyzed together.

(iii) *Criteria for a valid definitive test.* (A) Control mortality should not exceed 20 percent at the end of the test.

(B) The oxygen concentration shall have been >70 percent of the air saturation value throughout the test.

(C) The pH for the controls and for at least the most concentrated solutions shall be known throughout the test. The deviation from the initial value at the beginning of the test should be <0.3 units.

(D) The first young should have been born in the controls after a maximum of 9 days.

(E) The average cumulative number of young per female in the controls after three broods, should be >20 at a temperature of 20° ± 0.5°C.

(iv) *Definitive test procedures.* (A) At least 40 animals should be used at each test concentration, preferably divided into 4 batches of 10 animals each. The test concentrations are made up in a geometric series, and if possible, without any solvents.

(B) Every test shall include controls consisting of the same dilution water, conditions, procedures and daphnids from the same population (culture container), except that none of the chemical is added.

(C) The minimum duration of the test is 14 days, in which at least three broods of the F1 generation shall have appeared in the controls. If this is not the case, the test shall be continued until the third brood in the control is complete. If desired, the test can be continued for a total period of 3 to 4 weeks, even if three broods are born within 3 weeks.

(D) The live and dead daphnids of the "parental" generation (P) are counted and the dead specimens removed. This should preferably be carried out daily, but at least every 2 days, e.g., Monday, Wednesday and Friday.

(E) The presence of eggs in the brood pouch, males or epphipia shall be recorded. The condition and size of the parent generation should be visually compared with the controls.

(F) When the parental animals are about 7 days old, the first young daphnids emerge from the brood pouch. After this, a new batch appears every 2 to 3 days. These batches are called "broods" of the F1 generation.

(G) The newborn young of the F1 generation are counted at least every 2 days (Monday, Wednesday, and Friday) and their estimated condition (based on visual examination) is recorded. After counting and examination, the young are poured away. The presence of eggs from which no young have emerged (on the bottom of the test vessel) is recorded.

(5) [Reserved]

(6) *Analytical measurements.* (i) Deionized water should be used in making stock solutions of the test substance. Standard analytical methods should be used whenever available in performing the analyses. The analytical method used to measure the amount of test substance in a sample shall be validated before beginning the test by appropriate laboratory practices. An analytical method should not be used if likely degradation products of the test substance, such as hydrolysis and oxidation products, give positive or negative interferences which cannot be systematically identified and corrected mathematically.

(ii) Samples of the test substance should be taken at the beginning and during the test. The actual concentration shall not drop below 80 percent of the nominal concentration. Aeration of the test solutions is permissible, unless this would cause the actual concentration of the test substance to drop below 80 percent of the nominal concentration.

(iii) The oxygen concentration in all test solutions shall be checked once every 48 hours (if desired, every Monday, Wednesday and Friday).

(iv) The pH of the controls and of at least the most concentrated solution shall be checked before and after each renewal in the renewal test and once every 48 hours in a flow-through test.

(d) *Test conditions*—(1) *Test species*—(i) *Selection. Daphnia magna* less than 24 hours old at the beginning of the test, laboratory bred, free from known diseases and with a known history (breeding method, pretreatment) are used in this test. Other *Daphnia* species may be used provided that the relevant re-production parameters are comparable to those of *Daphnia magna.*

(ii) *Feeding.* The daphnids should be fed at least daily during the definitive test. In the chronic daphnid test, food (in any quantity) of any kind that meets the criteria of reproduction for validity of the test, is acceptable. Overloading of the test system with food should be avoided in order to minimize sorption of the test substance. Log-phase, unicellular green algae are generally suitable.

(2) *Facilities*—(i) *Apparatus.* (A) Normal laboratory apparatus and equipment should be used. Equipment which will come into contact with the test solutions should preferably be all glass. This glassware should be cleaned with solvents known to remove previously tested chemicals.

(B) This reproduction test should not be carried out in a static test system; either a renewal or flow-through system shall be used. The renewal period should be guided by the chemical analysis and (if applicable) the oxygen level in the test solution. The solutions shall be renewed at least once every 48 hours (if desired, on Monday, Wednesday, and Friday).

(C) Volatile compounds should be tested in completely filled closed containers, large enough to prevent the oxygen concentrations from falling below 70 percent of the saturation value. An almost-closed, flow-through system may also be used. When more than 20 percent of the test compound would be lost through volatility, the test should be carried out either in a flow-through system or in an enclosed container of sufficient size to ensure that the oxygen level does not fall below 70 percent of the saturation value.

(ii) *Cleaning.* If the renewal scheme is used, the glassware shall be emptied and food residues removed at renewal. It is recommended that the glassware be rinsed with deionized water and kept as a coded series for the following renewal. Each test unit therefore has two vessels which are used alternately. If flow-through systems are used, these should be cleaned twice a week.

(iii) *Dilution water.* Surface or ground water, reconstituted water, or dechlorinated tap water is acceptable as dilution water if daphnids will survive and reproduce in it for the duration of the culturing, acclimation, and testing periods without showing signs of stress. The quality of the dilution water should be constant and should meet the following specifications:

Substance	Maximum concentration
Particulate matter	20 mg/l.
Total organic carbon or	2 mg/l.
chemical oxygen demand	5 mg/l.
Un-ionized ammonia	20 μg/l.
Residual chlorine	1 μg/l.
Total organophosphorus pesticides.	50 ng/l.
Total organochlorine pesticides plus polychlorinated biphenyls (PCBs).	50 ng/l.
or organic chlorine	25 ng/l.

(3) *Test parameters*—(i) *Carriers.* If solvents, solubilizing agents, emulsifiers, etc., have to be used, they should be commonly used adjuvants and should not themselves be toxic at the concentrations used. They should also not interact to alter the toxicity of the substance under test. In no case should the concentration of an organic solvent exceed 0.1 ml/l.

(ii) *Dissolved oxygen.* The oxygen concentration shall be >70 percent of the air saturation value throughout the test.

(iii) *Lighting.* A light-dark cycle is necessary for the definitive test; 8 hours darkness and 16 hours light are recommended.

(iv) *Loading.* At least 40 ml of test solution should be provided for each animal in the definitive test.

(v) *Temperature.* The test temperature should be between 18 and 22 °C, but for each test it should be constant, within ±0.5 °C.

(e) *Reporting*—(i) *Test substance information:*

(A) Chemical designation.

(B) Additional designations, e.g., trade name.

(C) Empirical formula.

(D) Manufacturer.

(E) Batch number.

(F) Degree of purity.

(G) Date of sampling.

(H) Water solubility.

(I) Vapor pressure.

(J) Biodegradability.

(K) Chemical stability in water and daylight.

(L) *n*-octanol/water partition coefficient.

(ii) *General information:*

(A) Source of Daphnia, any pretreatment, breeding method (including source, kind and amount of food, feeding frequency), species identification and method of verification.

(B) Name and address of the testing laboratory, name of the person responsible for carrying out the test (study director).

(C) Name and address of sponsor.

(D) Dates of testing.

(E) Description of the test method or reference to the method used.

(iii) *Conditions of testing:*

(A) Carriers and/or additives used and their concentrations. If it is observed that the stability or homogeneity of the test substance cannot be maintained, then care should be taken in the interpretation of the results and note made that these may not be reproducible.

(B) Dilution water: source and chemical and physical characteristics including at least hardness, pH, Ca/Mg ratio, Na/K ratio, alkalinity.

(C) Test temperature.

(D) Light quality, intensity and periodicity.

(E) All measurements of pH and oxygen level made during the test, preferably in tabular form.

(F) Results and date of test performed with reference compound if available.

(G) Description of test vessels: volume of solution, number of test organisms per vessel, number of test vessels per concentration, conditioning of the test vessels, the introduction of the test substance in the dilution water.

(H) In case of renewal, the renewal procedure and scheme. In case of flow-through, the test substance delivery system, the flowrate, periodicity of cleaning and technique used.

(I) If measured, the actual concentrations of the test substance and the dates of measurement.

(J) Number and percentage of daphnids that showed any adverse effect in the controls and in each treatment at each observation period and a description of the nature of the effects observed, e.g., immobilization, mortality in tabular form.

(K) Description or reference to statistical procedures applied.

(L) Any other effects differentiating organisms in tests and controls.

(iv) *Specific range-finding and definitive test information:* (A) For the 24 hour EC_{50} (acute immobilization) phase also report:.

(1) The 24 hour EC_{50} preferably with 95 percent confidence limits, either by computation or graphically, and the method applied. The probit method is recommended.

(2) If possible, the slope of the concentration response curve with its 95 percent confidence limits.

(3) The highest tested concentration producing no immobile daphnids.

(4) The lowest tested concentration producing 100 percent immobile daphnids.

(5) Any other effect observed and the concentration at which it occurred.

(B) For the reproduction phase also report:

(1) The EC_{50} (immobilization) and LC_{50} values as far as possible at 24 hours, 48 hours, 96 hours, 7 days, 14 days and at the end of the test, preferably with 95 percent confidence limits, either by computation or graphically, and the method applied. For the determination, a probit method should be used.

(2) The length of time for the appearance of the first brood for each concentration.

(3) The number of young alive in each test vessel on given days when counts were made (the minimum requirement is for counts at 48 hour intervals on Mondays, Wednesdays, and Fridays).

(4) The number of dead young in each test vessel on given days when counts were made.

(5) Source, kind and amount of food; feeding frequency.

(6) If the recommended concentration scheme was followed and no effects on reproduction are detected, then the results may be reported as being greater than the highest concentration tested.

(7) For each of the observed effects a statistical analysis of the homogeneity of replicate results for each concentration should be made. If homogeneity is found, it should be determined through an appropriate statistical analysis, whether a significant difference exists between the control and the test concentrations.

(8) The highest concentration tested at which no significant difference is found compared to the controls with respect to mortality, reproduction and other observed effects.

(9) The lowest concentration tested with significant difference compared to the controls.

(10) Any other parameter can be reported at the option of the study director.

§ 797.1400 Fish acute toxicity test.

(a) *Purpose.* This guideline may be used to develop data on the acute toxicity of chemical substances and mixtures ("chemicals") subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94–469, 90 Stat. 2003, 15 U.S.C. 2601 *et seq.*). This guideline prescribes tests to be used to develop data on the acute toxicity of chemicals to fish. The United States Environmental Protection Agency (EPA) will use data from these tests in assessing the hazard of a chemical to the environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA), and the definitions in Part 792—Good Laboratory Practice Standards of this chapter apply to this test guideline. The following definitions also apply to this guideline:

(1) "Acclimation" means the physiological compensation by test organisms to new environmental conditions (e.g., temperature, hardness, pH).

(2) "Acute toxicity test" means a method used to determine the concentration of a substance that produces a toxic effect on a specified percentage of test organisms in a short period of time (e.g., 96 hours). In this guideline, death is used as the measure of toxicity.

(3) "Carrier" means a solvent used to dissolve a test substance prior to delivery to the test chamber.

(4) "Conditioning" means the exposure of construction materials, test chambers, and testing apparatus to dilution water or to test solutions prior to the start of a test in order to minimize the sorption of the test substance onto the test facilities or the leaching of substances from the test facilities into the dilution water or test solution.

(5) "Death" means the lack of opercular movement by a test fish.

(6) "Flow-through" means a continuous or an intermittent passage of test solution or dilution water through a test chamber, or a holding or acclimation tank with no recycling.

(7) "Incipient LC_{50}" means that test substance concentration, calculated from experimentally-derived mortality data, that is lethal to 50 percent of a test population when exposure to the test substance is continued until the mean increase in mortality does not exceed 10 percent in any concentration over a 24-hour period.

(8) "LC_{50}" means that test substance concentration, calculated from experimentally-derived mortality data, that is lethal to 50 percent of a test population during continuous exposure over a specified period of time.

(9) "Loading" means the ratio of fish biomass (grams, wet weight) to the volume (liters) of test solution in a test chamber or passing through it in a 24 hour period.

(10) "Static" means the test solution is not renewed during the period of the test.

(11) "Test solution" means the test substance and the dilution water in which the test substance is dissolved or suspended.

(c) *Test procedures*—(1) *Summary of the test.* (i) Test chambers are filled with appropriate volumes of dilution water. If a flow-through test is performed, the flow of dilution water through each chamber is adjusted to the rate desired.

(ii) The test substance is introduced into each test chamber. In a flow-through test, the amount of test substance which is added to the dilution water is adjusted to establish and maintain the desired concentration of test substance in each test chamber.

(iii) Test fish which have been acclimated in accordance with the test design are introduced into the test and control chambers by stratified random assignment.

(iv) Fish in the test and control chambers are observed periodically during the test; dead fish are removed at least twice each day and the findings are recorded.

(v) The dissolved oxygen concentration, pH, temperature and the concentration of test substance are measured at intervals in selected test chambers.

(vi) Concentration-response curves and LC_{50} values for the test substance are developed from the mortality data collected during the test.

(2) [Reserved]

(3) *Range finding test.* If the toxicity of the test substance is not already known, a range finding test should be performed to determine the range of concentrations to be used in the definitive test. The highest concentration of test substance for use in the range finding test should not exceed its solubility in water or the permissible amount of the carrier used.

(4) *Definitive test.* (i) A minimum of 20 fish should be exposed to each of five or more test substance concentrations. The range of concentrations to which the fish are exposed should be such that in 96 hours there are at least two partial mortality exposures bracketing 50 percent survival.

(ii) For exposure to each concentration of a test substance, an equal number of test fish should be placed in two or more replicate test chambers. The distribution of individual fish among the test chambers should be randomized.

(iii) Every test should include a control consisting of the same dilution water, conditions, procedures, and fish from the same group used in the test, except that none of the test substance is added.

(iv) Mortality data collected during the test are used to calculate a 96-hour LC_{50}. The 24-, 48-, and 72-hour values should be calculated whenever there is sufficient mortality data to determine such values. If the 96-hour LC_{50} is less than 50 percent of the estimated 48-hour LC_{50} in a flow-through test, the test should be continued until the mean increase in mortality at any test concentration does not exceed 10 percent over a 24-hour period or until 14 days.

(v) Test fish should not be fed while they are being exposed to the test substance under static conditions or during the first 96 hours of flow-through testing. If the test continues past 96 hours, the fish should be fed a suitable food at a maintenance level every other day beginning on test day 5. Any excess food and the fecal material should be removed when observed.

(5) *Test results.* (i) Death is the primary criterion used in this test guideline to evaluate the toxicity of the test substance.

(ii) In addition to death, any abnormal behavior such as, but not limited to, erratic swimming, loss of reflex, increased excitability, lethargy, or any changes in appearance or physiology such as discoloration, excessive mucous production, hyperventilation, opaque eyes, curved spine, or hemorrhaging should be recorded.

(iii) Observations on compound solubility should be recorded. The investigator should report the appearance of surface slicks, precipitates, or material adhering to the sides of the test chamber.

(iv) Each test and control chamber should be checked for dead fish and observations recorded at 24, 48, 72, and 96 hours after the beginning of the test or within one hour of the designated times. If the test is continued past 96 hours, additional observations should be made every 24 hours until termination.

(v) The mortality data is used to calculate LC_{50}'s and their 95 percent confidence limits, and to plot concentration-response curves for each time interval whenever sufficient data exists. The methods recommended for use in calculating LC_{50}'s include probit, logit, binomial, and moving average angle.

(vi) A test is unacceptable if more than 10 percent of the control fish die or exhibit abnormal behavior during a 96-hour test. If a flow-through test is continued past 96 hours, the maximum allowable additional mortality is 10 percent.

(6) *Analytical measurements*—(i) *Water quality analysis.* (A) The hardness, acidity, alkalinity, pH, conductivity, TOC or COD, and particulate matter of the dilution water should be measured at the beginning of each static test and at the beginning and end of each flow-through test. The month to month variation of the above values should be less than 10 percent and the pH should vary less than 0.4 units.

(B) During static tests, the dissolved oxygen concentration, temperature and pH should be measured in each test chamber at the beginning of the test and as often as needed thereafter to document changes from the initial levels. The test solution volume should not be reduced by more than 10 percent as a result of these measurements.

(C) During flow-through tests, dissolved oxygen, temperature and pH measurements should be made in each chamber at the beginning of the test and every 48 hours thereafter until the end of the test.

(ii) *Collection of samples for measurement of test substance.* Test solution samples to be analyzed for the test substance should be taken midway between the top, bottom, and sides of the test chamber. These samples should not include any surface scum or material dislodged from the bottom or sides. Samples should be analyzed immediately or handled and stored in a manner which minimizes loss of test substance through microbial degradation, photodegradation, chemical reaction, volatilization, or sorption.

(iii) *Measurement of test substance.* (A) For static tests, the concentration of dissolved test substance (that which passes through a 0.45 micron filter) should be measured at a minimum in each test chamber at the beginning (0-hour, before fish are added) and at the end of the test. During flow-through tests, the concentration of dissolved test substance should be measured as follows:

(*1*) In each chamber at 0-hour.

(*2*) In each chamber at 96-hours and every 4 days thereafter, as long as the test is continued.

(*3*) In at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system.

(B) Filters and their holders used for determining the dissolved test substance concentrations should be prewashed with several volumes of distilled water and undergo a final rinse with test solution. Glass or stainless steel filter holders are best for organic test substances, while plastic holders are best for metals. The sample should be filtered within 30 minutes after it is taken from the test chamber.

(C) The analytical methods used to measure the amount of test substance in a sample should be validated before beginning the test. The accuracy of a method should be verified by a method such as using known additions. This involves adding a known amount of the test substance to three water samples taken from a chamber containing dilution water and the same number and species of fish as are used in the test. The nominal concentration of the test substance in those samples should span the concentration range to be used in the test. Validation of the analytical method should be performed on at least two separate days prior to starting the test.

(D) An analytical method is not acceptable if likely degradation products of the test substance give positive or negative interferences, unless it is shown that such degradation products are not present in the test chambers during the test.

(E) In addition to analyzing samples of test solution, at least one reagent blank, containing all reagents used, should also be analyzed.

(F) If the measured concentrations of dissolved test substances are considerably lower (e.g., <50 percent) than the nominal concentrations, the total test substance concentration should be measured in the highest test concentration.

(G) Among replicate test chambers, the measured concentrations should not vary more than 20 percent. The measured concentration of the test substance in any chamber during the test should not vary more than 30 percent from the measured concentration prior to initiation of the test.

(H) The mean measured concentration of dissolved test substance should be used to calculate all LC_{50}'s and to plot all concentration-response curves.

(d) *Test conditions*—(1) *Test species.* (i) *Selection.* The test species for this test are the rainbow trout (*Salmo gairdneri*), bluegill (*Lepomis macrochirus*) and fathead minnow (*Pimephales promelas*). The particular species of fish to be used will be prescribed in the test rule.

(ii) *Age and condition of fish.* (A) Juvenile fish should be used. Fish used in a particular test should be the same age and be of normal size and appearance for their age. The longest fish should not be more than twice the length of the shortest.

(B) All newly acquired fish should be quarantined and observed for at least 14 days prior to use in a test.

(C) Fish should not be used for a test if they appear stressed or if more than five percent die during the 48 hours immediately prior to the test.

(iii) *Acclimation of test fish.* (A) If the holding water is not from the same source as the test dilution water, acclimation to the dilution water should be done gradually over a 48-hour period. The fish should then be held an additional 14 days in the dilution water prior to testing. Any changes in water temperature should not exceed 3 °C per day. Fish should be held for a minimum of 7 days at the test temperature prior to testing.

(B) During the final 48-hours of acclimation, fish should be maintained in facilities with background colors and light intensities similar to those of the testing area and should not be fed.

(2) *Facilities*—(i) *General.* Facilities needed to perform this test include:

(A) Flow-through tanks for holding and acclimating fish.

(B) A mechanism for controlling and maintaining the water temperature during the holding, acclimation and test periods.

(C) Apparatus for straining particulate matter, removing gas bubbles, or insufficient dissolved oxygen, respectively.

(D) Apparatus for providing a 16-hour light and 8-hour dark photoperiod with a 15- to 30-minute transition period.

(E) Chambers for exposing test fish to the test substance.

(F) A test substance delivery system for flow-through tests.

(ii) *Construction materials.* Construction materials and commercially purchased equipment that may contact the stock solution, test solution, or dilution water should not contain substances that can be leached or dissolved into aqueous solutions in quantities that can alter the test results. Materials and equipment that contact stock or test solutions should be chosen to minimize sorption of test chemicals. Glass, stainless steel, and perfluorocarbon plastic should be used whenever possible. Concrete, fiberglass, or plastic (e.g., PVC) may be used for holding tanks, acclimation tanks, and water supply systems, but they should be used to remove rust particles. Rubber, copper, brass, galvanized metal, epoxy glues, and lead should not come in contact with the dilution water, stock solution, or test solution.

(iii) *Test substance delivery system.* In flow-through tests, diluters, metering pump systems or other suitable devices should be used to deliver the test substance to the test chambers. The system used should be calibrated before each test. Calibration includes determining the flow rate through each chamber and the concentration of the test substance delivered to each chamber. The general operation of the test substance delivery system should be checked twice daily during a test. The 24-hour flow rate through a test chamber. During a test, the flow rates should not vary more than 10 percent from one test chamber to another or from one time to any other.

(iv) *Test chambers.* Test chambers made of stainless steel should be welded, not soldered. Test chambers made of glass should be fused or bonded using clear silicone adhesive. As little adhesive as possible should be left exposed in the interior of the chamber.

(v) *Cleaning of test system.* Test substance delivery systems and test chambers should be cleaned before each test. They should be washed with detergent and then rinsed in sequence with clean water, pesticide-free acetone, clean water, and 5 percent nitric acid, followed by two or more changes of dilution water.

(vi) *Dilution water.* (A) Clean surface or ground water reconstituted water, or dechlorinated tap water is acceptable as dilution water if the test fish will survive in it for the duration of the holding, acclimating, and testing periods without showing signs of stress, such as discoloration, hemorrhaging, disorientation or other unusual behavior. The quality of the dilution water should be constant and should meet the following specifications measured at least twice a year:

Substance	Maximum
Particulate matter	20 mg/liter.
Total organic carbon or	2 mg/liter.
chemical oxygen demand	5 mg/liter.
Un-ionized ammonia	1 µg/liter.
Residual chlorine	1 µg/liter.
Total organochloring pesticides	50 µg/liter.
Total organocholorine pesticides plus polychlorinated biphenyls (PCBs).	50 µg/liter.
or organic chlorine	25 µg/liter.

(B) The concentration of dissolved oxygen in the dilution water should be between 90 and 100 percent saturation; 9.8 to 10.9 mg/l for tests with trout, and 8.0 to 8.9 mg/l for tests with bluegill or fathead minnow at sea level. If necessary, the dilution water can be aerated before the addition of the test substance. All reconstituted water should be aerated before use. Buffered soft water should be aerated before but not after the addition of buffers.

(C) If disease organisms are present in the dilution water in sufficient numbers to cause infection, they should be killed or removed by suitable equipment.

(D) Glass distilled or carbon filtered deionized water with a conductivity less than 1 micromho/cm is acceptable for use in making reconstituted water. If the reconstituted water is prepared from a ground or surface water source, conductivity, and total organic carbon (TOC) or chemical oxygen demand (COD) should be measured on each batch.

(vii) *Carriers.* (A) Distilled water should be used in making stock solutions of the test substance. If the stock volume however is more than 10 percent of the test solution volume, dilution water should be used. If a carrier is absolutely necessary to dissolve the test substance, the volume used should not exceed the minimum volume necessary to dissolve or suspend the test substance in the test solution. If the test substance is a mixture, formulation, or commercial product, none of the ingredients is considered a carrier unless an extra amount is used to prepare the stock solution.

(B) Triethylene glycol and dimethyl formamide are the preferred carriers, but acetone can also be used. The concentration of triethylene glycol in the test solution should not exceed 80 mg/l. The concentration of dimethyl formamide or acetone in the test solution should not exceed 5/0 mg/l.

(3) *Test parameters*—(i) *Loading.* The number of fish placed in a test chamber should not be so great as to affect the results of the test. The loading should not be so great that the test substance concentrations are decreased by more than 20 percent due to uptake by the fish. In static tests, loading should not exceed 0.5 grams of fish per liter of solution in the test chamber at any one time. In flow-through tests loading should not exceed 0.5 grams of fish per liter of test solution passing through the chamber in 24 hours. These loading rates should be sufficient to maintain the dissolved oxygen concentration above the recommended levels and the ammonia concentration below 20 µg/l.

(ii) *Dissolved oxygen concentration.* (A) During static tests with rainbow trout the dissolved oxygen in each test chamber should be greater than 5.5 mg/1. In tests with bluegill and fathead minnows, the DO should be maintained above 4.5 mg/l.

(B) During flow-through tests the dissolved oxygen concentration should be maintained above 8.2 mg/1 in tests with trout and above 6.6 mg/l in tests with bluegills or fathead minnows.

(iii) *Temperature.* The test temperature should be 22 ± 1 °C for bluegill and fathead minnows, and 12±1 °C for rainbow trout. The temperature should be measured at least hourly in one test chamber.

(iv) *Light.* A 16-hour light and 8-hour dark photoperiod with a 15- to 30-minute transition period should be maintained.

(e) *Reporting.* The sponsor should submit to the EPA all data developed by the test that are suggestive or predictive of toxicity. In addition to the reporting requirements prescribed in Part 792—Good Laboratory Practice Standards of this chapter, the reported test data should include the following:

(1) The source of the dilution water, a description of any pretreatment, and the measured hardness, acidity, alkalinity, pH, conductivity, TOC or COD and particulate matter.

(2) A description of the test chambers, the depth and volume of solution in the chamber, the specific way the test was begun (e.g., conditioning, test substance additions), and for flow-through tests, a description of the test substance delivery system.

(3) Detailed information about the test fish, including the scientific name and method of verification, average weight (grams, wet weight), standard length, age, source, history, observed diseases, treatments, and mortalities, acclimation procedures, and food used.

(4) The number of replicates used, the number of organisms per replicate, the loading rate, and the flow rate for flow-through tests.

(5) The measured DO, pH and temperature and the lighting regime.

(6) The solvent used, the test substance concentration in the stock solution, the highest solvent concentration in the test solution and a description of the solubility determinations in water and solvents if used.

(7) The concentration of the test substance in each test chamber just before the start of the test and at all subsequent sampling periods.

(8) The number of dead and live tests organisms, the percentage of organisms that died, and the number that showed any abnormal effects in the control and in each test chamber at each observation period.

(9) The 96-hour LC_{50}, and when sufficient data have been generated, the 24-, 48-, 72-, and incipient LC_{50} values, their 95 percent confidence limits, and the methods used to calculate the LC_{50} values and their confidence limits.

(10) When observed, the observed no effect concentration (the highest concentration tested at which there were no mortalities or abnormal behavioral or physiological effects).

(11) The concentration-response curve at each observation period for which a LC_{50} was calculated.

(12) Methods and data records of all chemical analyses of water quality parameters and test substance concentrations, including method validations and reagent blanks.

§ 797.1440 Fish Acute Toxicity Test.

(a) *Purpose.* This guideline will be used in developing data on the acute toxicity of chemical substances and mixtures ("chemicals") to fish subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94–469, 90 Stat. 2003, 15 U.S.C. 2601 *et seq.*). The United States Environmental Protection Agency (EPA) will use data from these tests in assessing the hazard of a chemical to the environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA) and in Part 792—Good Laboratory Practice Standards of this chapter apply to this test guideline. The following definitions also apply:

(1) "Acute toxicity" is the discernible adverse effects induced in an organism within a short period of time (days) of exposure to a chemical. For aquatic animals this usually refers to continuous exposure to the chemical in water for a period of up to four days. The effects (lethal or sub-lethal) occurring may usually be observed within the period of exposure with aquatic organisms.

(2) "Acute lethal toxicity" is the lethal effect produced on an organism within a short period of time of exposure to a chemical.

(3) "Confidence limits" are the limits within which, at some specified level of probability, the true value of a result lies.

(4) "LC_{50}" is the median lethal concentration, i.e., that concentration of a chemical in air or water killing 50 percent of a test batch of organisms within a particular period of exposure (which should be stated).

(5) "Static test" is a toxicity test with aquatic organisms in which no flow of test solution occurs. (Solutions may remain unchanged throughout the duration of the test).

(6) "Semi-static test" is a test without flow of solution, but with occasional batchwise renewal of test solutions after prolonged periods (e.g., 24 hours).

(7) "Flow-through test" is a toxicity test in which water is renewed constantly in the test chambers, the chemical under test being transported with the water used to renew the test medium.

(8) "Time-response curve" is the curve relating cumulative percentage response of a test batch of organisms, exposed to a single dose or single concentration of a chemical, to a period of exposure.

(9) "Toxicity curve" is the curve produced from toxicity tests when LC_{50} values are plotted against duration of exposure. (This term is also used in aquatic toxicology, but in a less precise sense, to describe the curve produced when the median period of survival is plotted against test concentrations.)

(10) "Units" all concentrations are given in weight per volume (e.g., mg/liter).

(c) *Test procedures*—(1) *Summary of the test.* (i) The aqueous solubility and the vapor pressure of the test chemical should be known prior to testing. The structural formula of the test chemical, its purity, stability in water and light, *n*-octanol/water partition coeffecient, and pK_a value should be known. The results of a biodegradability test and the method of analysis for the quantification of the chemical in water should also be known.

(ii) The fish are exposed to a range of test substance concentrations preferably for a period of up to 96 hours. Mortalities are recorded at 24, 48, 72 and 96 hours and the concentrations which kill 50 percent of the fish (LC_{50}) are determined where possible.

(iii) The maximum concentration tested producing no mortality and the minimum concentration tested producing total mortality should be recorded.

(iv) For chemicals with limited solubility under the test conditions, it may not be possible to determine an LC_{50}.

(2) [Reserved].

(3) *Range-finding test.* It may be necessary to perform a range-finding test prior to a definitive test. It provides information about the range of concentrations to be used in the definitive test.

(4) *Definitive test.* (i) Fish should be exposed to at least five concentrations spaced by a constant factor not exceeding 1.8. A control and solvent control, when appropriate should also be tested.

(ii) Stock solutions of the required strength are prepared by dissolving the appropriate amount of the test substance in the required volume of dilution water. The pH value of the stock solution should be adjusted to the pH value of the dilution water unless there are specific reasons not to do so. The test should be carried out without adjustment of pH if there is evidence of marked change in the pH of the solution, and it is advised that the test be repeated with pH adjustment and the results reported. This pH adjustment should be made in such a way that the stock solution concentration is not changed to any significant extent and that no chemical reaction or physical precipitation of the test compound is caused. NCl or NaOH should be used to adjust the pH.

(iii) Stock solutions of substances of low aqueous solubility may be prepared by ultrasonic dispersion or, if necessary, by use of organic solvents, emulsifiers or dispersants of low toxicity to fish. When such auxiliary substances are used, the control fish should be exposed to the same concentration of the auxiliary substance as that used in the highest concentration of the test substance. The concentration of such auxiliaries should not exceed 0.1 ml/l.

(iv) The chosen test concentrations are prepared by dilution of the stock solution.

(v) For test to be valid, the following criteria apply:

(A) If it is observed that the stability or homogeneity of the test substance cannot be maintained, then care should be taken in the interpretation of the results and a note made that these results may not be reproducible.

(B) The mortality in the controls should not exceed 10 percent at the end of the test.

(C) The dissolved oxygen concentration should have been >60 percent of air saturation throughout the test.

(D) There should be evidence that the concentration of the substance being tested has been satisfactorily maintained (e.g., within 80 percent of the nominal concentration) over the test period.

(5) *Test results.* (i) The fish are inspected after 24, 48, 72, and 96 hours. Fish are considered dead if touching of the caudal peduncle produces no reaction. Dead fish are removed when observed, and mortalities are recorded. Observations after the first 3 hours and 6 hours are desirable.

(ii) Records are kept of visible abnormalities (e.g., loss of equilibrium, swimming behavior, respiratory function, pigmentation, etc.).

(iii) The cumulative percentage mortality for each recommended exposure period should be plotted against concentration on logarithmic-probability paper. A line is then fitted by eye to these points and the concentration corresponding to the 50 percent response point is read off. This is the LC_{50} for the appropriate exposure period. Median lethal concentrations also can be calculated using standard procedures given in any of the references cited in paragraph (f) of this section. Confidence limits (p=0.95) for the calculated LC_{50} values can be determined using the standard procedures. The LC_{50} value should be rounded off to two significant figures.

(iv) Where the data obtained are inadequate for the use of standard methods of calculating the LC_{50} (because most of the results are for either no deaths or total mortality, where a dilution ratio of 1.8 has been used) then the highest concentration causing no deaths and the lowest concentration producing 100 percent deaths should be used to determine the LC_{50} (this being taken as being the geometric mean of these two concentrations).

(6) [Reserved].

(d) *Test conditions*—(1) *Test species*—(i) *Selection.* (A) One of several species may be used, the selection being at the discretion of the testing laboratory. It is suggested that the species used be selected on the basis of such important practical criteria as: their ready availability throughout the year, their ease of maintenance, their convenience for testing, and any economic, biological or ecological factors which have bearing. The fish should be in good health and free from any apparent malformation. If other species fulfilling the above criteria are used, the test method should be adapted in such a way as to provide suitable test conditions.

(B) Examples of fish recommended for testing and their size are given in the following Table 1:

TABLE 1—RECOMMENDED SPECIES FOR ACUTE TESTING

Recommended species	Recommended total length (centimeter)
Brachydanio rerio (Teleostei, Cyprinidae) (Hamilton-Buchanan) Zebra-fish	2.0±1.0
Pimephales promelas (Teleostei, Cyprinidae) Fathead minnow	2.0±1.0
Cyprinus carpio (Teleostei, Cyprinidae) (Linne 1758) Common carp	3.0±1.0
Oryzias latipes (Teleostei, Poeciliidae) (Schlegel 1850) Red killifish	2.0±1.0
Poecilia reticulata (Teleostei, Poeciliidae) (Peters 1859) Guppy	2.0±1.0
Lepomis macrochirus (Teleostei, Centrarchidae) (Linnaeus 1758) Bluegill	2.0±1.0
Salmo gairdneri (Teleostei, Salmonidae) (Richardson 1836) Rainbow trout	5.0±1.0

(ii) *Collection or acquisition.* The fish mentioned above are easy to rear or are widely available throughout the year. They are capable of being bred and cultivated either in fish farms or in the laboratory under disease- and parasite-controlled conditions so that the test animal will be healthy and of known parentage.

(iii) *Holding and acclimation.* (A) Fish should be held for at least 12 to 15 days before testing. All fish should be maintained in water of the quality to be used in the test for at least 7 days before they are used.

(B) Coldwater fish should be held in tanks containing at least 300 l of water while warmwater fish should be held in tanks containing at least 100 l.

(C) The temperature of the holding water should be the same as that used for testing. The dissolved oxygen concentrations should be maintained above 80 percent of the air saturation value. A 12 to 16 hour photoperiod should be used.

(D) All fish should be fed three times per week or daily until 24 hours before the test is started.

(E) A batch of fish is acceptable for testing if the percentage mortality over the 7-day period prior to testing is less than five. If the mortality is between 5 and 10 percent acclimation should continue for seven 7 days. If the mortality is greater than 10 percent, the entire batch of fish should be rejected.

(2) *Test facilities*—(i) *Apparatus.* An oxygen meter, equipment for determination of water hardness, adequate apparatus for temperature control, test tanks made of chemically inert materials and other normal laboratory equipment are needed.

(ii) *Dilution water.* (A) Drinking water (dechlorinated if necessary), good quality natural water, or reconstituted water, with a total hardness of between 50 and 250 mg/l (as $CaCO)_3$ and with a pH of 6.0–8.5 are preferred.

(B) Reconstituted water should be prepared from deionized water or distilled water with a conductivity <10 Scm^{-1} One hundred liters of reconstituted water can be prepared by adding 2.5 l of the following solutions to a tank and bringing the solution to volume with deionized water:

11.76g $CACl_2 \cdot 2H_2O$/l
4.93g $MgSO_4 \cdot 7H_2O$/l
2.59g $NaHCO_3$/l
2.59g KCl/l

The sum of the calcium and magnesium ions in this solution is 2.5 mmol/l. The proportion of Ca:Mg-ions is 4.13 and of Na:K-ions is 10:1. The acid capacity of this solution is 0.8 mmol/l.

(C) The dilution water should be aerated until oxygen saturation is achieved and then stored for about 2 days without further aeration before use.

(3) *Test parameters.* (i) Constant conditions should be maintained as far as possible throughout the test and, if necessary, semi-static or flow-through procedures should be used.

(ii) The preparation and storage of the test material, the holding of the fish, and all operations and tests should be carried out in an environment free from harmful concentrations of dust, vapors, and gases and in such a way as to avoid cross-contamination. Any disturbances that may change the behavior of the fish should be avoided.

(iii) The following parameters are important:

(A) *Dissolved oxygen.* The dissolved oxygen concentrations should be at least 60 percent of the air saturation value.

(B) *Light.* A 12 to 16 hour photoperiod should be used.

(C) *Loading.* A maximum loading of 1.0 g/l for static and semi-static tests is recommended: for flow-through systems a higher loading can be acceptable.

(D) *Temperature.* Test temperatures of 15 ± 2 °C for rainbow trout and 22 ± 2 °C for carp are recommended. The other recommended species should be tested at 23 ± 2 °C. The temperature should be maintained within ±1 °C of the selected test temperature throughout the test period.

(E) *Feeding.* The fish should not be fed during the test.

(e) *Reporting.* (1) The sponsor should submit to the EPA all data developed by the test that are suggestive or predictive of toxicity.

(2) In addition to the reporting requirements prescribed in Part 792—Good Laboratory Practice Standards of this chapter the reported test data should include the following:

(i) Details of the test procedures used (e.g., static, semi-static, flow-through, aerated, etc.).

(ii) Information about the test organism (scientific name, strain, supplier, any pretreatment, etc.).

(iii) The concentrations tested.

(iv) The number of fish in each test chamber and the loading rate.

(v) The methods of preparation of stock and test solutions.

(vi) The dissolved oxygen concentrations, pH values, temperature, total hardness of the test solutions measured each 24 hours and any other available information on water quality.

(vii) Any available information on the concentrations of the test chemical in the test solutions.

(viii) The maximum concentration causing no mortality within the period of the test.

(ix) The minimum concentration causing 100 percent mortality within the period of the test.

(x) The cumulative mortality in each concentration according to the recommended observation times.

(xi) The LC_{50} values (based on nominal concentrations) at each of the recommended observation times (with 95 percent confidence limits, if possible).

(xii) A graph of the concentration-mortality curve at the end of the test.

(xiii) The statistical procedures used for determining the LC_{50} values.

(xiv) The mortality of the control animals.

(xv) Any incidents in the course of the test which might have influenced the results.

(xvi) Any abnormal responses of the fish.

(xvii) A statement that the test was carried out in agreement with the prescriptions of the Test Guideline given above (otherwise a description of any deviations occurring).

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) American Public Health Association. *Standard methods for the examination of water and wastewater.* 14th ed. (American Public Health Association: New York, 1975).

(2) U.S. Environmental Protection Agency, Committee on Methods for Toxicity Tests with Aquatic Organisms, "methods for acute toxicity tests with fish, macroinvertebrates and amphibians," EPA Report No. 660/3-75-009 (Corvallis, Oregon, 1975).

(3) Finney, A.J. *Statistical Methods in Biological Assay.* (Griffin Ltd.: Weycombe, U.K., 1978).

(4) Litchfield, J.T., Wilcoxon, F. "A simplified method of evaluating dose-effect experiments," *Journal of Pharmacology and Experimental Therapeutics,* 96:99–1113 (1947).

(5) U.S. Environmental Protection Agency, "Methods for measuring the acute toxicity of effluents to aquatic organisms," EPA Report No. 600/4-78-012 (W. Peltier: Cincinnati, Ohio, 1978).

(6) Sprague, J.B. "Measurement of pollutant toxicity to fish. I: Bioassay Methods for Acute Toxicity," *Water Research* 3:794–821 (1969).

(7) ASTM STP 634, Stephan, C.E. Methods for calculating an LC_{50}. Eds. Mayer, F.L., Hamelink, J.L. *Aquatic Toxicology and Hazard Evaluation.* American Society for Testing and Materials. (1977) pp.65–84.

(8) Tabata, K. "Quality control of Japanese rice fish for TLm-test;" *Water and Effluent,* 14:1297–1303 (1972).

§ 797.1520 Fish Bioconcentration Test.

(a) *Purpose.* This guideline is intended to be used for assessing the propensity of chemical substances to bioconcentrate in freshwater fish. This guideline describes a bioconcentration test procedure for the continuous exposure of fathead minnows (*Pimephales promelas*) to a test substance in a flow-through system. The United States Environmental Protection Agency (EPA) will use data from this test in assessing the hazard a chemical may present to the environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA) and the definitions in Part 792—Good Laboratory Practice Standards of this chapter, are applicable to this test guideline. The following definitions also apply:

(1) "Acclimation" is the physiological compensation by test organisms to new environmental conditions (e.g. temperature, hardness, pH).

(2) "Bioconcentration" is the net accumulation of a substance directly from water into and onto aquatic organisms.

(3) "Bioconcentration factor (BCF)" is the quotient of the concentration of a test substance in aquatic organisms at or over a discrete time period of exposure divided by the concentration in the test water at or during the same time period.

(4) "Carrier" is a solvent used to dissolve a test substance prior to delivery of the test substance to the test chamber.

(5) "Depuration" is the elimination of a test substance from a test organism.

(6) "Depuration phase" is the portion of a bioconcentration test after the uptake phase during which the organisms are in flowing water to which no test substance is added.

(7) "Dilution water" is the water to which the test substance is added and in which the organisms undergo exposure.

(8) "Loading" is the ratio of fish biomass (grams, wet weight) to the volume (liters) of test solution passing through the test chamber during a 24-hr. period.

(9) "Organic chlorine" is the chlorine associated with all chlorine-containing compounds that elute just before lindane to just after mirex during gas chromatographic analysis using a halogen detector.

(10) "Organochlorine pesticides" are those pesticides which contain carbon and chlorine such as aldrin, DDD, DDE, DDT, dieldrin, endrin, and heptachlor.

(11) "Steady-state" is the time period during which the amounts of test substance being taken up and depurated by the test organisms are equal, i.e., equilibrium.

(12) "Steady-state bioconcentration factor" is the mean concentration of the test substance in test organisms during steady-state divided by the mean

concentration in the test solution during the same period.

(13) "Stock solution" is the concentrated solution of the test substance which is dissolved and introduced into the dilution water.

(14) "Test chamber" is the container in which the test organisms are maintained during the test period.

(15) "Test solution" is dilution water containing the dissolved test substance to which test organisms are exposed.

(16) "Uptake" is the sorption of a test substance into and onto aquatic organisms during exposure.

(17) "Uptake phase" is the initial portion of a bioconcentration test during which the organisms are exposed to the test solution.

(c) *Test procedures*—(1) *Summary of the test.* (i) Fathead minnows are continuously exposed to at least one constant sublethal concentration of a test substance under flow-through conditions for a maximum of 28 days. During this time, test solution and fish are periodically sampled and analyzed using appropriate methods to quantify the test substance concentration. If prior to day 28, the tissue concentrations of the substance sampled over three consecutive sampling periods have been shown to be statistically similar (i.e., steady-state has been reached), the uptake phase of the test may be terminated and the remaining fish transferred to untreated flowing water until 95 percent of the accumulated residues have been eliminated, or for a maximum depuration period of 14 days.

(ii) The mean test substance concentration in the fish at steady-state is divided by the mean test solution concentration at the same time to estimate the bioconcentration factor (BCF).

(iii) If steady-state is not reached during 28 days of uptake, the steady-state BCF is calculated using non-linear parameter estimation methods.

(2) [Reserved]

(3) [Reserved]

(4) *Definitive test*—(i) *Background information.* The following data on the test substance should be known prior to testing:

(A) Its solubility in water.

(B) Its stability in water.

(C) Its octanol-water partition coefficient.

(D) Its acute toxicity to fathead minnows.

(E) The validity accuracy and minimum detection limits of the proposed analytical methods.

(ii) *Selection of test concentration.* (A) At least one concentration should be tested to assess the propensity of the compound to bioconcentrate. The concentration selected should not stress or adversely affect the fish and should be less than one-tenth the 96-hr or incipient LC_{50} determined from a flow-through test with fathead minnows. The test concentration should be less than the solubility limit of the compound in water and close to the potential or expected environmental concentration. The limiting factor of how low one can test is based on the detection limit of the analytical methods. The concentration of the test material in the test solution should be at least 3 times greater than the detection limit in water.

(B) If it is desired to document that the potential to bioconcentrate is independent of the test concentration, at least 2 concentrations should be tested that are at least a factor of 10 apart.

(iii) *Estimation of test duration.* (A) An estimate of the length of the uptake and depuration phases should be made prior to testing. This will allow the most effective sampling schedule to be determined. The uptake phase should continue until steady-state has been reached, but need not be longer than 28 days. The test should continue for at least 4 days.

(B)(*1*) The time to steady state (S in hours) can be estimated from the water solubility or the octanol-water partition coefficient using the following equations:

$S = 3.0/\text{antilog}\ (0.431 \log W - 2.11)$ or,

$S = 3.0/\text{antilog}\ (-0.414 \log P + 0.122)$

where

W = water solubility (mg/l) and

P = octanol-water partition coefficient

(*2*) Based upon the estimate of the time to steady state, one of the following sampling schemes may be used to generate the appropriate data.

TIME TO STEADY-STATE IN DAYS

Test period	$S<4$	$S>4<14$	$S>15<21$	$S>21$
	Sampling Days			
Exposure	[1]1	[1]4	1	1
	[1]6	1	3	3
	1	3	7	7
	2	7	10	10
	3	10	14	14
	4	12	18	21
		14	22	28
Depuration	[1]1	1	1	1
	[1]6	2	3	3
	[1]12	4	7	7
	1	6	10	14
				14

[1] = hours.

(C) The depuration phase should continue until at least 95 percent of the accumulated test substance and metabolites have been eliminated, but no longer than 14 days.

(iv) *Test initiation.* (A) The test should not be started until the test substance delivery system has been observed to be functioning properly for at least 48 hours. This time should be sufficient to allow the test substance concentration to become equilibrated with the test exposure system. Analyses of two sets of test solution samples taken prior to test initiation should document this equilibrium (i.e., the concentrations do not vary more than 20 percent from each other). At initiation (time 0), test solution samples should be collected immediately prior to the addition of fish to the test chambers.

(B) The appropriate number of fathead minnows should be impartially distributed to each test chamber up to five at a time until the appropriate numbers have been distributed. The exact number of test organisms depends upon the expected length of testing, sample size, and the number of additional specialized analyses to be performed at termination.

(v) *Feeding.* (A) Fish should be fed once a day throughout the uptake and depuration phases. Feeding should always be done just after sampling to minimize the effects of the test substance present in the gut when sampling. Fish should be fed the same food at a similar quantity as they received during holding and acclimation.

(B) Uneaten food and fecal material should be removed from the test aquaria within 30 minutes after feeding to minimize uptake of test substance by the food or feces.

(vi) *Observations.* (A) Observations on fish appearance and behavior should be made and recorded daily. Any abnormal behavior such as erratic swimming, lethargy, increased excitability, or any changes in appearances or physiology such as discoloration, hyperventilation or opaque eyes should be recorded.

(B) Observations on compound solubility should also be recorded. These include the appearance of surface slicks, precipitates, or material adsorbing to the test chamber.

(vii) *Water quality measurements.* The water temperature and dissolved oxygen concentration should be recorded at least daily and the pH twice weekly in each test chamber during uptake and depuration.

(viii) *Sampling procedures.* (A) At each of the designated sampling times, triplicate water samples and enough fish should be collected from the exposure chamber(s) to allow for at least four fish tissue analyses. A similar number of control fish should also be collected at each sample point, but only fish collected at the first sampling period and weekly thereafter should be analyzed. Triplicate control water samples will be collected at the time of test initiation and weekly thereafter. Test solution samples should be removed from the approximate center of the water column.

(B) At each sampling period, the appropriate number of fish is netted and removed from each test chamber. Care should be taken not to sample the weakest and consequently usually the smallest fish, especially during the first few sampling periods, to prevent biasing the test results. Each fish is pithed, blotted dry and then frozen at < -10 °C if not analyzed within 4 hours.

(C) At termination, an extra set of fish should be sampled and eviscerated for quantifying the residues in the viscera and carcass. If a radiolabelled test compound is used, a sufficient number of fish should be sampled at termination to permit identification and quantitation of any major (>10 percent of parent) metabolites present. It is crucial to determine how much of the activity present in the fish is directly attributable to the parent compound.

(5) *Test results*—(i) *Biological.* (A) The maximum allowable mortality of fish is 10 percent per week. If more than 10 percent of the fish in the control or test chamber(s) die during any week of testing, the test should be repeated.

(B) Steady-state has been reached when the mean concentrations of test substance in whole fish tissue taken on three consecutive sampling periods are statistically similar (F test, $P=0.05$). A BCF is then calculated by dividing the mean tissue residue concentration during steady-state by the mean test solution concentration during this same period. A 95 percent confidence interval should also be derived for the BCF. This can be done by calculating the mean fish tissue concentration at steady state (X_f) and its 97.5 percent confidence interval, $\pm t$ (S.E.), where t is the t statistic$=0.025$ and S.E. is one standard error of the mean. This calculation would yield lower and upper confidence limits (L_f and U_f). The same procedure can be used to calculate the mean and 97.5 percent confidence interval from the test solution concentrations at steady-state, $X_s \pm t$ (S.E.), and the resulting upper and lower confidence limits (L_s and U_s). The 95 percent confidence interval of the BCF would then be between L_f/U_s and U_f/L_s.

(C) If steady-state was not reached during the 28-day uptake period, the maximum BCF should be calculated using the mean tissue concentration from that day and the mean water concentration from that and the previous sampling day. An uptake rate constant should then be calculated using appropriate techniques, such as the BIOFAC program developed by Blau and Agin (1978) under paragraph (F)(1) of this section. This rate constant will allow the estimation of a steady state BCF and the estimated time to steady-state.

(D) If 95 percent elimination has not been observed after 14 days depuration, then a depuration rate constant should be calculated. This rate constant will allow estimation of the time to 95 percent elimination.

(ii) *Analytical.* (A) All samples should be analyzed using EPA methods and guidelines whenever feasible. The specific methodology used should be validated before the test is initiated. The accuracy of the method should be measured by the method of known additions. This involves adding a known amount of the test substance to three water samples taken from an aquarium containing dilution water and a number of fish equal to that to be used in the test. The nominal concentration of these samples should be the same as the concentration to be used in the test. Samples taken on two separate days should be analyzed. The accuracy and precision of the analytical method should be checked using reference or split samples or suitable corroborative methods of analysis. The accuracy of standard solutions should be checked against other standard solutions whenever possible.

(B) An analytical method is not acceptable if likely degradation products of the test substance, such as hydrolysis and oxidation products, give positive or negative interferences, unless it is shown that such degradation products are not present in the test chambers during the test. Atomic absorption spectrophotometric methods for metals and gas chromatographic methods for organic compounds are preferable to colorimetric methods.

(C) In addition to analyzing samples of test solution, at least one reagent blank should also be analyzed when a reagent is used in the analysis.

(D) When radiolabelled test compounds are used, total radioactivity should be measured in all samples. At the end of the uptake phase, water and tissue samples should be analyzed using appropriate methodology to identify and estimate the amount of any major (>10 percent of the parent compound) degradation products or metabolites that may be present.

(6) [Reserved]

(d) *Test conditions*—(1) *Test species*—(i) *Selection.* (A) The fathead minnow (*Pimephales promelas*) should be used as the test organism.

(B) Immature fish should be used. They should be young enough so as not to mature during the test. Fish used in the same test should be as similar in size as possible to reduce variability. The standard deviation of the weight should be less than 20 percent of the mean ($N=30$).

(C) Fish used in the same test should be from the same supplier or culture unit and from the same holding and acclimation tank(s).

(D) Fathead minnows should not be used if they appear diseased or otherwise stressed or if more than 5 percent die during the 48 hours prior to testing. Diseased fish should be discarded or treated and held for a minimum of 14 days before testing.

(ii) *Care and handling.* (A) Fish purchased from a commercial source should be attended to immediately upon arrival. Transfer of the fish from the shipping to the holding water should be gradual to reduce stress caused by differences in water quality characteristics and temperature. Fish should be quarantined and observed for at least 14 days prior to testing.

(B) During holding, the fish should not be crowded and the dissolved oxygen concentration should be above 60 percent saturation. Holding tanks should be kept clean and free of debris. Fish should be fed at least once a day with a food which will support their survival and growth.

(C) Fish should be handled as little as possible. When handling is necessary, it should be done as gently, carefully, and quickly as possible using dip nets made of small mesh nylon, silk, bolting cloth, plankton netting, or other similar knotless materials. Handling equipment should be sterilized between uses by autoclaving, treating with an iodophor or with 200 mg hypochlorite/liter.

(iii) *Acclimation.* If the holding water is not from the same source as the test dilution water, acclimation to the dilution water should be done gradually over a 48-hour period. The fish should then be held an additional 14 days in the dilution water prior to testing. Any changes in water temperature should not exceed 3 °C per day. Fish should be held for a minimum of 7 days at the test temperature prior to testing.

(iv) *Loading.* The number of fish placed in each test chamber and the flow rate through the test chamber should be such that the uptake of the test substance by fish upon introduction into the test solution does not reduce the measured concentration of the test solution by more than 20 percent of the concentration measured before the fish were introduced. The loading should not exceed 0.1 g fish per liter of test solution delivered over any 24-hour period, and the minimum turnover rate should be 6 aquaria volumes per 24 hours. For some compounds, loading rates less than 0.1g/l may be needed to prevent a substantial loss of test substance as a result of fish uptake.

(2) *Facilities*—(i) *Dilution water.* (A) A constant supply of good quality water should be available throughout the holding, acclimation and testing periods. Although unadulterated well water is recommended, dechlorinated tap water or reconstituted soft water may be used. A dilution water is acceptable if fathead minnows will survive and grow normally for 60 days without exhibiting signs of stress, i.e., discoloration, lack of feeding, poor response to external stimuli, or lethargy.

(B) The total hardness, alkalinity, pH, specific conductance, temperature and dissolved oxygen concentration of the dilution water should be determined weekly. The pH should not vary more than 0.4 units and the other parameters more than 10 percent on a monthly basis.

(C) Reconstituted soft water, if used, should be prepared by adding 4.8 g $NaHCO_3$, 3.0 g $CaSO_4\ 2H_2O$, 3.0 g $MgSO_4$, and 200 mg KCl to each 100 l of deionized or glass distilled water, or to dechlorinated tap water with a total residual chlorine concentration less than 1 µg/l. In all cases the specific conductance at 25 °C of the water source should be less than 1 micromho/cm.

(D) All water should be extensively aerated prior to use if the dissolved oxygen concentration is less than 90 percent of saturation. If the concentration of dissolved gases exceeds 110 percent of saturation, the excess gases should be removed using appropriate apparatus.

(E) The quality of the dilution water should be consistent and should meet the following specifications measured at least twice a year.

Substance	Maximum concentration
Particulate matter	20 mg/liter.
Total organic carbon	
or	2 mg/liter.
Chemical oxygen demand	5 mg/liter.
Un-ionized ammonia	1 µg/liter.
Residual chlorine	1 µg/liter.
Total organophosphorus pesticides	50 ng/liter.
Total organochlorine pesticides plus polychlorinated biphenyls (PCBs).	
or	50 ng/liter.
Organic chlorine	25 ng/liter.
Copper, cadmium or zinc	10 µg/liter.

(ii) *Construction materials.* Materials and equipment that contact dilution water, stock solutions or test solutions should not leach or absorb substances. Glass, stainless steel and perfluorocarbon plastics should be used whenever possible. Concrete, unplasticized plastics and fiberglass may be used for holding and acclimation tanks and in the water supply system, but they should be thoroughly conditioned before use by rinsing with a continuous flow of water >25 °C for 48 hours. The use of flexible tubing should be avoided as phthalate esters leach from these materials. Cast iron pipe may be used but filters will be needed to remove rust particles. Rubber, copper, brass, galvanized metal, and epoxy glue should not come in contact with dilution water, stock solutions, or test solutions.

(iii) *Fish holding and acclimation.* (A) Tanks are needed for holding and acclimating fathead minnows prior to testing. The number and size of tanks needed depends upon the amount of testing to be performed and the availability of fish of the right age. A constant supply of good quality dilution water should be supplied to all tanks. The volume required depends upon the holding temperature and the number of fish being held, but the flow should be great enough to maintain a dissolved oxygen concentration >60 percent of saturation.

(B) Temperature control apparatus are needed to maintain the desired holding and acclimation temperatures. Apparatus controls should be able to maintain temperatures within 1 °C of the appropriate temperature. If the water is heated, care should be taken to avoid supersaturation of gases in the water.

(iv) *Testing apparatus.* (A) Test chambers can be made from welded stainless steel or from double strength glass joined with clear silicone adhesive. The size, shape and depth of the test chambers are not important as long as they accommodate the loading requirements.

(B) The test substance delivery system used should accommodate the physical and chemical properties of the test substance and the selected exposure concentration. The apparatus used should accurately and precisely deliver the appropriate amount of stock solution and dilution water to the test chambers. The introduction of the test substance should be done in such a way as to maximize the homogeneous distribution of the test substance throughout the test chamber.

(C) The dilution water should be delivered to an elevated headbox from which it can flow by gravity to the test substance delivery system. Use of a headbox facilitates a constant delivery rate and heating or cooling of the water to the approximate test temperature prior to delivery. Water in the headbox may also be easily aerated or degassed as the situation dictates.

(v) *Cleaning of test apparatus.* Delivery systems and test chambers should be cleaned before and after each use. If there is obvious absorption of a test substance by the silicone adhesive, those applicable parts of the delivery system should be discarded.

(3) *Test parameters*—(i) *Dissolved oxygen.* The dissolved oxygen concentration in each chamber should be greater than 5.3 mg/l (60 percent of sea-level saturation at 22 °C) throughout testing.

(ii) *Temperature.* The test temperature should be 22 ± 1 °C. Temporary excursions (<8 hours) to 20 or 24 °C are permissible.

(iii) *Lighting.* A photoperiod of 12 hours light and 12 hours dark with a 15 to 30 minute transition period is recommended.

(iv) *Test substance.* The name and purity of the test substance to be tested will be specified in the test rule. Radiolabelled compounds should not be used unless there are no suitable, validated, analytical techniques to measure unlabelled test substance in fish, or the costs of these analytical techniques are very high.

(v) *Carrier use.* Whenever possible, the test substance should be added, directly to the dilution water or from a water stock solution. With compounds having a low water solubility, it may be necessary to prepare test solutions using a carrier. The carriers to be used, in order of preference are: triethylene glycol (TEG), dimethyl formamide (DMF) and acetone. The amount used should be kept to a minimum and should not exceed 80 mg/l in the test solution for TEG and 5.0 mg/l for DMF and acetone.

(e) *Reporting.* In addition to the information required in Part 792—Good Laboratory Practice Standards of this chapter, the report should contain the following:

(1) The source of the dilution water, its mean monthly chemical characteristics (total hardness, alkalinity, pH, specific conductance, temperature and D.O.) and a description of any pretreatment.

(2) Detailed information about the fathead minnows used, including age, mean and standard deviation wet weight (blotted dry) and standard length, source, history of disease, parasites and treatment, acclimation procedures, and food used.

(3) The number of organisms tested, loading rate and volume additions per 24 hours.

(4) The percentage mortality of control fish and fish in each exposure chamber

and any observed abnormal behavioral or physiological effects.

(5) The method of stock solution preparation including nominal and measured concentrations and solvent used.

(6) The mean, standard deviation and range of the temperature, dissolved oxygen concentration and pH during the test period.

(7) Photoperiod length and light intensity.

(8) Description of sampling and analytical methods for water and tissue analyses.

(9) The mean, standard deviation and range of the concentration of test compound in the test solution and fish tissue at each sampling period.

(10) The time to steady-state.

(11) The steady-state or maximum BCF and the 95 percent confidence limits.

(12) The time to 95 percent elimination of accumulated residues.

(f) *References.* For further background information on this test guideline the following references should be consulted:

(1) Blau, G.E. and Agin, C.L., "A users manual for BIOFAC: A computer program for characterizing the ratio of uptake and clearance of chemicals in aquatic organisms," (1978). *Dow Chemical Co.*, Mammalian Environmental Toxicology, Bldg. 1702, Midland, MI 48640.

(2) [Reserved]

§ 797.1560 Fish Bioconcentration Test.

(a) *Purpose.* This guideline is to be used for assessing the propensity of chemical substances to bioconcentrate in fish. This guideline describes a bioconcentration test procedure for the continuous exposure of fish to a test substance in a flow-through system. The United States Environmental Protection Agency (EPA) will use data from this test in assessing the hazard a chemical may present to the environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA) and in Part 792—Good Laboratory Practice Standards of this chapter are applicable to this test guideline. The following definitions also apply:

(1) "Bioconcentration" is the increase in concentration of test material in or on test organisms (or specified tissues thereof) relative to the concentration of test material in the ambient water.

(2) "Bioconcentration factor (BCF)" is the ratio of the test substance concentration in the test fish (C_f) to the concentration in the test water (C_w) at steady-state.

(3) "Depuration or clearance or elimination" is the process of losing test material from the test organisms.

(4) "Depuration rate constant (k_2) is the mathematically determined value that is used to define the depuration of test material from previously exposed test animals when placed in untreated dilution water, usually reported in units per hour.

(5) "Steady-state or apparent plateau" is a condition in which the amount of test material being taken up and depurated is equal at a given water concentration.

(6) "Uptake (u)" is the process of sorbing test material into and/or onto the test organisms.

(7) "Uptake phase" is the time during the test when test organisms are being exposed to the test material.

(8) "Uptake rate constant (k_1)" is the mathematically determined value that is used to define the uptake of test material by exposed test organisms, usually reported in units of liters/gram/hour.

(c) *Test procedures*—(1) *Summary of the test.* (i) The test compounds' water solubility, n-octanol/water partition coefficient and stability in water (hydrolysis, photolysis and microbial degradation) should be known prior to testing. The 24-hour and 96-hour LC_{50}'s for the fish species to be used in the study should also be known. These data should be based on test substance concentrations measured during a flow-through acute toxicity test.

(ii) The procedure proposed is applicable to organic chemicals that are not readily degradable in a microbial degradation test, relatively stable in the aquatic environment and soluble in water at <1 mg/l.

(iii) Before any biological experiments are carried out, the analytical method for the particular substance should be tested. It should be shown experimentally on both water and organisms that the recovery as well as the reproducibility are satisfactory. Blank samples (of water, solvents, etc.) should regularly be analyzed to ensure that no contamination occurs. The detection level should be determined and no quantification should be based on signals which are less than 2.5 times the instrument noise. Organisms and water samples should be removed in such a way that no contamination or losses by adsorption occur.

(iv) This Guideline describes a procedure for characterizing the bioconcentration potential of chemicals in aquatic biota. Parameters used to characterize the bioconcentration potential include the uptake rate constant (k_1), the depuration rate constant (k_2), and the steady-state bioconcentration factor, BCF (k_1/k_2).

(v) Each of three separate groups of test organisms of the same species is exposed to a different concentration of the test material in water: 0, x, and 10x mg/l, where x is defined by analytical and toxicological boundaries. The duration of the uptake phase (3 hours to 30 days) and depuration phase (6 hours to 60 days) varies according to the time required to reach the desirable percent of steady-state which is roughly estimated before the test starts. During both phases of the test, organisms and water are periodically removed from the test chambers and analyzed for the test material.

(vi) The uptake rate constant, depuration rate constant(s), bioconcentration factor, and their confidence limits are calculated from the model that best describes the measured concentrations of test material in the organisms and water at any point in time.

(2) [Reserved]

(3) [Reserved]

(4) *Definitive test*—(i) *Test solution preparation.* The test material should be added to the dilution water with minimal use of solvents or other carriers. Several systems adaptable to flow-through tests have been described for saturation of water with relatively insoluble test materials (Chadwick and Kugemgi (1968) under paragraph (f)(3) of this section, Borthwick et al. (1977) under paragraph (f)(2) of this section, Veith and Comstock (1975) under paragraph (f)(11) of this section. Acetone, dimethylformamide, ethanol, methanol, and triethylene glycol are the solvents recommended for use in preparing stock solutions. The concentration of solvent in any test solution should not exceed 0.1 ml/liter in flow-through tests.

(ii) *Exposure concentrations.* Test fish should be exposed to two or more concentrations of test material in water under flow-through conditions. As a guidance, the highest concentration should be less than one-tenth of the threshold or incipient LC_{50} for the test species and at least 10 times higher than the detection limit in water and, if possible, each exposure concentration should differ from another by a factor of 10.

(iii) *Test duration*—(A) *Estimation of the uptake phase.* (*1*) As a guideline, the statistically optimum duration of the uptake phase (u) is near the midpoint of an uptake curve plotted on semi-log paper, or $u=1.6/k_2$, but not more than $3.0/k_2$, which is equivalent to 95 percent of steady-state (Reilly et al. (1977) under paragraph (f)(7) of this section. A pre-test estimate of k_2 may be obtained from:

(*i*) A test with the same compound and a different species.

(*ii*) A test with a similar material.

(*iii*) The results of a preliminary range-finding test.

(*iv*) Water solubility data.

$\log k_2 = 0.43 \log(s) - 2.11$

where (s) is the aqueous solubility in ppm.

(*v*) *n*-octanol/water partition coefficient data.

$\log k_2 = -0.414 \log (K_{ow}) + 0.122$

where (K_{ow}) is the n-octanol/water partition coefficient.

(*2*) The duration of the uptake phase (u) for a test material with $\log K_{ow}=3$ would be:

$\log k_2 = 0.414\ (3) + 0.122 = -1.12$

$k_2 = 0.0759$

$u = 1.6/0.0759 = 21$ hours

Similarly, for a test material having a $\log K_{ow}=6$, the duration of the uptake phase (u) would be:

$\log k_2 = -0.414\ (6) + 0.122 = -2.362$

$k_2 = 0.0043$

$u = 1.6/0.0043 = 372$ hours (16 days)

(B) *Estimation of the depuration phase.* Two times u is usually sufficient time for about 95 percent removal of the body burden ($t_{1/2} = 0.69/k_2$), but several biological or analytical factors may suggest equally acceptable guidelines. Some compounds follow more complex uptake/depuration behavior than a simple two compartment (C_w and C_f), two parameter (k_1 and k_2) model. For these compounds, longer depuration periods are advisable. On the other hand, the depuration time will most likely be restricted by the lower limit of analytical detection for fish.

(iv) *Sampling schedule.* As a guideline, no fewer than four uptake sampling times and five depuration sampling times should be spaced throughout the duration of the experiment according to the following fractions of the total time (T_t): first at 0.0278 T_t, second at 0.0556 T_t, third at 0.1111 T_t, fourth at 0.2222 T_t, fifth at 0.3333 T_t (this is the optimum change-over time), sixth at 0.5000 T_t, seventh at 0.6667 T_t, eighth at 0.8333 T_t, and ninth at 1.000 T_t. The following Table I contains examples of acceptable sampling schedules for bioconcentration tests with test materials with a $\log K_{ow}=3.0$ and $\text{Log } K_{ow}=6.0$:

TABLE I.—ACCEPTABLE SAMPLING SCHEDULES FOR BIOCONCENTRATION TESTS

Test phase	Action	Sampling times		Number of water samples	Number of fish samples
		log Kow=3 Hours	log Kow=6 Days		
	Start test	−1	−2		
	Add fish	0	−1	2[1]	
	0.5	0	2		
	1.0	0.5	2		
	1.5	1.0	2		
Uptake	2	1.4	2	4	
	4	2.8	2	4	
	7	5.7	2	4	
	13	11	2	4	
	20	17	2	6	
	Transfer fish to untreated water 24.	20	2		
	30	26	2	4	
Depuration.	40	34	2	4	
	50	43	2	4	
Terminate test.	60	51	2	6	
Total			28	40	

[1] Samples taken after a minimum of 3 tank volumes have been delivered.

(v) *Sampling procedures.* (A) It is advisable to analyze both water and organism samples as soon as possible after they have been collected to prevent degradation or loss of test material and to determine approximate uptake and depuration rate constants as the test proceeds. If samples cannot be analyzed immediately, it is sometimes appropriate to extract the test material into a solvent, rendering it inert or easier to store until it can be analyzed.

(B) Water samples should be obtained by siphoning through glass tubing from the most central point in the test tank. The sample vessel and siphon should be rinsed with the test solution before collecting the sample.

(C) Water samples are best collected directly into glass vessels of appropriate volume from which the test material can be extracted or analyzed. These vessels might include separatory funnels in the case of organic compounds, or scintillation vials for radioactive test materials.

(D) If significant amounts of particulate matter are present in the water sampled, a second sample should be taken and analyzed after centrifuging to determine whether test material was adsorbed on the particulate matter rather than dissolved.

(E) Water samples containing highly persistent test materials can be stored frozen in plastic containers for later analysis. Care should be exercised to avoid use of containers which could sorb or contaminate samples. With most organic test materials, and especially those tending to degrade easily, a better practice is to extract them from the water and store them under refrigeration in solvent in tightly sealed glass vials.

(F) When removing test organisms for analysis, they should be netted or trapped in a random manner with as little disturbance as possible. If two or more test material concentrations are present, separate nets should be used for each concentration. Organisms should be rinsed with dilution water if accompanied by extraneous matter, blotted dry, and killed by pithing the brain with a dissecting needle or by severing the spinal cord above the opercular region with scissors. They should then be individually weighed and a record made to permit association of the weight with the sample.

(G) Fish may be analyzed as whole fish or as portions, e.g., edible portion (muscle), viscera, remaining carcass, etc. Specific organs may also be analyzed if sufficient biomass is available. If results based on body portions are desired, after the fish is killed it should be eviscerated, taking care not to puncture any parts of the visceral portion which could leak body fluids and possibly cause contamination of the remaining portions. The edible portion or muscle may be removed with a scalpel, blotted dry, and weighed before storing or analysis. The remaining carcass should be weighed before being stored or analyzed. It is necessary to record data for each portion for each individual fish so that whole body data can be reconstructed based on the sum of the parts.

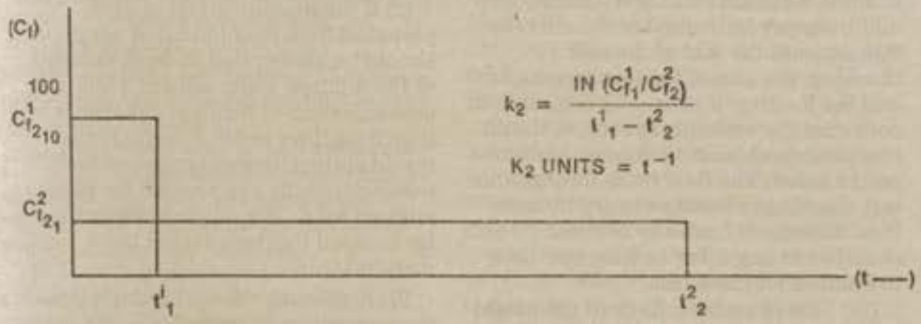
(H) After weighing, the sample is usually ground or homogenized to promote extraction of test material or to enhance solution of the tissue. Procedures for grinding, extraction, separation of impurities, determination of lipid content, etc., are described in the U.S. Food and Drug Administration's Pesticide Analytical Manual (1975) under paragraph (f)(10) of this section or the U.S. Environmental Protection Agency's Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental Samples (1974) under paragraph (f)(9) of this section.

(I) When determining the bioconcentration of test materials which concentrate in lipids, it is often desirable to determine the percent of the total tissue weight made up by lipids. Results between samples are frequently less variable when based on lipid weight rather than on total weight (Reinert 1970, under paragraph (f)(8) of this section).

(J) Organism samples can be wrapped in acetone-rinsed foil, placed in glass jars and frozen if they are not to be analyzed immediately.

(5) *Test results.* (i) Most bioconcentration data can reasonably be described with a simple two-compartment/two-parameter model as shown by a straight line depuration profile plotted on semi-log paper. If the depuration profile does not appear to be a straight line, then more complex models can be employed (Blau et al. 1975 under paragraph (f)(1) of this section). Typical variations from the simple model include a third parameter to describe the rate of metabolism of the parent compound or two additional parameters to describe redistribution of the parent compound within the body of the fish. If the best model is in question, it may be worthwhile to estimate parameters for the models in question and to compare the likelihood index of each model according to statistical tests (Blau et al. 1975 under paragraph (f)(1) of this section).

(ii) *Graph paper method for depuration rate constant.* Plot each concentration of the test material found in fish at each sampling time on semi-log paper. The slope of that line is K_2:



(iii) *Graph paper method for uptake rate constant.* Given k_2, calculate k_1 as follows:

$$k_1 = \frac{C_{f\,k2}}{C_w\,(1-e^{-k_2t})}$$

The value of C_f is read from the smooth uptake/depuration curve near the uptake mid-point on semi-log paper.

(iv) *Computer method for calculating uptake and depuration rate constant.* The preferred means for obtaining the bioconcentration factor and k_1 and k_2 rate constants is to use nonlinear parameter estimation methods on a digital computer. Two such programs are BIOFAC (Dow) and NONLIN (Procter and Gamble). These programs find values for k_1 and k_2 given a set of sequential time concentration data and the model:

$$C_f = \begin{cases} C_w \cdot \frac{k_1}{k_2}\,(1-e^{-k_2t}) & 0 \le t \le t_c \\ C_w \cdot \frac{k_1}{k_2}\,[e^{-k_2(t-t_c)} - e^{-k_2t_c}] & t_c \le t \end{cases}$$

This approach provides standard deviation estimates of k_1 and k_2, and BIOFAC statistically weights the analytical and biological variation of the fish concentration data. These and other nonlinear parameter estimation programs are readily available for most computers accepting the Fortran IV language or can be made available from a time-sharing service bureau; they are currently being used by many bioconcentration testing laboratories.

(v) *Validity of the test results.* (A) Scientific judgment rather than rigid criteria should be exercised in accepting or rejecting bioconcentration test results.

(B) Calculated BCF values based on an octanol/water partition coefficient have a very wide confidence margin (greater than ±100 percent), but the quality of the value may be better (narrower confidence margin) than an experimental value from a poorly designed study. Generally, the confidence margins for well designed studies approach ±20 percent. Acceptable bioconcentration data should be reported with confidence margins.

(C) Other criteria for judging the quality of bioconcentration data include the following guidelines:

(*1*) Percent mortality or adverse effect in control or treated organisms (suggested guideline, 10 percent).

(*2*) Percent effect of dose on uptake/depuration rate constants (suggested guideline 20 percent).

(*3*) Percent variation in C_w (suggested guideline, 20 percent) except for the initial dip that may approach 50 percent during the first few days of exposure.

(*4*) Temperature and dissolved oxygen should not vary more than ± 1 °C and ±2 mg/liter.

(*5*) The importance of actually visualizing an apparent plateau has been a subject of recent debate. It is suggested that 80 percent of steady-state (k_1/k_2) in any tissue with a confidence margin of ±20 percent is more than sufficient to estimate high quality rate constants for compounds with BCF <10,000. For compounds with BCF >10,000 it may be desirable and acceptable to terminate the uptake phase after a few days not to exceed 28 days even though <80 percent of steady-state was reached.

(*6*) A clearly defined uptake/depuration profile is an indicator of high quality bioconcentration data.

(6) *Analytical measurements.* (i) Prior to analyzing fish or water for the test substance, control samples should be spiked with several different concentrations of the test substance and then analyzed. Final values of C_w and C_f should be corrected for recoveries and background.

(ii) Analytical detection limits of test substance in both fish and water should be determined before the bioconcentration test begins and should be documented in the protocol. As a guideline, the limit of detection may be defined as a signal 2.5 times higher than the background noise level.

(iii) If possible, results reported as "not detected at the limit of detection" should be minimized by pre-test method development and experimental design.

These results cannot be used for rate constant calculations. The units C_w and C_f should both be expressed either as ppm or ppb.

(d) *Test conditions—Test species*—(1) *Selection.* (i) The procedures regarding selection of which species to test, their source, handling, holding, disease treatment, acclimation, and quality assurance prior to and during testing should be those given in Committee on Methods for Toxicity Tests with Aquatic Organisms (1975), under paragraph (b)(6) of this section.

(ii) The freshwater fish species used most frequently in bioconcentration tests have been rainbow trout, bluegill, and fathead minnows. The most commonly used marine fish have been spot, sheepshead minnows, silversides, shiner perch, English sole, staghorn sculpin, and 3-spine sticklebacks. These species are more readily available than most others and can be obtained in convenient sizes.

(2) *Facilities*—(i) *Construction materials.* Construction materials and commercially purchased equipment that may contact any water into which test organisms are placed should not contain any substances that can be leached or dissolved by the water. Glass, #316 stainless steel, and perfluorocarbon plastics should be used whenever possible to minimize leaching, dissolution, and sorption. Some will be more suitable than others for use with specific test materials. Unplasticized plastics, cast iron, and concrete can be used for holding and acclimation tanks and in the water supply system. Rubber, copper, brass, galvanized metal, and lead should not come into contact with dilution water, stock solutions, effluent samples or test solutions.

(ii) *Toxicant delivery system.* (A) One of several toxicant delivery systems can be used successfully, including the proportional diluter (Lemke et al. 1977), under paragraph (f)(6) of this section. Diluters are accurate over extended periods of time, are relatively trouble-free, and have fail-safe provisions. However, proportional diluters often require that laboratories have more than 8 feet of headroom. A small chamber to promote mixing of test material-bearing and dilution water should be used between the diluter and test chambers for each concentration. Design alterations, such as modification to deliver duplicates of only two or three concentrations, are easy to make (Jarvinen et al. 1977, under paragraph (f)(5) of this section).

(B) Pump systems are relatively simple to understand and use, require little space, and can be quite accurate. Some investigators have found metering pumps to maintain less variable test concentrations than piston operated pumps.

(C) The performance of the toxicant delivery system should be checked before and during each test. This should include determination of the flow rate through each test chamber and measurement of either the concentration of toxicant in each test chamber or the volumes delivered by each portion of the delivery system. The general operation of the toxicant delivery system should be checked daily during the test.

(D) The flow rate through the test chambers should be at least five volume additions per 24 hours, but should take into account the size of the test chamber, the size of the test organisms and the loading. It is usually desirable to construct the metering system so that it can provide at least 10 volume additions per 24 hours. The flow rates through the test chambers should not vary by more than 20 percent from any one test chamber to any other or from one time to another within a test.

(iii) *Test chambers.* Each of the height and width dimensions of the test chamber should be at least 1.5 times the largest horizontal dimension of the test organism. A minimum volume of one liter per fish is crowded but satisfactory for fish up to 15 g; large volumes to fish weight ratios are preferred to minimize the initial C_w dip and to help maintain the dissolved oxygen concentration.

(iv) *Cleaning.* Metering systems, test chambers, and equipment used to prepare and store dilution water, stock solutions, and test solutions should be cleaned before use. New equipment should be washed with detergent and rinsed with water, pesticide-free acetone, water, acid (such as 5 percent concentrated nitric acid), and twice with distilled water. At the end of every test, all items that are to be used again should be immediately emptied, rinsed with water, cleaned by a procedure appropriate for removing the test material (e.g., acid to remove metals and bases; detergent, organic solvent, or activated carbon to remove organic compounds), and rinsed twice with distilled water. Acid is useful for removing mineral deposits, and 200 mg of hypochlorite/liter is useful for removing organic matter and for disinfection. A solution containing 200 mg hypochlorite per liter is conveniently prepared by adding 6 ml of liquid household chlorine bleach to 1 liter of water. However, acid and hypochlorite should not be mixed because hazardous fumes may be produced. Metering systems and test chambers should be rinsed with dilution water just before use.

(v) *Dilution water*—(A) *General requirements.* (*1*) An adequate supply of dilution water that is acceptable to the test organisms and to the purpose of the test should be available. A minimum criterion for an acceptable dilution water is that healthy test organisms will survive in it for the duration of acclimation and testing without showing signs of stress, such as discoloration or unusual behavior. A better criterion for an acceptable freshwater dillution water is that test organisms will survive, grow, and reproduce satisfactorily in it.

(*2*) If the dilution water is or is prepared from dechlorinated water, it should be shown that in fresh samples of the dilution water either (*i*) the concentration of residual chlorine is less than 3 mg/liter or (*ii*) *Acartia tonsa*, mysid shrimp, oyster larvae, or first instar daphnids can survive for 48 hours without food. The dilution water should be assayed for the selected test material.

(B) *Freshwater.* Because daphnids are more sensitive to many toxicants than most other readily available freshwater aquatic animals, water in which first instar daphnids will survive for 48 hours without food is probably acceptable for most short-term tests with freshwater animals. Water in which daphnids will survive, grow, and reproduce satisfactorily should be an acceptable dilution water for longer tests with freshwater animals.

(C) *Estuarine and marine water.* Because *Acartia tonsa*, mysid shrimp, and oyster larvae are more sensitive to many toxicants than most other estuarine and marine aquatic animals, water in which they will survive for 48 hours without food is probably acceptable for most short-term tests with estuarine and marine animals. Water in which *Acartia tonsa* or mysid shrimp will survive, grow, and reproduce satisfactorily should be an acceptable dilution water for longer tests with estuarine and marine animals.

(e) *Reporting.* In addition to the reporting requirements prescribed in Part 792—Good Laboratory Practice Standards of this chapter, the test report should include the following information:

(1) A detailed description of the test material, including its source, lot number, composition (identity and concentration of major ingredients and major impurities), known physical and chemical properties, and identity and concentration of any carriers (solvents) or other additives used.

(2) The source of the dilution water, its chemical characteristics, and a description of any pretreatment.

(3) Detailed information about the test organisms, including scientific name and how verified (and strain for salmonids when appropriate), weight (wet, blotted dry), standard length of fish, height of bivalve molluscs, age, life stage, source, history, observed diseases, treatments, acclimation procedure, and food used.

(4) A description of the experimental design and metering system.

(5) Description of tissue and water samples analyzed, and methods used to obtain, prepare, and store them.

(6) Methods used for, and results (with standard deviation) of all chemical analyses of water quality and concentration of test material in tissue and water, including validation studies and reagent blanks.

(7) The steady-state bioconcentration factor, the uptake and depuration rate constants, the confidence margins (± standard deviation) and the method of computations/data analysis.

(8) Anything unusual about the test, any deviation from these procedures, and any other relevant information.

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Blau, G.E., Neely, W.B., Branson, D.R. "Ecokinetics: a study of the fate and distribution of chemicals in laboratory ecosystems," *American Institute of Chemical Engineering Journal,* 21:854–861 (1975).

(2) Borthwick, P.W., Tagatz, M.E., Forester, J. "A gravity-flow column to provide pesticide-laden water for aquatic bioassays," *Bulletin of Environmental Contamination and Toxicology,* 13:183–187 (1977).

(3) Chadwick, G.C., Kugemagi, V. "Toxicity evaluation of a technique for introducing dieldrin into water," *Journal of the Water Pollution Control Federation,* 40: 76–82 (1968).

(4) U.S. Environmental Protection Agency, Committee on Methods for Toxicity Tests with Aquatic Organisms. "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians," EPA Report 660/3–75–009 (Corvallis, Oregon, 1975).

(5) U.S. Environmental Protection Agency, Jarvinen, A.W., Hoffman, M.J., Thorslund, T.W. "Toxicity of DDT food and water exposure to fathead minnows" U.S. Environmental Protection Agency, EPA Report No. 600/3–76–114 (Duluth, Minnesota, 1977).

(6) U.S. Environmental Protection Agency, Lemke, A.E., Brungs, W.A., Halligan, B.J. "Manual for construction and operation of toxicity testing proportional diluters," EPA Report 600/3–78–072 (Duluth, Minnesota, 1978).

(7) Reilly, P.M., Bajramovic, R., Blau, G.E., Branson, D.R., Sauerhoff, M.W. "Guidelines for the optimal design of experiments to estimate parameters in first order kinetic models," *Canadian Journal of Chemical Engineering,* 55:614–622 (1977).

(8) Reinert, R.E. "Pesticide concentrations in Great Lakes fish," *Pesticides Monitoring Journal,* 3(4): 233–240 (1970).

(9) U.S. Environmental Protection Agency. "Analysis of human or animal adipose tissue," Ed. Thompson, J.F., *Analyses of Pesticide Residues in Human and Environmental Samples,* 1974, available from U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

(10) U.S. Food and Drug Administration. *Pesticide Analytical Manual.* Vol. 1, 1975, available from the Food and Drug Administration, 5600 Fishers Lane, Rockville, MD 20852.

(11) Veith, G.D., Comstock, V.M. "Apparatus for continuously saturating water with hydrophobic organic chemicals" *Journal of Fishery Research Board of Canada,* 32: 1849–1851 (1975).

§ 797.1600 Fish early life stage toxicity test.

(a) *Purpose.* This guideline is intended to be used for assessing the propensity of chemical substances to produce adverse effects to fish during the early stages of their growth and development. This guideline describes the conditions and procedures for the continuous exposure of several representative species to a chemical substance during egg, fry and early juvenile life stages. The Environmental Protection Agency (EPA) will use data from this test in assessing the potential hazard of the test substance to the aquatic environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA) and the definitions in Part 792—Good Laboratory Practice Standards, apply to this section. In addition, the following definitions are applicable to this specific test guideline:

(1) "Acclimation" physiological or behavioral adaptation of organisms to one or more environmental conditions associated with the test method (e.g., temperature, hardness, pH).

(2) "Carrier" solvent or other agent used to dissolve or improve the solubility of the test substance in dilution water.

(3) "Conditioning" exposure of construction materials, test chambers, and testing apparatus to dilution water or to the test solution prior to the start of the test in order to minimize the sorption of test substance onto the test facilities or the leachig of substances from test facilities into the dilution water or the test solution.

(4) "Control" an exposure of test organisms to dilution water only or dilution water containing the test solvent or carrier (no toxic agent is intentionally or inadvertently added).

(5) "Dilution water" the water used to produce the flow-through conditions of the test to which the test substance is added and to which the test species is exposed.

(6) "Early life stage toxicity test" a test to determine the minimum concentration of a substance which produces a statistically significant observable effect on hatching, survival, development and/or growth of a fish species continuously exposed during the period of their early development.

(7) "Embryo cup" a small glass jar or similar container with a screened bottom in which the embryos of some species (i.e., minnow) are placed during the incubation period and which is normally oscillated to ensure a flow of water through the cup.

(8) "Flow through" refers to the continuous or very frequent passage of fresh test solution through a test chamber with no recycling.

(9) "Hardness" the total concentration of the calcium and magnesium ions in water expressed as calcium carbonate (mg $CaCO_3$/liter).

(10) "Loading" the ratio of biomass (grams of fish, wet weight) to the volume (liters) of test solution passing through the test chamber during a specific interval (normally a 24-hr. period).

(11) "No observed effect concentration (NOEC)" the highest tested concentration in an acceptable early life stage test: (i) which did not cause the occurrence of any specified adverse effect (statistically different from the control at the 95 percent level); and (ii) below which no tested concentration caused such an occurrence.

(12) "Observed effect concentration (OEC)" the lowest tested concentration in an acceptable early life stage test: (i) which caused the occurrence of any specified adverse effect (statistically different from the control at the 95 percent level); and (ii) above which all tested concentrations caused such an occurrence.

(13) "Replicate" two or more duplicate tests, samples, organisms, concentrations, or exposure chambers.

(14) "Stock solution" the source of the test solution prepared by dissolving the test substance in dilution water or a carrier which is then added to dilution

water at a specified, selected concentration by means of the test substance delivery system.

(15) "Test chamber" the individual containers in which test organisms are maintained during exposure to test solution.

(16) "Test solution" dilution water with a test substance dissolved or suspended in it.

(17) "Test substance" the specific form of a chemical substance or mixture that is used to develop data.

(c) *Test Procedures*—(1) *Summary of test.* (i) The early life stage toxicity test with fish involves exposure of newly fertilized embryos to various concentrations of a test substance. Exposure continues for 28 days post hatch for the minnows and 60 days post hatch for the trout species. During this time various observations and measurements are made in a specific manner and schedule in order to determine the lowest effect and highest no-effect concentrations of the test substance.

(ii) A minimum of five exposure (treatment) concentrations of a test substance and one control are required to conduct an early life stage toxicity test. The concentration of the test substance in each treatment is usually 50 percent of that in the next higher treatment level.

(iii) For each exposure concentration of the test substance and for each control (i.e., regular control and carrier control is required) there should be:

(A) At least two replicate test chambers, each containing one or more embryo incubation trays or cups; and there should be no water connections between the replicate test chambers;

(B) At least 60 embryos divided equally, through randomization, between the embryo incubation trays or cups for each test concentration and control (i.e., 30 per embryo cup with 2 replicates);

(C) All surviving larvae divided equally between the test chambers for each test concentration and control (e.g., 30 larvae per test chamber with 2 replicates).

(iv) *Duration.* (A) For fathead minnow and sheepshead minnow a test begins when the newly fertilized minnow embryos (less than 48-hours old) are placed in the embryo cups and are exposed to the test solution concentrations. The test terminates following 28 days of post-hatch exposure, i.e., 28 days after the newly hatched fry are transferred from the embryo cups into the test chambers.

(B) For brook trout and rainbow trout a test begins when newly fertilized trout embryos (less than 96-hours old) are placed in the embryo trays or cups and are exposed to the test solution concentrations. The test terminates following 60 days of post-hatch exposure (for an approximate total exposure period of 90 days).

(C) For silverside a test begins with newly fertilized embryos (less than or equal to 48 hours old) and is terminated 28 days after hatching. The chorionic fibrils should be cut before randomly placing the embryos in the egg incubation cups.

(2) [Reserved]

(3) *Range-finding test.* (i) A range finding test is normally performed with the test substance to determine the test concentrations to be used in the early life stage toxicity test, especially when the toxicity is unknown. It is recommended that the test substance concentrations be selected based on information gained from a 4- to 10-day flow-through toxicity test with juveniles of the selected test species.

(ii) The highest concentration selected for the early life stage toxicity test should approximate the lowest concentration indicated in any previous testing to cause a significant reduction in survival. The range of concentrations selected is expected to include both observed effect and no-observed effect levels. The dilution factor between concentrations is normally 0.50, however, other dilution factors may be used as necessary.

(4) *Definitive test*—(i) *General.* (A) A test should not be initiated until after the test conditions have been met and the test substance delivery system has been observed functioning properly for 48-hours. This includes temperature stability, flow requirements of dilution water, lighting requirements, and the function of strainers and air traps included in the water-supply system, and other conditions as specified previously.

(B) New holding and test facilities should be tested with sensitive organisms (i.e., juvenile test species or daphnids) before use to assure that the facilities or substances possibly leaching from the equipment will not adversely affect the test organisms during an actual test.

(C) Embryos should be acclimated for as long as practical to the test temperature and dilution water prior to the initiation of the test.

(D) When embryos are received from an outside culture source (i.e., rainbow and brook trout) at a temperature at variance with the recommended test temperature they should be acclimated to the test temperature. When eggs are received, they should be immediately unpacked and the temperature of the surrounding water determined. Sudden temperature changes should be avoided. Acclimation to the appropriate test temperature should be accomplished within a period of six hours, and should incorporate the use of dilution water.

(E) Embryos should be visually inspected prior to placement in the embryo cups or screen trays. All dead embryos should be discarded. Dead embryos can be discerned by a change in coloration from that of living embryos (e.g. trout embryos turn white when dead). During visual inspection, empty shells, opaque embryos and embryos with fungus or partial shells attached should be removed and discarded. If less than 50 percent of the eggs to be used appear to be healthy, all embryos in such a lot should be discarded.

(ii) *Embryo incubation procedures.* (A) Embryos can be distributed to the embryo cups or screen trays using a pipette with a large bore or a similar apparatus. Newly-hatched silverside fry are very sensitive to handling; the egg incubation cups should not be handled at all the first 5 days after hatching begins. Just before hatching is expected to begin, the embryos should be transferred to clean incubation cups. Trout embryos can be distributed by using a small container which has been precalibrated to determine the approximate number of embryos it can hold; embryos are measured volumetrically in this manner, and are then poured onto the screen tray (or embryo cup). Trout embryos should be separated on the screen tray so that they are not in contact with each other. A final count will ensure the actual number on the screen tray. After random assignment, the screen trays or embryo cups are placed in the test chambers.

(B) Each day until hatch the embryos are visually examined. Minnow embryos may be examined with the aid of a magnifying viewer. Trout embryos should not be touched. Trout embryos should be maintained in low intensity light or in darkness until one-week post hatch, and are usually examined with the aid of a flashlight or under low intensity light. Dead embryos should be removed and discarded. Live embryos which are heavily infected with fungus should be discarded, but should be subtracted from the initial number of embryos used as a basis for the calculations of percentage hatch.

(C) When embryos begin to hatch they should not be handled.

(iii) *Initiation of fry exposure.* (A) Forty-eight hours after the first hatch in each treatment level, or when hatching is completed, the live young fish should be counted and transferred from each embryo cup into the appropriate test chamber. For silverside, all surviving fry are not counted until six days after hatching and are not transferred to embryo cups. All of the normal and abnormal fry should be gently released into the test chamber by allowing the fry to swim out of each embryo cup; nets should not be used. The trout embryos incubated on screen trays will hatch out in the test chambers, therefore handling of fish is not necessary.

(B) If necessary, fry can be transferred from one replicate embryo cup to the other replicate within a test concentration to achieve equal numbers in each replicate chamber.

(C) The number of live fry, live normal fry, live embryos, dead embryos and unaccounted for embryos for each cup should be recorded when hatching is deemed complete. Those fry which are visibly (without the use of a dissecting scope or magnifying viewer) lethargic or grossly abnormal (either in swimming behavior or physical appearance) should be counted. Late hatching embryos should be left in the embryo cups to determine if they will eventually hatch or not. The range of time-to-hatch (to the nearest day) for each cup should be recorded.

(iv) *Time to first feeding.* (A) The first feeding for the fathead and sheepshead minnow fry should begin shortly after transfer of the fry from the embryo cups to the test chambers. Silversides are fed the first day after hatch. Trout species initiate feeding at swim-up. The trout fry should be fed trout starter mash three times a day *ad libitum*, with excess food siphoned off daily. The minnow fry should be fed live newly-hatched brine shrimp nauplii (*Artemia salina*) at least three times a day.

(B) For the first seven days, feeding should be done at minimum intervals of four hours (i.e., 8 am, 12 noon, and 4 pm); thereafter the fry should be fed as indicated below.

(v) *Feeding.* (A) The fathead and sheepshead minnow fry should be fed newly-hatched brine shrimp nauplii for the duration of the test at approximately 4-hour intervals three times a day during the week and twice on the weekend after the first week. Trout fry should be fed at similar intervals, and may receive live brine shrimp nauplii in addition to the trout starter food after the first week. Between days 1 and 8 after first hatching, silverside fry are fed the rotifer, *Brachionus plicatilis*, three times daily at a concentration of 5,000-10,000 organisms per egg cup (based on 15 fish/cup). From days 9-11, the fry should be fed approximately 2,500 newly-hatched brine shrimp (*Artemia*) nauplii and 5,000-10,000 rotifers twice daily. For the remainder of the test, the fish will be fed brine shrimp exclusively. The number of organisms used should be gradually increased to approximately 5,000 nauplii by test day 28.

(B) An identical amount of food should be provided to each chamber. Fish should be fed *ad libitum* for 30 minutes with excess food siphoned off the bottom once daily if necessary.

(C) Fish should not be fed for the last 24 hours prior to termination of the test.

(vi) *Carriers.* Water should be used in making up the test stock solutions. If carriers other than water are absolutely necessary, the amount used should be the minimum necessary to achieve solution of the test substance. Triethylene glycol and dimethyl formamide are preferred, but ethanol and acetone can be used if necessary. Carrier concentrations selected should be kept constant at all treatment levels.

(vii) *Controls.* Every test requires a control that consists of the same dilution water, conditions, procedures, and test organisms from the same group used in the other test chambers, except that none of the test substance is added. If a carrier (solvent) is used, a separate carrier control is required in addition to the regular control. The carrier control should be identical to the regular control except that the highest amount of carrier present in any treatment is added to this control. If the test substance is a mixture, formulation, or commercial product, none of the ingredients is considered a carrier unless an extra amount is used to prepare the stock solution.

(viii) *Randomization.* The location of all test chambers and species within the test system should be randomized. A representative sample of the test embryos should be impartially distributed by adding to each cup or screen tray no more than 20 percent of the number of embryos to be placed in each cup or screen tray and repeating the process until each cup or screen tray contains the specified number of embryos. Alternatively, the embryos can be assigned by random assignment of a small group (e.g., 1-5) of embryos to each embryo cup or screen tray, followed by random assignment of a second group of equal number to each cup or tray, which is continued until the appropriate number of embryos are contained in each embryo cup or screen tray. The method of randomization used should be reported in detail.

(ix) *Observations.* During the embryo exposure period observations should be made to check for mortality. During the exposure period of the fry, observations should be made to check for mortality and to note the physical appearance and behavior of the young fish. The biological responses are used in combination with physical and chemical data in evaluating the overall lethal and sublethal effects of the test substance. Additional information on the specific methodology for the data obtained during the test procedure are discussed in the following sections.

(x) *Biological data.* (A) Death of embryos should be recorded daily and dead embryos removed when discovered to prevent the spread of fungal contamination.

(B) When hatching commences, daily records of the number of embryos remaining in each embryo cup are required. This information is necessary to quantify the hatching success. A record of all deformed larvae should be kept throughout the entire post-hatch exposure. Time to swim-up should be recorded for the trout. Upon transfer of fry from the embryo cups to the test chambers, daily counts of the number of live fish should be made. At a minimum, live fish should be counted on days 4, 11, 18, 25 and (weekly thereafter for the trout species) finally on termination of the test.

(C) The criteria for death of young fish is usually immobility, especially absence of respiratory movement, and lack of reaction to gentle prodding. Deaths should be recorded daily and dead fish removed when discovered.

(D) Daily and at termination of the test, the number of fish that appear (without the use of a magnifying viewer) to be abnormal in behavior (e.g., swimming erratic or uncoordinated, obviously lethargic, hyperventilating, or over excited, etc.) or in physical appearance (e.g., hemorrhaging, producing excessive mucous, or are discolored, deformed, etc.) should be recorded and reported in detail.

(E) All physical abnormalities (e.g., stunted bodies, scoliosis, etc.) should be photographed and the deformed fish which die, or are sacrificed at the termination of the test, should be preserved for possible future pathological examination.

(F) At termination, all surviving fish should be measured for growth. Standard length measurements should be made directly with a caliper, but may be measured photographically. Measurements should be made to the nearest millimeter (0.1mm is desirable). Weight measurements should also be made for each fish alive at termination (wet, blotted dry and to the nearest 0.01g for the minnows and 0.1g for the trout). If the fish exposed to the toxicant appear to be edematous compared to control fish, determination of dry, rather than wet, weight is recommended.

(G) Special physiological, biochemical and histological investigations on embryos, fry, and juveniles may be deemed appropriate and should be performed on a case by case basis.

(5) *Test results.* (i) Data from toxicity tests are usually either continuous (e.g. length or weight measurements) or dichotomous (e.g. number hatching or surviving) in nature. Several methods are available and acceptable for statistical analysis of data derived from early life stage toxicity tests; however, the actual statistical methodology to analyze and interpret the test results should be reported in detail.

(ii) The significance level for all statistical testing should be a minimum of $P = 0.05$ (95 percent confidence level).

(A) *Example of statistical analysis.* (*1*) Mortality data for the embryonic stage, fry stage and for both stages in replicate exposure chambers should first be analyzed using a two-way analysis of variance (ANOVA) with interaction model. This analysis will determine if replicates are significantly different from each other. If a significant difference between replicates or a significant interaction exists, cause for the difference should be determined. Modification should then be made in the test apparatus or in handling procedures for future toxicity tests. Further calculations should incorporate the separation of replicates. If no significant difference is observed, replicates may be pooled in further analyses.

(*2*) After consideration of replicate responses, mortality data should then be subjected to one-way ANOVA. The purpose of this analysis is to determine if a significant difference exists in the percentage mortality between control fish and those exposed to the test material.

(*3*) If the one-way ANOVA results in a F ratio that is significant, it would be acceptable to perform t-tests on the control versus each concentration. A second technique is to identify treatment means that are significantly different; this method should involve the additional assumption that the true mean response decreases generally with increasing concentration. The researcher may also be interested in determining significant differences between concentrations.

(*4*) Growth data should also be analyzed by one-way ANOVA with the inclusion of a covariate to account for possible differences in growth of surviving fry in embryo cup(s) that contain fewer individuals. This condition can occur in cases when the same amount of food is given to each test chamber regardless of the number of survivors.

(B) *Test data to be analyzed.* Data to be statistically analyzed are:

(*1*) Percentage of healthy, fertile embryos at 40–48 hours after initiation of the test. Percentage is based upon initial number used.

(*2*) Percentage of embryos that produce live fry for release into test chambers. Percentage is based on number of embryos remaining after thinning.

(*3*) Percentage of embryos that produce live, normal fry for release into test chambers. Percentage is based upon number of embryos remaining after thinning.

(*4*) Percentage of fry survival at swim-up for trout. Percentage is based upon number of embryos remaining after thinning.

(*5*) Percentage of embryos that produce live fish at end of test. Percentage is based upon number of embryos remaining after thinning.

(*6*) Percentage of embryos that produce live, normal fish at end of test. Percentage is based upon number of embryos remaining after thinning.

(*7*) Weights and lengths of individual fish alive at the end of the test.

(C) It is important that fish length and weight measurements be associated with individual test chambers since the density of the fish and available food should be considered in the growth of the organism.

(iii) *Acceptability criteria.* (A) An early life stage toxicity test is not acceptable unless at least one of the following criteria is significantly different ($p = 0.05$) from control organisms when compared with treated organisms, and the responses are concentration-dependent: mortality of embryos, hatching success, mortality of fry (at swim-up for trout), total mortality throughout the test, and growth (i.e. weight). If no significant effects occur, but the concentrations tested were the highest possible due to solubility or other physio-chemical limitations, the data will be considered for acceptance.

(B) In addition to obtaining significant effects on the exposed test species, a measure of acceptability in the response of control fish is also required.

(C) A test is not acceptable if the average survival of the control fish at the end of the test is less than 80 percent or if survival in any one control chamber is less than 70 percent. For silversides, a test is not acceptable if the average overall survival of the control embryos and fish at the end of the test is less than 60 percent.

(D) If a carrier is used, the criteria for effect (mortality of embryos and fry, growth, etc.) used in the comparison of control and exposed test organisms should also be applied to the control and control with carrier chambers. For the test to be considered acceptable, no significant difference should exist between these criteria.

(E) A test is not acceptable if the relative standard deviation ($RSD = 100$ times the standard deviation divided by the mean) of the weights of the fish that were alive at the end of the test in any control test chamber is greater than 40 percent.

(6) *Analytical measurements*—(i) *Analysis of water quality.* Measurement of certain dilution water quality parameters should be performed every 6 months, to determine the consistency of the dilution water quality. In addition, if data in 30 day increments are not available to show that freshwater dilution water is constant, measurements of hardness, alkalinity, pH, acidity, conductivity, TOC or COD and particulate matter should be conducted once a week in the control and once a week in the highest test substance concentration. Measurement of calcium, magnesium, sodium, potassium, chloride, and sulfate is desirable.

(ii) *Dissolved oxygen measurement.* The dissolved oxygen concentration should be measured in each test chamber at the beginning of the test and at least once daily thereafter (as long as live organisms are present) in one replicate of the control and the high, medium, and low test substance concentrations.

(iii) *Temperature measurement.* Temperatures should be recorded in all test chambers at the beginning of the test, once weekly thereafter and at least hourly in one test chamber. When possible, the hourly measurement should be alternated between test chambers and between replicates.

(iv) *Test substance measurement.* (A) Prior to the addition of the test substance to the dilution water, it is recommended that the test substance stock solution be analyzed to verify the concentration. After addition of the test substance, the concentration of test substance should be measured at the beginning of the test in each test concentration (including both replicates) and control(s), and in one replicate at each test concentration at least once a week thereafter. Replicates should be alternated each week. If a malfunction in the delivery system is discovered, water samples should be taken from the affected test chambers immediately and analyzed.

(B) The measured concentration of test substance in any chamber should be no more than 30 percent higher or lower than the concentration calculated from the composition of the stock solution and the calibration of the test substance delivery system. If the difference is more than 30 percent, the concentration of test substance in the solution flowing into the exposure chamber (influent) should be analyzed. These results will indicate whether the problem is in the stock solution, the test substance delivery system or in the test chamber. Measurement of degradation products of the test substance is recommended if a reduction of the test substance concentration occurs in the test chamber.

(v) *Sampling and analysis methodology.* (A) Generally, total test substance measurements are sufficient; however, the chemical characteristics of the test substance may require both dissolved and suspended test substance measurements.

(B) For measurement of dissolved or suspended test substance or both, water samples should be taken midway between the top, bottom, and sides of the test chamber and should not include any surface scum or material stirred up from the bottom of sides. For measurement of total test substance, a large volume of the solution in the test chamber should be collected and used as the sample. Samples of test solutions should be handled and stored appropriately to minimize loss of test substance by microbial degradation, photodegradation, chemical reaction, volatilization, or sorption.

(C) Chemical and physical analyses should be performed using standardized methods whenever possible. The analytical method used to measure the concentration of the test substance in the test solution should be validated before the beginning of the test. At a minimum, a measure of the accuracy of the method should be obtained, on each of two separate days by using the method of known additions, and using dilution water from a tank containing test organisms. Three samples should be analyzed at the next to lowest test substance concentration. It is also desirable to study the accuracy and precision of the analytical method for test guideline determination by use of reference (split) samples, or interlaboratory studies, and by comparsion with alternative, reference or corroborative methods of analysis.

(D) An analytical method is not acceptable if likely degradation products of the test substance, such as hydrolysis and oxidation products, give positive or negative interferences, unless it is shown that such degradation products are not present in the test chambers during the test. In general, atomic absorption spectrophotometric methods for metals and gas chromatographic methods for organic compounds are preferable to colorimetric methods.

(E) In addition to analyzing samples of test solution, at least one reagent blank also should be analyzed when a reagent is used in the analysis. Also, at least one sample for the method of known additions should be prepared by adding test substance at the concentration used in the toxicity test.

(d) *Test conditions*—(1) *Test species.* (i) One or more of the recommended test species will be specified in rules under Part 799 of this chapter requiring testing of specific chemicals. The recommended test species are:

(A) Fathead minnow (*Pimephales promelas* Rafinesque).

(B) Sheepshead minnow (*Cyprinodon variegatus*).

(C) Brook trout (*Salvelinus fontinalis*).

(D) Rainbow trout (*Salmo gairdneri*).

(E) Atlantic silverside (*Menidia menidia*).

(F) Tidewater silverside (*Menidia peninsulae*).

(ii) Embryos used to initiate the early life stage test should be less than 48 hours old for the fathead and sheepshead minnows, silversides, and less than 96 hours old for the brook trout and rainbow trout. In addition, the following requirements should be met:

(A) All embryos used in the test should be from the same source. Embryos should be obtained from a stock cultured in-house when possible, and maintained under the same parameters as specified for the test conditions. When it is necessary to obtain embryos from an external source, caution should be exercised to ensure embryo viability and to minimize the possibility of fungal growth. A description of the brood stock history or embryo source should be made available to EPA upon request.

(B) Test species should be cared for and handled properly in order to avoid unnecessary stress. To maintain test species in good condition and to maximize growth, crowding should be prevented, and the dissolved oxygen level should be maintained near saturation.

(C) Embryos and fish should be handled as little as possible. Embryos should be counted and periodically inspected until hatching begins. When larvae begin to hatch, they should not be handled. Transfer of minnow larvae from embryo cups to test chambers should not involve the use of nets. No handling is necessary following introduction into the test chambers until termination of the test.

(D) If fathead minnow embryos are obtained from in-house culture units, the embryos should be gently removed from the spawning substrate. The method for separating the fertilized eggs from the substrate is important and can affect the viability of the embryos; therefore the finger-rolling procedure is recommended.

(E) Disease treatment. Chemical treatments to cure or prevent diseases should not be used before, and should not be used during a test. All prior treatments of brood stock should be reported in detail. Severely diseased organisms should be destroyed.

(2) *Test facilities*—(i) *Construction materials.* Construction materials and equipment that contact stock solutions, test solutions, or dilution water into which test embryos or fish are placed should not contain any substances that can be leached or dissolved into aqueous solutions in quantities that can affect test results. Materials and equipment that contact stock or test solutions should be chosen to minimize sorption of test chemicals from dilution water. Glass, #316 stainless steel, nylon screen and perfluorocarbon plastic (e.g., Teflon®) are acceptable materials. Concrete or rigid (unplasticized) plastic may be used for holding and acclimation tanks, and for water supply systems, but they should be thoroughly conditioned before use. If cast iron pipe is used in freshwater supply systems, colloidal iron may leach into the dilution water and strainers should be used to remove rust particles. Natural rubber, copper, brass, galvanized metal, epoxy glues, and flexible tubing should not come in contact with dilution water, stock solutions, or test solutions.

(ii) *Test chambers* (exposure chambers). (A) Stainless steel test chambers should be welded or glued with silicone adhesive, and not soldered. Glass should be fused or bonded using clear silicone adhesive. Epoxy glues are not recommended, but if used ample curing time should be allowed prior to use. As little adhesive as possible should be in contact with the water.

(B) Many different sizes of test chambers have been used successfully. The size, shape and depth of the test chamber is acceptable if the specified flow rate and loading requirements can be achieved.

(C) The actual arrangement of the test chambers can be important to the statistical analysis of the test data. Test chambers can be arranged totally on one level (tier) side by side, or on two levels with each level having one of the replicate test substance concentrations or controls. Regardless of the arrangement, it should be reported in detail and considered in the data analysis.

(iii) *Embryo incubation apparatus.* (A) Recommended embryo incubation apparatus include embryo cups for the minnow species and screen trays for the trout species, although embryo cups can be used for the trout species. Embryo cups are normally constructed from approximately 4–5 cm inside diameter, 7–8 cm high, glass jars with the end cut off or similar sized sections of polyethylene tubing. One end of the jar or tubing is covered with stainless steel or nylon screen (approximately 40 meshes per inch is recommended). Embryo cups for silversides are normally constructed by using silicone adhesive to glue a 10-cm high, 363-um nylon mesh tube inside a 9-cm I.D. glass Petri dish bottom. The embryo cups should be appropriately labeled and then suspended in the test chamber in such a manner as to ensure that the test solution regularly flows through the cup and that the embryos are always submerged but are not agitated too vigorously. Cups may be oscillated by a rocker arm apparatus with a low rpm motor (e.g., 2 rpm) to maintain the required flow of test water. The vertical-travel distance of the rocker arm apparatus during oscillation is normally 2.5–4.0cm. The water level in the test chambers may also be varied by means of a self-starting siphon in order to ensure exchange of water in the embryo cups.

(B) The trout embryo incubation trays can be made from stainless steel screen (or other acceptable material such as plastic) of about 3–4 mm mesh. The screen tray should be supported above the bottom of the test chamber by two folds of screen or other devices which function as legs or supports. The edges of the screen tray should be turned up to prevent bump spills and to prevent the embryos from rolling off in the event of excessive turbulence. Suspending or supporting the screen tray off the bottom ensures adequate water circulation around the embryos and avoids contact of embryos with possible bottom debris.

(iv) *Test substance delivery system.* (A) The choice of a specific delivery system depends upon the specific properties and requirements of the test substance. The apparatus used should accurately and precisely deliver the appropriate amount of stock solution and dilution water to the test chambers. The system selected should be calibrated before each test. Calibration includes determining the flow rate through each chamber, and the proportion of stock solution to dilution water delivered to each chamber. The generated operation of the test substance delivery system should be checked twice daily for normal operation throughout the test. A minimum of five test substance concentrations and one control should be used for each test.

(B) The proportional diluter and modified proportional diluter systems and metering pump systems have proven suitable and have received extensive use.

(C) Mixing chambers should be used between the diluter and the test chamber(s). This may be a small container or flow-splitting chamber to promote mixing of test substance stock solution and dilution water, and is positioned between the diluter and the test chambers for each concentration. If a proportional diluter is used, separate delivery tubes should run from the flow-splitting chamber to each replicate test chamber. Daily checks on this latter system should be made.

(D) Silverside fry are injured easily and are susceptible to impingement on the mesh of the incubation cups. Consequently, water flow into and out of the cups when counting fry must be at a slow rate. This can be accomplished by using small diameter (e.g., 2 mm I.D.) capillary tubes to drain the test solution from spitter boxes into the replicate test chambers. The use of a self-starting siphon to gradually lower (i.e., less than or equal to 1 min.) the water level approximately 2 cm in the test chamber is recommended. A minimum water depth of 5 cm should be maintained in the cups. Although it may be satisfactory, a rocker-arm type apparatus has not yet been used with silversides.

(v) *Other equipment required.* (A) An apparatus for removing undesirable organisms, particulate matter and air bubbles.

(B) An apparatus for aerating water.

(C) A suitable magnifying viewer for examination of minnow embryos.

(D) A suitable apparatus for the precise measurement of growth of the fish, including both length (e.g., with metric or ruler caliper or photographic equipment) and weight.

(E) Facilities for providing a continuous supply of live brine shrimp nauplii (*Artemia salina*).

(F) For silversides, facilities for providing a supply of rotifers (*Brachionus plicatilis*) for approximately 11 days.

(G) Facilities (or access to facilities) for performing the required water chemistry analyses.

(vi) *Cleaning of equipment.* (A) Test substance delivery systems and test chambers should be cleaned before use. Test chambers should be cleaned during the test as needed to maintain the dissolved oxygen concentration, and to prevent clogging of the embryo cup screens and narrow flow passages.

(B) Debris can be removed with a rubber bulb and large pipette or by siphoning with a glass tube attached to a flexible hose. Debris should be run into a bucket light enough to observe that no live fish are accidentally discarded.

(vii) *Dilution water*—(A) *General.* (*1*) A constant supply of acceptable dilution water should be available for use throughout the test. Dilution water should be of a minimum quality such that the test species selected will survive in it for the duration of testing without showing signs of stress (e.g., loss of pigmentation, disorientation, poor response to external stimuli, excessive mucous secretion, lethargy, lack of feeding or other unusual behavior). A better criterion for an acceptable dilution water for tests on early life stages should be such that the species selected for testing will survive, grow and reproduce satisfactorily in it.

(*2*) The concentration of dissolved oxygen in the dilution water (fresh or salt) should be between 90% and 100% saturation. When necessary, dilution water should be aerated by means of airstones, surface aerators, or screen tubes before the introduction of the test substance.

(*3*) Water that is contaminated with undesirable microorganisms (e.g., fish pathogens) should not be used. If such contamination is suspected, the water should be passed through a properly maintained ultraviolet sterilizer equipped with an intensity meter before use. Efficacy of the sterilizer can be determined by using standard plate count method.

(B) *Freshwater.* (*1*) Natural water (clean surface or ground water) is preferred, however, dechlorinated tap water may be used as a last resort. Reconstituted freshwater is not recommended as a practical dilution water for the early life stage toxicity test because of the large volume of water required.

(*2*) Particulate and dissolved substance concentrations should be measured at least twice a year and should meet the following specifications.

Substance	Concentration maximum
Particulate matter	<20 mg/liter.
Total organic carbon (TOC)	<2 mg/liter.
Chemical oxygen demand (COD)	<5 mg/liter.
Un-ionized ammonia	<1 µg/liter.
Residual chlorine	<1 µg/liter.
Total organoposphorus pesticides	<50 ng/liter.
Total organochlorine pesticides plus polychlorinated biphenyls (PCBs).	<50 ng/liter.
Total organic chlorine	<25 ng/liter.

(*3*) During any one month, freshwater dilution water should not vary more than 10 percent from the respective monthly averages of hardness, alkalinity and specific conductance; the monthly pH range should be less than 0.4 pH units.

(C) *Saltwater.* (*1*) Marine dilution water is considered to be of constant quality if the minimum salinity is greater than 15 ‰ and the weekly range of the salinity is less than 15 ‰. The monthly range of pH should be less than 0.8 pH units. Saltwater should be filtered to remove larval predators. A pore size of <20 micrometers (um) is recommended. For silversides, the recommended salinity is 20 ppt and should be maintained between 15 and 25 ppt throughout testing.

(*2*) Artificial sea salts may be added to natural seawater during periods of low salinity to maintain salinity above 15 ‰.

(3) *Test parameters*—(i) *Dissolved oxygen concentration.* It is recommended that the dissolved oxygen concentration be maintained between 90 and 100 percent saturation; but it should be no less than 75 percent saturation at all times for both minnow species and between 90 and 100 percent saturation for the trout species in all test chambers. Dilution water in the head box may be aerated, but the test solution itself should not be aerated.

(ii) *Loading and flow rate.* (A) The loading in test chambers should not exceed 0.1 grams of fish per liter of test solution passing through the test chamber in 24 hours. The flow rate to each chamber should be a minimum of 6 tank volumes per 24 hours. During a test, the flow rates should not vary more than 10 percent from any one test chamber to any other.

(B) A lower loading or higher flow rate or both should be used if necessary to meet the following three criteria at all times during the test in each chamber containing live test organisms: (*1*) the concentration of dissolved oxygen should not fall below 75 percent saturation for the fathead and sheepshead minnows and 90 percent for the rainbow and brook trout; (*2*) the concentration of un-ionized ammonia should not exceed 1 µg/liter; and (*3*) the concentration of toxicant should not be lowered (i.e., caused by uptake by the test organisms and/or materials on the sides and bottoms of the chambers) more than 20 percent of the mean measured concentration.

(iii) *Temperature.* (A) The recommended test temperatures are:

(*1*) Fathead minnow——25 °C for all life stages.

(*2*) Sheepshead minnow——30 °C for all life stages.

(*3*) Rainbow and brook trout——10 °C for embryos. 12 °C for fry and alevins.

(*4*) Atlantic and tidewater silversides——25 °C for all life stages.

(B) The actual test temperature during the duration of the test should remain within 1.5 °C of the selected test temperature. It is recommended that the test system be equipped with an automatic alarm system to alert staff of instantaneous temperature changes in excess of 2 °C. If the water is heated (i.e., for minnow species), precautions should be taken to ensure that supersaturation of dissolved gases is avoided. Temperatures should be recorded in all test chambers at the beginning of the test and weekly thereafter. The temperature should be recorded at least hourly in one test chamber throughout the test.

(iv) *Light.* (A) Brook and rainbow trout embryos should be maintained in darkness or very low light intensity through one week post-hatch, at which time a 14-hour light and 10-hour dark photoperiod should be provided.

(B) For fathead and sheepshead minnows, a 16-hour light and 8-hour dark (or 12:12) photoperiod should be used throughout the test period.

(C) For silversides, a 14-hour light and 10-hour dark photoperiod should be used throughout the test period.

(D) A 15-minute to 30-minute transition period between light and dark is optional.

(F) Light intensities ranging from 30 to 100 lumens at the water surface should be provided; the intensity selected should be duplicated as closely as possible for all test chambers.

(E) Light intensities ranging from 30 to of an early life stage toxicity test should include the following:

(1) Name of test, sponsor, investigator, laboratory, and dates of test duration.

(2) Detailed description of the test substance including its source, lot number, composition (identity and concentration of major ingredients and major impurities), known physical and chemical properties, and any carriers (solvents) or other additives used.

(3) The source of the dilution water, its chemical characteristics, and a description of any pretreatment.

(4) Detailed information about the test organisms including scientific name and how verified and source history, observed diseases, treatments, acclimation procedure, and concentration of any contaminants and the method of measurement.

(5) A description of the experimental design and the test chambers, the depth and volume of the solution in the chambers, the way the test was begun, the number of organisms per treatment, the number of replicates, the loading, the lighting, a description of the test substance delivery system, and the flow rate as volume additions per 24 hours.

(6) Detailed information on feeding of fish during the toxicity test, including type of food used, its source, feeding frequency and results of analysis (i.e., concentrations) for contaminants.

(7) Number of embryos hatched, number of healthy embryos, time to hatch, mortality of embryos and fry, measurements of growth (weight and length), incidence of pathological or histological effects and observations of other effects or clinical signs, number of healthy fish at end of test.

(8) Number of organisms that died or showed an effect in the control and the results of analysis for concentration(s) of any contaminant in the control(s) should mortality occur.

(9) Methods used for, and the results of (with standard deviation), all chemical analyses of water quality and test substance concentration, including validation studies and reagent blanks; the average and range of the test temperature(s).

(10) Anything unusual about the test, any deviation from these procedures, and any other relevant information.

(11) A description of any abnormal effects and the number of fish which were affected during each period between observations in each chamber, and the average concentration of test substance in each test chamber.

(12) Reference to the raw data location.

§ 797.1800 Oyster acute toxicity test.

(a) *Purpose.* This guideline will be used in developing data on the acute toxicity of chemical substances and mixtures ("chemicals") subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94–469, 90 Stat. 2003, 15 U.S.C. 2601 *et. seq.*). This guideline prescribes tests to be used to develop data on the acute toxicity of chemicals to Eastern oysters, *Crassostrea virginica* (Gmelin). The United States Environmental Protection Agency (U.S. EPA) will use data from these tests in assessing the hazard of a chemical to the environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA) and in Part 792—Good Laboratory Practice Standards of this chapter are applicable to this test guideline. The following definitions also apply:

(1) "Acute toxicity" is the discernible adverse effects induced in an organism within a short period of time (days) of exposure to a chemical. For aquatic animals this usually refers to continuous exposure to the chemical in water for a period of up to four days. The effects (lethal or sublethal) occurring may usually be observed within the period of exposure with aquatic organisms. In this test guideline, shell deposition is used as the measure of toxicity.

(2) "EC_{50}" is that experimentally derived concentration of a chemical in water that is calculated to induce shell deposition 50 percent less than that of the controls in a test batch of organisms during continuous exposure within a particular exposure period which should be stated.

(3) "Shell deposition" is the measured length of shell growth that occurs between the time the shell is ground at test initiation and test termination 96 hours later.

(4) "Umbo" means the narrow end (apex) of the oyster shell.

(5) "Valve height" means the greatest linear dimension of the oyster as measured from the umbo to the ventral edge of the valves (the farthest distance from the umbo).

(c) *Test procedures*—(1) *Summary of the test.* (i) The water solubility and the vapor pressure of the test chemical should be known. Prior to testing, the structural formula of the test chemical, its purity, stability in water and light, n-octanol/water partition coefficient, and pK_a values should be known prior to testing. The results of a biodegradability test and the method of analysis for the quantification of the chemical in water should also be known.

(ii) For chemicals with limited solubility under the test conditions, it may not be possible to determine an EC_{50}. If it is observed that the stability or homogeneity of the test chemical cannot be maintained, then care should be taken in the interpretation of the results and a note made that these results may not be reproducible.

(iii) Test chambers are filled with appropriate volumes of dilution water. The flow of dilution water through each chamber is adjusted to the rate desired. The test chemical is introduced into each test chamber and the flow-rate adjusted to establish and maintain the desired concentration in each test chamber. Test oysters which have been acclimated and prepared by grinding away a portion of the shell periphery are randomly introduced into the test and control chambers. Oysters in the test and control chambers are observed daily during the test for evidence of feeding or unusual conditions, such as shell gaping, excessive mucus production or formation of fungal growths in the test chambers. The observations are recorded and dead oysters removed. At the end of 96 hours the increments of new shell growth are measured in all oysters. The concentration-response curve and EC_{50} value for the test chemical are developed from these data.

(2) [Reserved]

(3) *Range-finding test.* A range-finding test should be conducted to establish test chemical concentrations for the definitive test. The test is conducted in the same way as the definitive test except a widely spaced chemical concentration series (i.e., log-interval) is used.

(4) *Definitive test.* (i) Oysters which meet condition criteria (age, size, reproductive status, health) and which have been acclimated to test conditions should have approximately 3 to 5 mm of the shell periphery, at the rounded (ventral) end, ground away with a small electric disc grinder or other appropriate device, taking care to uniformly remove the shell rim to produce a smooth, rounded blunt profile. The oyster's valves should be held together tightly during grinding to avoid vibrating the shell and injuring the adductor muscle. Oysters of which so much of the shell rim has been removed that an opening into the shell cavity is visible should not be used.

(ii) It is desirable to have shell growth values for the low and high concentrations relatively close to, but different from, 0 and 100 percent. Therefore, the range of concentrations to which the oysters are exposed should be such that in 96 hours relative to the controls, very little shell growth occurs in oysters exposed to the highest concentration and shell growth is slightly less than controls at the lowest concentration. Oysters in the remaining concentrations should have increments of shell growth, such that ideally, the concentration producing 50 percent shell growth relative to the growth is bracketed with at least one concentration above and one below it.

(iii) The test should be carried out without adjustment of pH unless there is evidence of marked change in the pH of the solution. Then it is advised that the test be repeated with pH adjustment to that of the dilution water and the results reported.

(iv) The test begins when at least 20 prepared oysters are placed in each of the test chambers containing the appropriate concentrations of test substance and controls. The steady-state flows and test chemical concentrations should be documented. At least 5 test chemical concentrations should be used. The dilution factor between concentrations should not exceed 1.8.

(v) The distribution of individual oysters among the test chambers should be randomized. The oysters should be spread out equidistantly from one another so that the entire test chamber is used. The oysters should also be placed with the left (cupped) valve down and the open, unhinged ends all oriented in the same direction facing the incoming flow of test solution.

(vi) The oysters are inspected at least after 24, 48, 72 and 96 hours. Oysters are considered dead if touching of the gaping shell produces no reaction. Dead oysters are removed when observed and mortalities are recorded. Observations at 3 hours and 6 hours are also desirable.

(vii) Shell growth is the primary criterion used in this test guideline to evaluate the toxicity of the test chemical. Shell growth increments in all oysters should be measured after 96 hours exposure. Record the length of the longest "finger" of new shell growth to the nearest 0.5 mm. Oysters should be handled very gently at this stage to prevent damage to the new shell growth.

(viii) Records should be kept of visible abnormalities such as loss of feeding activity (failure to deposit feces), excessive mucus production (stringy material floating suspended from oysters), spawning or appearance of shell (closure or gaping).

(ix) The criteria for a valid definitive test are:

(A) The mortality in the controls should not exceed 10 percent at the end of the test.

(B) The dissolved oxygen concentration should be at least 60 percent of air saturation throughout the test.

(C) Oysters should not spawn during test. If they do the test should be repeated with prespawn oysters.

(D) There should be evidence that the concentration of the substance being tested has been satisfactorily maintained (e.g., within 80 percent of the nominal concentration) over the test period. The total concentration of test substance (i.e., both dissolved and suspended undissolved particulates) should be measured: (*1*) in each chamber at 0-hour, (*2*) in each chamber at 96-hours and (*3*) in at least one appropriate chamber whenever a malfunction is detected in any part of the test chemical delivery system.

(E) Dissolved oxygen, temperature, salinity and pH measurements should be made at the beginning of the test, at 48 hours, and at the end of the test in the control chambers and in those test chambers containing the highest, lowest and a middle concentration of the test substance.

(5) *Test results*. (i) At the end of the test, a one-way analysis of variance followed with an appropriate ad hoc test (the Studentized Neuman-Keul's or Duncan's multiple range tests; or Dunnetts' or Williams' pairwise comparison tests) should be conducted on the oyster shell deposition test data. The probit transformation should then be applied to the response variable and then regressed, using least squares regression, on dose or log-dose. An F Test for linearity should be conducted to determine whether the chosen regression technique adequately describes the experimental data.

(ii) Calculate the ratio of the mean shell growth for each group of test oysters (exposed to each of the test chemical concentrations) to the mean shell growth of the group of control oysters. From these data the concentration-response curve is drawn and an EC_{50} along with the 95 percent confidence limits on the value are determined from the curves. The mean measured concentration of test chemical should be used to calculate the EC_{50} and to plot the concentration-response curve.

(6) [Reserved]

(d) *Test conditions*—(1) *Test species*—(i) *Selection*. (A) The Eastern oyster, *Crassostrea virginica*, should be used as the test organism.

(B) Oysters used in the same test should be 30 to 50 millimeters in valve height and should be as similar in age and/or size as possible to reduce variability. The standard deviation of the valve height should be less than 20 percent of the mean.

(C) Oysters used in the same test should be from the same source and from the same holding and acclimation tank(s).

(D) Oysters should be in a prespawn condition of gonadal development prior to and during the test as determined by direct or histological observation of the gonadal tissue for the presence of gametes.

(ii) *Acquisition*. Oysters may be cultured in the laboratory, purchased from culture facilities or commercial harvesters, or collected from a natural population in an unpolluted area free from epizootic disease.

(iii) *Acclimation*. (A) Oysters should be attended to immediately upon arrival. Oyster shells should be brushed clean of fouling organisms and the transfer of the oysters to the holding water should be gradual to reduce stress caused by differences in water quality characteristics and temperature. Oysters should be held for at least 12 to 15 days before testing. All oysters should be maintained in water of the quality to be used in the test for at least 7 days before they are used.

(B) During holding, the oysters should not be crowded and the dissolved oxygen concentration should be above 60 percent saturation. The temperature of the holding water should be the same as that used for testing. Holding tanks should be kept clean and free of debris. Cultured algae may be added to dilution water sparingly, as necessary to support life and growth and such that test results are not affected as confirmed by previous testing.

(C) Oysters should be handled as little as possible. When handling is necessary, it should be done as gently, carefully, and quickly as possible.

(D) A batch of oysters is acceptable for testing if the percentage mortality over the 7 day period prior to testing is less than 5 percent. If the mortality is between 5 and 10 percent, acclimation should continue for seven additional days. If the mortality is greater than 10 percent, the entire batch of oysters should be rejected. Oysters should not be used which appear diseased or otherwise stressed. Oysters infested with mudworms (*Polydora* sp.), boring sponges (*Cliona cellata*) or which have cracked, chipped, bored, or gaping shells should not be used.

(2) *Test facilities*—(i) *Apparatus*. (A) In addition to normal laboratory equipment, an oxygen meter, equipment for delivering the test chemical, adequate apparatus for temperature control, and test tanks made of chemically inert material are needed.

(B) Constant conditions in the test facilities should be maintained as much as possible throughout the test. The preparation and storage of the test material, the holding of the oysters and all operations and tests should be carried out in an environment free from harmful concentrations of dust, vapors and gases and in such a way as to avoid cross-contamination. Any disturbances that may change the behavior of the oysters should be avoided.

(ii) *Dilution water*. A constant supply of good quality unfiltered seawater should be available throughout the holding, acclimation and testing periods. Natural seawater is recommended, although artificial seawater with food added may be used. In either case, to ensure each oyster is provided equal amounts of food, the water should come from a thoroughly mixed common source and should be delivered at a flowrate of at least one and preferably five liters per hour per oyster. The flowrate should be ±10 percent of the nominal flow. A dilution water is acceptable if oyster will survive and grow normally for 14 days without exhibiting signs of stress; i.e., excessive mucus production (stringy material floating suspended from oysters), lack of feeding, shell gaping, poor shell closing in response to prodding, or excessive mortality. The dilution water should have a salinity in excess of 12 parts per thousand, and should be similar to that in the environment from which the test oysters originated. A natural seawater should have a weekly range in salinity of less than 10 parts per thousand and a monthly range in pH of less than 0.8 units. Artificial seawater salinity should not vary more than 2 parts per thousand nor more than 0.5 pH units. Oysters should be tested in dilution water from the same origin.

(3) *Test parameters*—(i) *Carriers*. Stock solutions of substances of low aqueous solubility may be prepared by ultrasonic dispersion or, if necessary, by use of organic solvents, emulsifiers or dispersants of low toxicity to oysters. When such carriers are used the control oysters should be exposed to the same concentration of the carrier as that used in the highest concentration of the test substance. The concentration of such carriers should not exceed 0.1 ml/l.

(ii) *Dissolved oxygen*. The dissolved oxygen concentrations should be at least 60 percent of the saturation value and should be recorded daily.

(iii) *Loading*. The loading rate should not crowd oysters and should permit adequate circulation of water while avoiding physical agitation of oysters by water current.

(iv) *Temperature*. The test temperature is 20 °C ± 1 °C. Temporary fluctuations (less than 8 hours) within 15 °C to 25 °C are permissible. Temperature should be recorded continuously.

(v) *pH*. The pH should be recorded twice weekly in each test chamber.

(e) *Reporting*. In addition to the reporting requirements prescribed in Part 792—Good Laboratory Practice Standards of this chapter, the report should contain the following:

(1) The source of the dilution water, the mean, standard deviation and range

of the salinity, pH, temperature, and dissolved oxygen during the test period.

(2) A description of the test procedures used (e.g., the flow-through system, test chambers, chemical delivery system, aeration, etc.).

(3) Detailed information about the oysters used, including the age and/or size (i.e., height), source, history, method of confirmation of prespawn condition, acclimation procedures, and food used.

(4) The number of organisms tested, the loading rate, and the flowrate.

(5) The methods of preparation of stock and test solutions, and the test chemical concentrations used.

(6) The number of dead and live test organisms, the percentage of organisms that died, and the number that showed any abnormal effects in the control and in each test chamber at each observation period.

(7) The 96-hour shell growth measurements of each oyster; the mean, standard deviation and range of the measured shell growth at 96 hours of oysters in each concentration of test substance and control.

(8) The calculated 96 hour EC_{50} and its 95 percent confidence limits and the statistical methods used to calculate these values.

(9) When observed, the 96 hour observed no-effect concentration (the highest concentration tested at which there were no mortalities, abnormal behavioral or physiological effects and at which shell growth did not differ from controls).

(10) A graph of the concentration-response curve based on the 96 hour chemical concentration and shell growth measurements upon which the EC_{50} was calculated.

(11) Methods and data records of all chemical analyses of water quality parameters and test substance concentrations, including method validations and reagent blanks.

(12) Any incidents in the course of the test which might have influenced the results.

(13) A statement that the test was carried out in agreement with the prescriptions of the test guideline given above (otherwise a description of any deviations occurring).

§ 797.1830 Oyster bioconcentration test.

(a) *Purpose.* This guideline is to be used for assessing the propensity of chemical substances to bioconcentrate in tissues of estuarine and marine molluscs. This guideline describes a bioconcentration test procedure for the continuous exposure of Eastern oysters (*Crassostrea virginica*) to a test substance in a flow-through system. The United States Environmental Protection Agency (U.S. EPA) will use data from this test in assessing the hazard a chemical may present to the environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA) and in Part 792—Good Laboratory Practice Standards of this chapter are applicable to this test guideline. The following definitions also apply:

(1) "Acclimation" is the physiological compensation by test organisms to new environmental conditions (e.g., temperature, salinity, pH).

(2) "Bioconcentration" is the net accumulation of a chemical directly from water into and onto aquatic organisms.

(3) "Bioconcentration factor (BCF)" is the quotient of the concentration of a test chemical in tissues of aquatic organisms at or over a discrete time period of exposure divided by the concentration of test chemical in the test water at or during the same time period.

(4) "Depuration" is the elimination of a test chemical from a test organism.

(5) "Depuration phase" is the portion of a bioconcentration test after the uptake phase during which the organisms are in flowing water to which no test chemical is added.

(6) "EC_{50}" is that experimentally derived concentration of a chemical in water that is calculated to induce shell deposition 50 percent less than that of the controls in a test batch of organisms during continuous exposure within a particular period of exposure (which should be stated).

(7) "Loading" is the ratio of the number of oysters to the volume (liters) of test solution passing through the test chamber per hour.

(8) "Steady-state" is the time period during which the amounts of test chemical being taken up and depurated by the test oysters are equal, i.e., equilibrium.

(9) "Steady-state bioconcentration factor" is the mean concentration of the test chemical in test organisms during steady-state divided by the mean concentration of the test chemical in the test solution during the same period.

(10) "Umbo" is the narrow end (apex) of the oyster shell.

(11) "Uptake" is the sorption of a test chemical into and onto aquatic organisms during exposure.

(12) "Uptake phase" is the initial portion of a bioconcentration test during which the organisms are exposed to the test solution.

(13) "Valve height" is the greatest linear dimension of the oyster as measured from the umbo to the ventral edge of the valves (the farthest distance from the umbo).

(c) *Test procedures*— (1) *Summary of the test.* Oysters are continuously exposed to a minimum of one constant, sublethal concentration of a test chemical under flow-through conditions for a maximum of 28 days. During this time, test solution and oysters are periodically sampled and analyzed using appropriate methods to quantify the test chemical concentration. If, prior to day 28, the tissue concentrations of the chemical sampled over three consecutive sampling periods have been shown to be statistically similar (i.e., steady-state has been reached), the uptake phase of the test is terminated, and the remaining oysters are transferred to untreated flowing water until 95 percent of the accumulated chemical residues have been eliminated, or for a maximum depuration period of 14 days. The mean test chemical concentration in the oysters at steady-state is divided by the mean test solution concentration at the same time to determine the bioconcentration factor (BCF). If steady-state is not reached during 28 days of uptake, the steady-state BCF should be calculated using non-linear parameter estimation methods.

(2) [Reserved]

(3) *Range-finding test.* The oyster acute toxicity test is used to determine the concentration levels to be used in the oyster bioconcentration test.

(4) *Definitive test.* (i) The following data on the test chemical should be known prior to testing:

(A) Solubility in water.

(B) Stability in water.

(C) Octanol-water partition coefficient.

(D) Acute toxicity (e.g., propensity to inhibit shell deposition) to oysters.

(E) The validity, accuracy and minimum detection limits of selected analytical methods.

(ii) At least one or more concentrations should be tested to assess the propensity of the compound to bioconcentrate. The concentrations selected should not stress or adversely affect the oysters and should be less than one-tenth the EC_{50} determined in either the range-finding or 96-hour definitive test under § 797.1800. The test concentration should be less than the solubility limit of the test substance in water and should be close to the potential or expected environmental concentration. The limiting factor of how low one can test is based on the detection limits of the analytical methods. The concentration of the test material in the test solution should be at least 10 times greater than the detection limit in water.

(iii) If it is desirable to document that the potential to bioconcentrate is independent of the test chemical concentration, at least two concentrations should be tested which are at least a factor of 10 apart.

(iv) To determine the duration of this test, an estimation of the uptake phase should be made prior to testing based upon the water solubility or octanol-water partition coefficient of the test chemical. This estimate should also be used to designate a sampling schedule.

(v) The following criteria should be met for a valid test:

(A) If it is observed that the stability or homogeneity of the test chemical cannot be maintained, then care should be taken in the interpretation of the results and a note made that these results may not be reproducible.

(B) The mortality in the controls should not exceed 10 percent at the end of the test.

(C) The dissolved oxygen concentration should be > 60 percent of saturation throughout the test.

(D) There should be evidence that the concentration of the chemical being tested has been satisfactorily maintained (e.g., within 80 percent of the nominal concentration) over the test period.

(E) Results are invalid and the test should be repeated if the oysters spawn during the test.

(F) Temperature variations from 20 °C should be held to a minimum.

(vi) The following methodology should be followed:

(A) The test should not be started until the test chemical delivery system has been observed to be functioning properly and the test chemical concentrations have equilibrated (i.e., the concentration does not vary more than 20 percent). Analyses of two sets of test solution samples taken prior to test initiation should document this equilibrium. At initiation (time 0), test solution samples should be collected immediately prior to the addition of oysters to the test chambers.

(B) The appropriate number of oysters should be brushed clean and should be randomly distributed to each test chamber. The oysters should be spread out equidistant from one another and placed with the left (cupped) valve down and the unhinged ends (opposite from umbo) all oriented in the same direction facing the incoming flow.

(C) Oysters should be exposed to the test chemical during the uptake phase until steady state has been reached or for a maximum of 28 days. The uptake phase should continue for at least 4 days. Then the remaining oysters should be transferred to untreated flowing water and sampled periodically to determine if depuration of the test chemical occurs. Every test should include a control consisting of the same dilution water, conditions, procedures, and oysters from the same group used in the test, except that none of the test chemical is added. If a carrier is present in the test chamber, a separate carrier control is required.

(D) Oysters should be observed (and data recorded) at least daily for feeding activity (deposition of feces) or any unusual conditions such as excessive mucus production (stringy material floating suspended from oysters), spawning, or appearance of shell (closure or gaping). If gaping is noted, the oyster(s) should be prodded. Oysters which fail to make any shell movements when prodded are to be considered dead, and should be removed promptly with as little disturbance as possible to the test chamber(s) and remaining live oysters.

(E) For oysters sampled, careful examination of all the tissues should be made at the time of shucking for any unusual conditions, such as a watery appearance or differences in color from the controls.

(F) Observations on compound solubility should also be recorded. These include the appearance of surface slicks, precipitates, or material adsorbing to the test chamber.

(vii) *Sampling.* (A) At each of the designated sampling times, triplicate water samples and enough oysters should be collected from the test chamber(s) to allow for tissue analyses of at least four oysters. The concentration of test chemical should be determined in a minimum of four oysters analyzed individually at each sampling period. If individual analysis is not possible, due to limitations of the sensitivity of the analytical methods, then pairs, triplicates or more oysters may be pooled to constitute a sample for measurement. A similar number of control oysters should also be collected at each sample point, but only those collected at the first sampling period and weekly thereafter, should be analyzed. Triplicate control water samples should be collected at the time of test initiation and weekly thereafter. Test solution samples should be removed from the approximate center of the water column.

(B) At each sampling period the appropriate numbers of oysters are removed and treated as follows:

(*1*) The valve height of each oyster should be measured.

(*2*) Oysters should be shucked as soon as practical after removal and should never be refrigerated or frozen in the shell. The shell should be opened at the hinge, the adductor muscle severed and the top valve removed. The remaining adductor muscle should be severed where it attaches to the lower valve and the entire oyster removed.

(*3*) The shucked oysters should then be drained 3 minutes, blotted dry, weighed and analyzed immediately for the test chemical. If analyses are delayed, the shucked oysters should be wrapped individually in aluminum foil (for organic analysis) or placed in plastic or glass containers (for metal analysis) and frozen.

(C) If a radiolabelled test compound is used, a sufficient number of oysters should also be sampled at termination to permit identification and quantitation of any major (greater than 10 percent of parent) metabolites present. It is crucial to determine how much of the activity present in the oyster is directly attributable to the parent compound.

(5) *Test results* (i) Steady-state has been reached when the mean concentrations of test chemical in whole oyster tissue for three consecutive sampling periods are statistically similar (F test, $P=0.05$). A BCF is then calculated by dividing the mean tissue residue concentration during steady-state by the mean test solution concentration during the same period. A 95 percent confidence interval should also be derived from the BCF. This should be done by calculating the mean oyster tissue concentration at steady-state (X_o) and its 97.5 percent confidence interval $X_o \pm t$ (S.E.) where t is the t statistic at $P=0.025$ and S.E. is the one standard error of the mean. This calculation would yield lower and upper confidence limits (L_o and U_o). The same procedure should be used to calculate the mean and 97.5 percent confidence interval for the test solution concentrations at steady-state, $XS \pm t$ (S.E.), and the resulting upper and lower confidence limits (L_s and U_s). The 95 percent confidence interval of the BCF would then be between L_o/U_s and U_o/L_s. If steady-state was not reached during the maximum 28-day uptake period, the maximum BCF should be calculated using the mean tissue concentration from that and the previous sampling day. An uptake rate constant should then be calculated using appropriate techniques. This rate constant is used to estimate the steady-state BCF and the time to steady-state.

(ii) If 95 percent elimination has not been observed after 14 days depuration then a depuration rate constant should also be calculated. This rate constant is used to estimate the time to 95 percent elimination.

(iii) Oysters used in the same test should be 30 to 50 millimeters in valve height and should be as similar in age and/or size as possible to reduce variability. The standard deviation of the height should be less than 20 percent of the mean (N=30).

(6) *Analytical measurements.* (i) All samples should be analyzed using USEPA methods and guidelines whenever feasible. The specific methodology used should be validated before the test is initiated. The accuracy of the method should be measured by the method of known additions. This involves adding a known amount of the test chemical to three water samples taken from an aquarium containing dilution water and a number of oysters equal to that to be used in the test. The nominal concentration of these samples should be the same as the concentration to be used in the test. Samples taken on two separate days should be analyzed. The accuracy and precision of the analytical method should be checked using reference or split samples or suitable corroborative methods of analysis. The accuracy of standard solutions should be checked against other standard solutions whenever possible.

(ii) An analytical method should not be used if likely degradation products of the test chemical, such as hydrolysis and oxidation products, give positive or negative interferences, unless it is shown that such degradation products are not present in the test chambers during the test. Atomic absorption spectrophotometric methods for metal and gas chromatographic methods for organic compounds are preferable to colorimetric methods.

(iii) In addition to analyzing samples of test solution at least one reagent blank should also be analyzed when a reagent is used in the analysis.

(iv) When radiolabelled test compounds are used, total radioactivity should be measured in all samples. At the end of the uptake phase, water and tissue samples should be analyzed using appropriate methodology to identify and estimate the amount of any major (at least 10 percent of the parent compound) degradation products or metabolites that may be present.

(d) *Test conditions*—(1) *Test species.* (i) The Eastern oyster, *Crassostrea virginica*, should be used as the test organism.

(ii) Oysters used in the same test should be 30 to 50 millimeters in valve height and should be as similar in age and/or size as possible to reduce variability. The standard deviation of the valve height should be less than 20 percent of the mean.

(iii) Oysters used in the same test should be from the same source and from the same holding and acclimation tank(s).

(iv) Oysters should be in a prespawn condition of gonadal development prior to and during the test as determined by direct or histological observation of the gonadal tissue for the presence of gametes.

(v) Oysters may be cultured in the laboratory, purchased from culture facilities or commercial harvesters, or collected from a natural population in an unpolluted area free from epizootic disease.

(vi) The holding and acclimation of the oysters should be as follows:

(A) Oysters should be attended to immediately upon arrival. Oyster shells should be brushed clean of fouling organisms and the transfer of the oysters to the holding water should be gradual to reduce stress caused by differences in water quality characteristics and temperature. Oysters should be held for at least 12 to 15 days before testing. All oysters should be maintained in water of the quality to be used in the test for at least 7 days before they are used.

(B) During holding, the oyster should not be crowded and the dissolved oxygen concentration should be above 60 percent saturation. The temperature of the holding waters should be the same as that used for testing. Holding tanks should be kept clean and free of debris. Cultured algae may be added to dilution water sparingly, as necessary to support life and growth, such that test results are not affected, as confirmed by previous testing. Oysters should be handled as little as possible. When handling is necessary, it should be done as gently, carefully, and quickly as possible.

(C) A batch of oysters is acceptable for testing if the percentage mortality over the 7 day period prior to testing is less than 5 percent. If the mortality is between 5 and 10 percent, acclimation should continue for 7 additional days. If the mortality is greater than 10 percent, the entire batch of oysters should be rejected. Oysters should not be used which appear diseased or otherwise stressed. Oysters infested with mudworms (*Polydora* sp.), boring sponges (*Cliona cellata*) or which have cracked, chipped, bored, or gaping shells should not be used.

(2) *Facilities*—(i) *Apparatus.* (A) An oxygen meter, equipment for delivering the test chemical, adequate apparatus for temperature control, test tanks made of chemically inert material and other normal laboratory equipment are needed.

(B) Constant conditions in the test facilities should be maintained as much as possible throughout the test. The preparation and storage of the test material, the holding of the oysters and all operations and tests should be carried out in an environment free from harmful concentrations of dust, vapors and gases and in such a way as to avoid cross-contamination. Any disturbances that may change the behavior of the oysters should be avoided.

(ii) *Dilution water.* A constant supply of good quality unfiltered seawater should be available throughout the holding, acclimation, and testing periods. Natural seawater is recommended, although artificial seawater with food (algae) added may be used. In either case, to ensure each oyster is provided equal amounts of food, the water should come from a thoroughly mixed common source and should be delivered at a flow rate of at least one, and preferably 5 liters per hour per oyster. The flowrate should be ±10 percent of the nominal flow. A dilution water is acceptable if oysters will survive and grow normally over the period in which the test is conducted without exhibiting signs of stress, i.e., excessive mucus production (stringy material floating suspended from oysters), lack of feeding, shell gaping, poor shell closing in response to prodding, or excessive mortality. The dilution water should have a salinity in excess of 12 parts per thousand, and should be similiar to that in the environment from which the test oysters originated. A natural seawater should have a weekly range in salinity of less than 10 parts per thousand and a monthly range in pH of less than 0.8 units. Artificial seawater should not vary more than 2 parts per thousand nor more than 0.5 pH units. Oysters should be tested in dilution water from the same origin.

(3) *Test parameters*—(i) *Carriers.* Stock solutions of substances of low aqueous solubility may be prepared by ultrasonic dispersion or, if necessary, by use of organic solvents, emulsifiers or dispersants of low toxicity to oysters. When such carriers are used, the control oysters should be exposed to the same concentration of the carrier as that used in the highest concentration of the test substance. The concentration of such carriers should not exceed 0.1 ml/l.

(ii) *Dissolved oxygen.* The dissolved oxygen concentrations should be at least 60 percent of the air saturation value and should be recorded daily.

(iii) *Loading.* The loading rate should not crowd oysters and should permit adequate circulation of water while avoiding physical agitation of oysters by water current.

(iv) *Temperature.* The test temperature should be 20 °C ±1 °C. Temporary excursions (less than 8 hours) within 15 °C to 25 °C are permissible. Temperature should be recorded continuously.

(v) *pH.* The pH should be recorded twice weekly in each test chamber.

(e) *Reporting.* In addition to the reporting requirements prescribed in Part 792—Good Laboratory Practice Standards of this chapter, the report should contain the following:

(1) The source of the dilution water, the mean, standard deviation and range of the salinity, pH, temperature and dissolved oxygen during the test period.

(2) A description of the test procedures used (e.g., the flow-through system, test chambers, chemical delivery system, aeration, etc.).

(3) Detailed information about the oysters used, including age, and/or size (i.e., height), weight (blotted dry), source, history, method of confirmation of prespawn condition, acclimation procedures, and food used.

(4) The number of organisms tested, loading rate and flowrate.

(5) The methods of preparation of stock and test solutions and the test chemical concentrations used.

(6) The number of dead and live organisms, the percentage of oysters that died and the number that showed any abnormal effects in the control and in each test chamber at each observation period.

(7) Methods and data records of all chemical analyses of water quality parameters and test chemical concentrations, including method validations and reagent blanks.

(8) Description of sampling, sample storage (if required) and analytical methods of water and tissue analyses for the test chemical.

(9) The mean, standard deviation and range of the concentration of test chemical in the test solution and oyster tissue at each sampling period.

(10) The time to steady-state.

(11) The steady-state or maximum BCF and the 95 percent confidence limits.

(12) The time to 95 percent elimination of accumulated residues of the test chemical from test oysters.

(13) Any incidents in the course of the test which might have influenced the results.

(14) If the test was not done in accordance with the prescribed conditions and procedures, all deviations should be described in full.

§ 797.1930 Mysid shrimp acute toxicity test.

(a) *Purpose.* This guideline is intended for use in developing data on the acute toxicity of chemical substances and mixtures ("chemicals") subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94-469, 90 Stat. 2003, 15 U.S.C. 2601 *et seq.*). This guideline prescribes a test using mysid shrimp as test organisms to develop data on the acute toxicity of chemicals. The United States Environmental Protection Agency (EPA) will use data from these tests in assessing the hazard of a chemical to the aquatic environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA) and in Part 792—Good Laboratory Practice Standards of this chapter, apply to this test guideline. The following definitions also apply to this guideline.

(1) "Death" means the lack of reaction of a test organism to gentle prodding.

(2) "Flow-through" means a continuous or an intermittent passage of test solution or dilution water through a test chamber or a holding or acclimation tank, with no recycling.

(3) "LC_{50}" means that experimentally derived concentration of test substance that is calculated to kill 50 percent of a test population during continuous exposure over a specified period of time.

(4) "Loading" means the ratio of test organisms biomass (grams, wet weight) to the volume (liters) of test solution in a test chamber.

(5) "Retention chamber" means a structure within a flow-through test chamber which confines the test organisms, facilitating observation of test organisms and eliminating loss of organisms in outflow water.

(6) "Static system" means a test chamber in which the test solution is not renewed during the period of the test.

(c) *Test procedures*—(1) *Summary of the test.* In preparation for the test, test chambers are filled with appropriate volumes of dilution water. If a flow-through test is performed, the flow of dilution water through each chamber is adjusted to the rate desired. The test substance is introduced into each test chamber. In a flow-through test, the rate at which the test substance is added is adjusted to establish and maintain the desired concentration of test substance in each test chamber. The test is started by randomly introducing mysids acclimated in accordance with the test design into the test chambers. Mysids in the test chambers are observed periodically during the test, the dead mysids removed and the findings recorded. Dissolved oxygen concentration, pH, temperature, salinity, the concentration of test substance, and other water quality characteristics are measured at specified intervals in test chambers. Data collected during the test are used to develop concentration-response curves and LC_{50} values for the test substance.

(2) [Reserved]

(3) *Range-finding test.* (i) A range-finding test should be conducted to determine:

(A) Which life stage (juvenile or young adult) is to be utilized in the definitive test.

(B) The test solution concentrations for the definitive test.

(ii) The mysids should be exposed to a series of widely spaced concentrations of test substance (e.g., 1, 10, 100 mg/l, etc.), usually under static conditions.

(iii) This test should be conducted with both newly-hatched juvenile (<24 hours old) and young adult (5 to 6 days old) mysids. For each age class (juvenile or young adult), a minimum of 10 mysids should be exposed to each concentration of test substance for up to 96 hours. The exposure period may be shortened if data suitable for the purpose of the range-finding test can be obtained in less time. The age class which is most sensitive to the test substance in the range-finding test should be utilized in the definitive test. When no apparent difference in sensitivity of the two life stages is found, juveniles should be utilized in the definitive test. No replicates are required and nominal concentrations of the chemical are acceptable.

(4) *Definitive test.* (i) The purpose of the definitive test is to determine the concentration-response curves and the 48- and 96-hour LC_{50} values with the minimum amount of testing beyond the range-finding test.

(ii) The definitive test should be conducted on the mysid life stage (juveniles or young adults) which is most sensitive to the test substance being evaluated.

(iii) A minimum of 20 mysids per concentration should be exposed to five or more concentrations of the chemical chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 2, 4, 8, 16, 32, and 64 mg/l). An equal number of mysids should be placed in two or more replicates. If solvents, solubilizing agents or emulsifiers have to be used, they should be commonly used carriers and should not possess a synergistic or antagonistic effect on the toxicity of the test substance. The concentration of solvent should not exceed 0.1 mg/l. The concentration ranges should be selected to determine the concentration-response curves and LC_{50} values at 48 and 96 hours. The concentration of test substance in test solutions should be analyzed prior to use.

(iv) Every test should include controls consisting of the same dilution water, conditions, procedures, and mysids from the same population or culture container, except that none of the chemical is added.

(v) The dissolved oxygen concentration, temperature, salinity, and pH should be measured at the beginning of the test and at 24, 48, 72, and 96 hours in each chamber.

(vi) The test duration is 96 hours. The test is unacceptable if more than 10 percent of the control organisms die or exhibit abnormal behavior during the 96 hour test period. Each test chamber should be checked for dead mysids at 3, 6, 12, 24, 48, 72, and 96 hours after the beginning of the test. Concentration-response curves and 48- and 96-hour LC_{50} values should be determined along with their 95 percent confidence limits.

(vii) In addition to death, any abnormal behavior or appearance should also be reported.

(viii) Distribution of mysids among test chambers should be randomized. In addition, test chambers within the testing area should be positioned in a random manner or in a way in which appropriate statistical analyses can be used to determine the variation due to placement.

(ix) The concentration of dissolved test substance (that which passes through a 0.45 micron filter) in the chambers should be measured as often as is feasible during the test. At a minimum, during static tests, the concentration of test substance should be measured in each chamber at the beginning and at the end of the test. During the flow-through test, the concentration of test substance should be measured (A) in each chamber at the beginning of the test and at 48 and 96 hours after the start of the test; (B) in at least one chamber containing the next to the lowest test substance concentration at least once every 24 hours during the test; and (C) in at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system. Among replicate test chambers of a treatment concentration, the measured concentration of the test substance should not vary more than 20 percent.

(5) [Reserved]

(6) *Analytical measurements*—(i) *Test chemical.* Deionized water should be used in making stock solutions of the test substance. Standard analytical methods should be used whenever available in performing the analyses. The analytical method used to measure the amount of test substance in a sample should be validated before beginning the test by appropriate laboratory practices. An analytical method is not acceptable if likely degradation products of the test substance, such as hydrolysis and oxidation products, give positive or negative interferences which cannot be systematically identified and corrected mathematically.

(ii) *Numerical.* The number of dead mysids should be counted during each definitive test. Appropriate statistical analyses should provide a goodness-of-fit determination for the concentration-response curves. A 48- and 96-hour LC_{50} and corresponding 95 percent interval should be calculated.

(d) *Test conditions*—(1) *Test species*—(i) *Selection.* (A) The mysid shrimp, *Mysidopsis bahia*, is the organism specified for these tests. Either juvenile (<24 hours old) or young adult (5 to 6 days old) mysids are to be used to start the test.

(B) Mysids to be used in acute toxicity tests should originate from laboratory cultures in order to assure that the individuals are of similar age and experiential history. Mysids used for establishing laboratory cultures may be purchased commercially or collected from appropriate natural areas. Because of similarities with other mysid species, taxonomic verification should be obtained from the commercial supplier or through an appropriate systematic key.

(C) Mysids used in a particular test should be of similar age and be of normal size and appearance for their age. Mysids should not be used for a test if they exhibit abnormal behavior or if they have been used in a previous test, either in a treatment or in a control group.

(ii) *Acclimation.* (A) Any change in the temperature and chemistry of the dilution water used for holding or culturing the test organisms to those of the test should be gradual. Within a 24-hour period, changes in water temperature should not exceed 1 °C, while salinity changes should not exceed 5 percent.

(B) During acclimation mysids should be maintained in facilities with background colors and light intensities similar to those of the testing areas.

(iii) *Care and handling.* Methods for the care and handling of mysids such as those described in paragraph (f)(1) of this section can be used during holding, culturing and testing periods.

(iv) *Feeding.* Mysids should be fed during testing. Any food utilized should support survival, growth and reproduction of the mysids. A recommended food is live *Artemia* spp. (48-hour-old nauplii).

(2) *Facilities*—(i) *Apparatus.* (A) Facilities which may be needed to perform this test include: (*1*) flow-through or recirculating tanks for holding and acclimating mysids; (*2*) a mechanism for controlling and maintaining the water temperature during the holding, acclimation and test periods; (*3*) apparatus for straining particulate matter, removing gas bubbles, or aerating the water, as necessary; and (*4*) an apparatus for providing a 14-hour light and 10-hour dark photoperiod with a 15 to 30 minute transition period. In addition, for flow-through tests, flow-through chambers and a test substance delivery system are required. Furthermore, it is recommended that mysids be held in retention chambers within test chambers to facilitate observations and eliminate loss of test organisms through outflow water. For static tests, suitable chambers for exposing test mysids to the test substance are required. Facilities should be well ventilated and free of fumes and disturbances that may affect the test organisms.

(B) Test chambers should be loosely covered to reduce the loss of test solution or dilution water due to evaporation and to minimize the entry of dust or other particulates into the solutions.

(ii) *Cleaning.* Test substance delivery systems and test chambers should be cleaned before each test following standard laboratory practices.

(iii) *Construction materials.* (A) Materials and equipment that contact test solutions should be chosen to minimize sorption of test chemicals from dilution water and should not contain substances that can be leached into aqueous solution in quantities that can affect test results.

(B) For use in the flow-through test, retention chambers utilized for confinement of test organisms can be constructed with netting material of appropriate mesh size.

(iv) *Dilution water.* (A) Natural or artificial seawater is acceptable as dilution water if mysids will survive and successfully reproduce in it for the duration of the holding, acclimating and testing periods without showing signs of stress, such as reduced growth and fecundity. Mysids should be cultured and tested in dilution water from the same origin.

(B) Natural seawater should be filtered through a filter with a pore size of <20 microns prior to use in a test.

(C) Artificial seawater can be prepared by adding commercially available formulations or by adding specific amounts of reagent-grade chemicals to deionized water. Deionized water with a conductivity less than 1 μ ohm/cm at 12 °C is acceptable for making artificial seawater. When deionized water is prepared from a ground or surface water source, conductivity and total organic carbon (or chemical oxygen demand) should be measured on each batch.

(v) *Test substance delivery system.* In flow-through tests, proportional diluters, metering pumps or other suitable systems should be used to deliver test substance to the test chambers. The system used should be calibrated before each test. Calibration includes determining the flow rate through each chamber and the concentration of the test substance in each chamber. The general operation of the test substance delivery system should be checked twice daily during a test. The 24-hour flow through a test chamber should be equal to at least 5 times the volume of the test chamber. During a test, the flow rates should not vary more than 10 percent among test chambers or across time.

(3) *Test parameters.* Environmental parameters of the water contained in test chambers should be maintained as specified below:

(i) Temperature of 25 ± 2 °C.

(ii) Dissolved oxygen concentration between 60 and 105 percent saturation. Aeration, if needed to achieve this level, should be done before the addition of the test substance. All treatment and control chambers should be given the same aeration treatment.

(iii) The number of mysids placed in a test solution should not be so great as to affect results of the test. Thirty mysids per liter is the recommended level of loading for a static test. Loading requirements for the flow-through test will vary depending on the flow rate of dilution water. The loading should not cause the dissolved oxygen concentration to fall below the recommended levels.

(iv) Photoperiod of 14 hours light and 10 hours darkness, with a 15 to 30 minute transition period.

(v) Salinity of 20 parts per thousand ±3%.

(e) *Reporting.* The sponsor should submit to the EPA all data developed during the test that are suggestive or predictive of acute toxicity and all concomitant toxicologic manifestations. In addition to the general reporting requirements prescribed in Part 792—Good Laboratory Practice Standards of this chapter, the reporting of test data should include the following:

(1) The source of the dilution water, its chemical characteristics (e.g., salinity, pH, etc.) and a description of any pretreatment.

(2) Detailed information about the test organisms, including the scientific name and method of verification, age, source, history, abnormal behavior, acclimation procedures and food used.

(3) A description of the test chambers, the depth and volume of solution in the chamber, the way the test was begun (e.g., conditioning, test substance additions, etc.), the number of organisms per treatment, the number of replicates, the loading, the lighting, the test substance delivery system and the flow rate expressed as volume additions per 24 hours.

(4) The measured concentration of test substance in test chambers at the times designated.

(5) The number and percentage of organisms that died or showed any other adverse effects in the control and in each treatment at each observation period.

(6) Concentration-response curves should be fitted to mortality data collected at 24, 48, 72, and 96 hours. A statistical test of goodness-of-fit should be performed and the results reported.

(7) The 48- and 96-hour LC$_{50}$, and when sufficient data have been generated, the 24-hour and 72-hour LC$_{50}$'s and the corresponding 95 percent confidence limits and the methods used to calculate the values. These calculations should be made using the average measured concentration of the test substance.

(8) Methods and data records of all chemical analyses of water quality and test substance concentrations, including method validations and reagent blanks.

(9) The data records of the holding, acclimation and test temperature and salinity.

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) U.S. Environmental Protection Agency, "Bioassay Procedures for the Ocean Disposal Permit Program," EPA Report No. 600-9-78-010 (Gulf Breeze, Florida, 1978).

(2) [Reserved]

§ 797.1950 Mysid Shrimp Chronic Toxicity Test.

(a) *Purpose.* This guideline is intended for use in developing data on the chronic toxicity of chemical substances and mixtures ("chemicals") subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94-469, 90 Stat. 2003, 15 U.S.C. 2601 *et seq.*). This guideline prescribes tests using mysids as test organisms to develop data on the chronic toxicity of chemicals. The United States Environmental Protection Agency (EPA) will use data from these tests in assessing the hazard of a chemical to the aquatic environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA) and in Part 792—Good Laboratory Practice Standards of this chapter apply to this test guideline. The following definitions also apply to this guideline:

(1) "Chronic toxicity test" means a method used to determine the concentration of a substance that produces an adverse effect from prolonged exposure of an organism to that substance. In this test, mortality, number of young per female and growth are used as measures of chronic toxicity.

(2) "Death" means the lack of reaction of a test organism to gentle prodding.

(3) "Flow-through" means a continuous or an intermittent passage of test solution or dilution water through a test chamber or a holding or acclimation tank, with no recycling.

(4) "G1 (Generation 1)" means those mysids which are used to begin the test, also referred to as adults; G2 (Generation 2) are the young produced by G1.

(5) "LC$_{50}$"means that experimentally derived concentration of test substance that is calculated to kill 50 percent of a test population during continuous exposure over a specified period of time.

(6) "Loading" means the ratio of test organism biomass (gram, wet weight) to the volume (liters) of test solution in a test chamber.

(7) "MATC" (Maximum Acceptable Toxicant Concentration) means the maximum concentration at which a chemical can be present and not be toxic to the test organism.

(8) "Retention chamber" means a structure within a flow-through test chamber which confines the test organisms, facilitating observation of test organisms and eliminating washout from test chambers.

(c) *Test procedures*—(1) *Summary of the test.* (i) In preparation for the test, the flow of test solution through each chamber is adjusted to the rate desired. The test substance is introduced into each test chamber. The rate at which the test substance is added is adjusted to establish and maintain the desired concentration of test substance in each test chamber. The test is started by randomly introducing mysids acclimated in accordance with the test design into retention chambers within the test and the control chambers. Mysids in the test and control chambers are observed periodically during the test, the dead mysids removed and the findings reported.

(ii) Dissolved oxygen concentration, pH, temperature, salinity, the concentration of test substance and other water quality characteristics are measured at specified intervals in selected test chambers.

(iii) Data collected during the test are used to develop a MATC (Maximum Acceptable Toxicant Concentration) and quantify effects on specific chronic parameters.

(2) [Reserved]

(3) *Range-finding test.* (i) A range-finding test should be conducted to establish test solution concentrations for the definitive test.

(ii) The mysids should be exposed to a series of widely spaced concentrations of the test substance (e.g., 1, 10, 100 mg/l), usually under static conditions.

(iii) A minimum of 10 mysids should be exposed to each concentration of test substance for a period of time which allows estimation of appropriate chronic test concentrations. No replicates are required and nominal concentrations of the chemical are acceptable.

(4) *Definitive test.* (i) The purpose of the definitive test is to determine concentration-response curves, LC_{50} values, and effects of a chemical on growth and reproduction during chronic exposure.

(ii) A minimum of 40 mysids per concentration should be exposed to four or more concentrations of the chemical chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 2, 4, 8, 16, 32 and 64 mg/l). An equal number of mysids should be placed in two or more replicates. If solvents, solubilizing agents or emulsifiers have to be used, they should be commonly used carriers and should not possess a synergistic or antagonistic effect on the toxicity of the test substance. The concentration of solvent should not exceed 0.1 ml/l. The concentration ranges should be selected to determine the concentration-response curves, LC_{50} values and MATC. Concentration of test substance in test solutions should be analyzed prior to use.

(iii) Every test should include controls consisting of the same dilution water, conditions, procedures and mysids from the same population or culture container, except that none of the chemical is added.

(iv) The dissolved oxygen concentration, temperature, salinity and pH should be measured at the beginning of the test and on days 7, 14, 21, and 28 in each chamber.

(v) The test duration is 28 days. The test is unacceptable if more than 20 percent of the control organisms die, appear stressed or are diseased during the test. The number of dead mysids in each chamber should be recorded on days 7, 14, 21, and 28 of the test. At the time when sexual characteristics are discernible in the mysids (approximately 10 to 12 days in controls; possible delays may occur in mysids exposed to test substances), the number of males and females (identified by ventral brood pouch) in each chamber should be recorded. Body length (as measured by total midline body length, from the anterior tip of the carapace to the posterior margin of the uropod) should be recorded for males and females at the time when sex can be determined simultaneously for all mysids in control and treatment groups. This time cannot be specified because of possible delays in sexual maturation of mysids exposed to test substances. A second observation of male and female body lengths should be conducted on day 28 of the test. To reduce stress on the mysids, body lengths can be recorded by photography through a stereomicroscope with appropriate scaling information. As offspring are produced by the G1 mysids (approximately 13 to 16 days in controls), the young should be counted and separated into retention chambers at the same test substance concentration as the chambers where they originated. If available prior to termination of the test, observations on the mortality, number of males and females and male and female body length should be recorded for the G2 mysids. Concentration-response curves, LC_{50} values and associated 95 percent confidence limits for the number of dead mysids (G1) should be determined for days 7, 14, 21, and 28. An MATC should be determined for the most sensitive test criteria measured (cumulative mortality of adult mysids, number of young per female, and body lengths of adult males and females).

(vi) In addition to death, any abnormal behavior or appearance should also be reported.

(vii) Distribution of mysids among test chambers should be randomized. In addition, test chambers within the testing area should be positioned in a random manner or in a way in which appropriate statistical analyses can be used to determined the variation due to placement.

(viii) The concentration of dissolved test substance (that which passes through a 0.45 micron filter) in the chambers should be measured as often as is feasible during the test. The concentration of test substance should be measured: (A) in each chamber at the beginning of the test and on days 7, 14, 21, and 28; and (B) in at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system. Among replicate test chambers of a treatment concentration, the measured concentration of the test substance should not vary more than 20 percent.

(5) [Reserved]

(6) *Analytical measurements*—(i) *Test chemical.* Deionized water should be used in making stock solutions of the test substance. Standard analytical methods should be employed whenever available in performing the analyses. The analytical method used to measure the amount of test substance in a sample should be validated before beginning the test by appropriate laboratory practices. An analytical method is not acceptable if likely degradation products of the test substance, such as hydrolysis and oxidation products, give positive or negative interferences which cannot be systematically identified and corrected mathematically.

(ii) *Numerical.* (A) The number of dead mysids, cumulative young per female and body lengths of male and female mysids should be recorded during each definitive test. Appropriate statistical analyses should provide a goodness-of-fit determination for the day 7, 14, 21 and 28 adult (G1) death concentration-response curves.

(B) A 7-, 14-, 21- and 28-day LC_{50}, based on adult (G1) death, and corresponding 95 percent confidence intervals should be calculated. Appropriate statistical tests (e.g., analysis of variance, mean separation test) should be used to test for significant chemical effects on chronic test criteria (cumulative mortality of adults, cumulative number of young per female and body lengths of adult male and females) on designated days. An MATC should be calculated using these chronic tests criteria.

(d) *Test conditions*—(1) *Test species*—(i) *Selection.* (A) The mysid shrimp, *Mysidopsis bahia,* is the organism specified for these tests. Juvenile mysids, ≤24 hours old, are to be used to start the test.

(B) Mysids to be used in chronic toxicity tests should originate from laboratory cultures in order to ensure the individuals are of similar age and experimental history. Mysids used for establishing laboratory cultures may be purchased commercially or collected from appropriate natural areas. Because of similarities with other mysid species, taxonomic determinations should be verified by the commercial supplier or by an appropriate individual.

(C) Mysids used in a particular test should be of similar age and be of normal size and appearance for their age.

(D) Mysids should not be used for a test if they exhibit abnormal behavior, or if they have been used in a previous test, either in a treatment or in a control group.

(ii) *Acclimation.* (A) Any change in the temperature and chemistry of the water used for holding or culturing the test organisms to those of the test should be gradual. Within a 24-hour period, changes in water temperature should not exceed 1 °C, while salinity changes should not exceed 5 percent.

(B) During acclimation mysids should be maintained in facilities with background colors and light intensities similar to those of the testing areas.

(iii) *Care and handling.* Methods for the care and handling of mysids such as those described in paragraph (f)(1) of this section can be used during holding, culturing and testing periods.

(iv) *Feeding.* Mysids should be fed during testing. Any food utilized should support survival, growth and reproduction of the mysids. A recommended food is live *Artemia* spp. nauplii (approximately 48 hours old).

(2) *Facilities*—(i) *Apparatus.* (A) Facilities which may be needed to perform this test include: (*1*) flow-through or recirculating tanks for holding and acclimating mysids; (*2*) a mechanism for controlling and maintaining the water temperature during the holding, acclimation and test periods; (*3*) apparatus for straining particulate matter, removing gas bubbles, or aerating the water, as necessary; and (*4*) an apparatus for providing a 14-hour light and 10-hour dark photoperiod with a 15- to 30-minute transition period. In addition, flow-through chambers and a test substance delivery system are required. It is recommended that mysids be held in retention chambers within test chambers to facilitate observations and eliminate loss through outflow water.

(B) Facilities should be well ventilated and free of fumes and disturbances that may affect test organisms.

(C) Test chambers should be loosely covered to reduce the loss of test solution or dilution water due to evaporation and to minimize the entry of dust or other particulates into the solutions.

(ii) *Cleaning.* Test substance delivery systems and test chambers should be cleaned before each test following standard laboratory practices.

(iii) *Construction materials.* (A) Materials and equipment that contact test solutions should be chosen to minimize sorption of test chemicals from the dilution water and should not contain substances that can be leached into aqueous solution in quantities that can affect the test results.

(B) Retention chambers utilized for confinement of test organisms can be constructed with netting material of appropriate mesh size.

(iv) *Dilution water.* (A) Natural or artificial seawater is acceptable as dilution water if mysids will survive and successfully reproduce in it for the duration of the holding, acclimating and testing periods without showing signs of stress, such as reduced growth and fecundity. Mysids should be cultured and tested in dilution water from the same origin.

(B) Natural seawater should be filtered through a filter with a pore size of >20 microns prior to use in a test.

(C) Artificial seawater can be prepared by adding commercially available formulations or by adding specific amounts of reagent-grade chemicals to deionized or glass-distilled water. Deionized water with a conductivity less than 1 μ ohm/cm at 12 °C is acceptable as the diluent for making artificial seawater. When deionized water is prepared from a ground or surface water source, conductivity and total organic carbon (or chemical oxygen demand) should be measured on each batch.

(v) *Test substance delivery system.* Proportional diluters, metering pumps or other suitable systems should be used to deliver test substance to the test chambers. The system used should be calibrated before each test. Calibration includes determining the flow rate and the concentration of the test substance in each chamber. The general operation of the test substance delivery system should be checked twice daily during a test. The 24-hour flow rate through a chamber should be equal to at least 5 times the volume of the chamber. The flow rates should not vary more than 10 percent among chambers or across time.

(3) *Test parameters.* Environmental parameters of the water contained in test chambers should be maintained as specified below:

(i) Temperature of 25 ± 2 °C.

(ii) Dissolved oxygen concentration between 60 and 105 percent saturation. Aeration, if needed to achieve this level, should be done before the addition of the test substance. All treatment and control chambers should be given the same aeration treatment.

(iii) The number of mysids placed in a test solution should not be so great as to affect results of the test. Loading requirements for the test will vary depending on the flow rate of dilution water. The loading should not cause the dissolved oxygen concentration to fall below the recommended levels.

(iv) Photoperiod of 14 hours light and 10 hours darkness, with a 15–30 minute transition period.

(v) Salinity of 20 parts per thousand ±3 percent.

(e) *Reporting.* The sponsor should submit to the EPA all data developed by the test that are suggestive or predictive of chronic toxicity and all concomitant toxicologic manifestations. In addition to the general reporting requirements prescribed in Part 792—Good Laboratory Practice Standards of this chapter, the reporting of test data should include the following:

(1) The source of the dilution water, its chemical characteristics (e.g., salinity, pH, etc.) and a description of any pretreatment.

(2) Detailed information about the test organisms, including the scientific name and method of verification, average length, age, source, history, observed diseases, treatments, acclimation procedures and food used.

(3) A description of the test chambers, the depth and volume of solution in the chamber, the way the test was begun (e.g., conditioning, test substance additions, etc.), the number of organisms per treatment, the number of replicates, the loading, the lighting, the test substance delivery system, and the flow rate expressed as volume additions per 24 hours.

(4) The measured concentration of test substance in test chambers at the times designated.

(5) The first time (day) that sexual characteristics can be observed in controls and in each test substance concentration.

(6) The length of time for the appearance of the first brood for each concentration.

(7) The means (average of replicates) and respective 95 percent confidence intervals for:

(A) Body length of males and females at the first observation day (depending on time of sexual maturation) and on day 28.

(B) Cumulative number of young produced per female on day 28.

(C) Cumulative number of dead adults on day 7, 14, 21 and 28.

(D) If available prior to test termination (day 28), effects on G2 mysids (number of males and females, body length of males and females and cumulative mortality).

(8) The MATC is calculated as the geometric mean between the lowest measured test substance concentration that had a significant ($P<0.05$) effect and the highest measured test substance concentration that had no significant ($P>0.05$) effect in the chronic test. The most sensitive of the test criteria for adult (G1) mysids (cumulative number of dead mysids, body lengths of males and females or the number of young per female) is used to calculate the MATC. The criterion selected for MATC computation is the one which exhibits an effect (a statistically significant difference between treatment and control groups; $P<0.05$) at the lowest test substance concentration for the shortest period of exposure. Appropriate statistical tests (analysis of variance, mean separation test) should be used to test for significant chemical effects. The statistical tests employed and the results of these tests should be reported.

(9) Concentration-response curves should be fitted to the cumulative number of adult dead for days 7, 14, 21 and 28. A statistical test of goodness-of-fit should be performed and the results reported.

(10) An LC_{50} value based on the number of dead adults with corresponding 95 percent confidence intervals for days 7, 14, 21 and 28. These calculations should be made using the average measured concentration of the test substance.

(11) Methods and data records of all chemical analyses of water quality and test substance concentrations, including method validations and reagent blanks.

(12) The data records of the holding, acclimation and test temperature and salinity.

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) U.S. Environmental Protection Agency, "Bioassay Procedures for the Ocean Disposal Permit Program," EPA Report No. 600/9-78-010 (Gulf Breeze, Florida, 1978).

(2) [Reserved]

§ 797.1970 Penaeid Shrimp Acute Toxicity Test.

(a) *Purpose.* This guideline is intended for use in developing data on the acute toxicity of chemical substances and mixtures ("chemicals") subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94-469, 90 Stat. 2003, 15 U.S.C. 2601 *et seq.*). This guideline prescribes tests using penaeid shrimp as test organisms to develop data on the acute toxicity of chemicals. The United States Environmental Protection Agency (EPA) will use data from these tests in assessing the hazard of a chemical to the aquatic environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA), and in Part 792—Good Laboratory Practice Standards of this chapter apply to this test guideline. The following definitions also apply to this guideline:

(1) "Death" means the lack of reaction of a test organism to gentle prodding.

(2) "Flow-through" means a continuous passage of test solution or dilution water through a test chamber, holding or acclimation tank with no recycling.

(3) "LC_{50}" means that experimentally derived concentration of test substance that is calculated to have killed 50 percent of a test population during continuous exposure over a specified period of time.

(4) "Loading" means the ratio of test organism biomass (grams, wet weight) to the volume (liters) of test solution in a test chamber.

(c) *Test procedures*—(1) *Summary of the test.* Prior to testing, the bottoms of the test chambers are covered with 2 to 3 cm of sand and then filled with appropriate volumes of dilution water. The flow is adjusted to the rate desired to achieve loading requirements. Penaeid shrimp are introduced into the test chambers according to the experimental design. The shrimp are maintained in the test chambers for a period of 3 to 7 days prior to the beginning of the test. The test begins when the test substance is introduced into the test chambers. The rate of flow is adjusted to maintain the desired test substance concentration in each chamber. The shrimp are observed during the test; dead shrimp are counted, removed, and the findings recorded. Dissolved oxygen concentration, pH, temperature, salinity, test substance concentration and other water quality characteristics are measured at specified intervals in selected test chambers. Data collected during the test are used to develop concentration-response curves and LC_{50} values for the test substance.

(2) [Reserved]

(3) *Range-finding test.* (i) A range-finding test should be conducted to determine the test substance concentrations to be used for the definitive test.

(ii) The shrimp should be exposed to a series of widely spaced concentrations of test substance (e.g., 1, 10, 100 mg/l, etc.).

(iii) A minimum of five penaeid shrimp should be exposed to each concentration of test substance for up to 96 hours. No replicates are required and nominal concentrations of the chemical are acceptable.

(4) *Definitive test.* (i) The purpose of the definitive test is to determine the concentration-response curves and the 48- and 96-hour LC_{50} values with the minimum amount of testing beyond the range-finding test.

(ii) A minimum of 20 shrimp per concentration should be exposed to five or more concentrations of the chemical chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 2, 4, 8, 16, 32 and 64 mg/l). An equal number of shrimp should be placed in two or more replicates. If solvents, solubilizing agents or emulsifiers have to be used, they should be commonly used carriers and should not possess a synergistic or antagonistic effect on the toxicity of the test substance. The concentration of solvent should not exceed 0.1 ml/l. The concentration ranges should be selected to determine the requested concentration-response curves and LC_{50} values. The concentration of test substance in test solutions should be analyzed for chemical concentration prior to use and at designated times.

(iii) Every test should include controls consisting of the same dilution water, conditions, procedures and shrimp from the same population or culture container, except that none of the chemical is added.

(iv) The dissolved oxygen concentration, temperature, salinity and pH should be measured at the beginning of the test and at 24, 48, 72, and 96 hours in each test chamber.

(v) The test duration is 96 hours. The test is unacceptable if more than 10 percent of the control organisms die or appear to be stressed or diseased during the 96 hour test period. Each test chamber should be checked for dead shrimp at 3, 6, 12, 24, 48, 72, and 96 hours after the beginning of the test. Concentration-response curves and 48- and 96-hour LC_{50} values should be determined along with their 95 percent confidence limits.

(vi) In addition to death, any abnormal behavior or appearance should also be reported.

(vii) Distribution of shrimp among test chambers should be randomized. In addition, test chambers within the testing area should be positioned in a random manner or in a way in which appropriate statistical analyses can be used to determine the variation due to placement.

(viii) The concentration of dissolved test substance (that which passes through a 0.45 micron filter) in the test chambers should be measured as often as is feasible during the test. The concentration of test substance should be measured:

(A) In each chamber at the beginning of the test and at 48 and 96 hours after the start of the test.

(B) In at least one chamber containing the next to the lowest test substance concentration at least once every 24 hours during the test.

(C) In at least one appropriate chamber whenever a malfunction is detected in any part of the test substance delivery system. Among replicate test chambers of a treatment concentration, the measured concentration of the test substance should not vary more than 20 percent.

(5) [Reserved]

(6) *Analytical measurements*—(i) *Test chemical.* Deionized water should be used in making stock solutions of the test substance. Standard analytical methods should be used whenever available in performing the analyses. The analytical method used to measure the amount of test substance in a sample should be validated before beginning the test by appropriate laboratory practices. An analytical method is not acceptable if likely degradation products of the test substance, such as hydrolysis and oxidation products, give positive or negative interferences which cannot be systematically identified and corrected mathematically.

(ii) *Numerical.* The number of dead shrimp should be counted during each definitive test. Appropriate statistical analyses should provide a goodness-of-fit determination for the concentration-response curves. A 48- and 96-hour LC_{50} and corresponding 95 percent intervals should be calculated.

(d) *Test conditions*—(1) *Test species*—(i) *Selection.* This test should be conducted using one of three species of penaeid shrimp: *Penaeus aztecus* (brown shrimp), *Penaeus duorarum* (pink shrimp), or *Penaeus setiferus* (white shrimp). Post-larval juvenile shrimp should be utilized. Shrimp may be reared from eggs in the laboratory or obtained directly as juveniles or adults. Shrimp used in a particular test should be of similar age and be of normal size and appearance. Shrimp should not be used for a test if they exhibit abnormal behavior or if they have been used in a previous test, either in a treatment or in a control group.

(ii) *Acclimation.* During acclimation, shrimp should be maintained in facilities with background colors and light intensities similar to those of the testing areas. In addition, any change in the temperature and chemistry of the dilution water used for holding and acclimating the test organisms to those of the test should be gradual. Within a 24-hour period, changes in water temperature should not exceed 1 °C, while salinity changes should not exceed 2 percent.

(iii) *Care and handling.* Upon arrival at the test facility, the shrimp should be transferred to water closely matching the temperature and salinity of the transporting medium. Shrimp should be held in glass tanks of 30 liter capacity or larger. No more than 22 to 24 shrimp should be placed in a 30 liter tank unless the flow-through apparatus can maintain dissolved oxygen levels above 60 percent of saturation. With species of the genus *Penaeus*, a minimum flow rate of 7.5 1/g body weight day should be provided. Larger flows, up to 22 1/g body weight day, may be desirable to ensure dissolve oxygen concentrations above 60 percent of saturation and the removal of metabolic products. The period of acclimation to ambient laboratory conditions should be at least 4 to 7 days.

(iv) *Feeding.* Penaeid shrimp should not be fed during testing. Every 2 or 3 days during the acclimation period, shrimp should be fed fish pieces approximately 1 cm^2. Uneaten food should be removed daily.

(2) *Facilities*—(i) *Apparatus.* (A) Facilities which may be needed to perform this test include: flow-through tanks for holding and acclimating penaeid shrimp; a mechanisn for controlling and maintaining the water temperature and salinity during the holding period; apparatus for straining particulate matter, removing air bubbles, or aerating water when necessitated by water quality requirements; and an apparatus providing a 12-hour light and 12-hour dark photoperiod with a 15 to 30 minute transition period. Facilities should be well ventilated, free of fumes and free of all other disturbances that may affect test organisms.

(B) Two to three centimeters of acid-washed sand, free of excess organic matter, should be placed in the bottom of test chambers.

(C) Test chambers should be loosely covered to reduce the loss of test solution or dilution water due to evaporation, minimize entry of dust and other particles and prevent escape of the shrimp.

(ii) *Cleaning.* Test substance delivery systems and test chambers should be cleaned before each test following standard laboratory practices.

(iii) *Construction materials.* Materials and equipment that contact test solutions should be chosen to minimize sorption of test chemicals from dilution water and should not contain substances that can be leached into aqueous solution in quantities that can affect test results.

(iv) *Dilution water.* (A) Natural or artificial seawater is acceptable as dilution water if shrimp will survive in it without signs of stress, such as unusual behavior or discoloration. Shrimp should be acclimated and tested in dilution water from the same origin.

(B) Natural seawater should be filtered through a 5 micrometer filter with a pore size <20 microns prior to use in a test.

(C) Artificial seawater can be prepared by adding commercially available formulations or by adding specific amounts of reagent-grade chemicals to deionized water. Deionized water with a conductivity less than 1μ ohm/cm at 12 °C is acceptable for making artificial seawater. When deionized water is prepared from a ground or surface water source, conductivity and total organic carbon (or chemical oxygen demand) should be measured on each batch.

(v) *Test substance delivery system.* Proportional diluters, metering pumps or other suitable systems should be used to deliver test substance to the test chambers. The system used should be calibrated before each test. Calibration includes determining the flow rate through each chamber and the concentration of the test substance in each chamber. The general operation of the test substance delivery system should be checked twice daily during a test. The 24-hour flow through a test chamber should be equal to a least 5 times the volume of the test chamber. During a test, the flow rates should not vary more than 10 percent among test chambers or across time.

(3) *Test parameters.* Environmental parameters of the water contained in test chambers should be as specified below:

(i) Temperature of 23 ± 1 °C.

(ii) Dissolved oxygen concentration between 60 and 105 percent saturation. Aeration, if needed to achieve this level, should be done before the addition of the test substance. All treatment and control chambers should be given the same aeration treatment.

(iii) The number of shrimp placed in a test solution should not be so great as to affect results of the test. Loading requirements will vary depending on the flow rate of dilution water. The loading should not cause the dissolved oxygen concentration to fall below the recommended levels.

(iv) Photoperiod of 12 hours light and 12 hours darkness, with a 15 to 30 minute transition period.

(v) Salinity of 20 parts per thousand ±3 percent.

(e) *Reporting.* The sponsor should submit to the EPA all data developed by the test that are suggestive or predictive of acute toxicity and all other toxicological manifestations. In addition to the general reporting requirements prescribed in Part 792—Good Laboratory Practice Standards of this chapter, the reporting of test data should include the following:

(1) The nature of the test, laboratory, name of the investigator, test substance and dates of test should be supplied.

(2) A detailed description of the test substances should be provided. This information should include the source, lot number, composition, physical and chemical properties and any carrier or additives used.

(3) Detailed information about the shrimp should be provided: common and scientific names, source of supply, age, history, weight, acclimation procedure and feeding history should be reported.

(4) A description of the experimental design including the number of test solution concentrations, number of replicates and number of shrimp per replicate should be provided.

(5) The source of the dilution water, its chemical characteristics (e.g., salinity) and a description of any pretreatment.

(6) A description of the test chambers, the depth and volume of solution in the chamber, the number of organisms per treatment, the number of replicates, the loading, the lighting, the test substance delivery system and flow rate expressed as volume additions per 24 hours.

(7) The concentration of the test substance in each test chamber before the start of the test and at the end.

(8) The number of dead shrimp and measurements of water temperature, salinity, and dissolved oxygen concentration in each test chamber should be recorded at the designated times.

(9) Methods and data records of all chemical analyses of water quality and test substance concentrations, including method validations and reagent blanks.

(10) Recorded data for the holding and acclimation period (temperature, salinity, etc.).

(11) Concentration-response curves should be fitted to mortality data collected at 24, 48, 72, and 96 hours. A statistical test of goodness-of-fit should be performed.

(12) For each set of mortality data, the 48- and 96-hour LC_{50} and 95 percent confidence limits should be calculated on the basis of the average measured concentration of the test substance. When data permits, LC_{50} values with 95 percent confidence limits should be computed for 24- and 72-hour observations.

(13) The methods used in calculating the concentration-response curves and the LC_{50} values should be fully described.

Subpart C—Terrestrial Guidelines

§ 797.2050 Avian Dietary Toxicity Test.

(a) *Purpose.* The guideline in this subpart is designed to develop data on the dietary toxicity to bobwhite and mallard of chemical substances and mixtures subject to acute environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94–469, 90 Stat. 2003 15 U.S.C. 2601 *et seq.).* The Agency will use these and other data to assess the acute hazard to birds and to provide an indication of potential chronic hazard that these chemicals may present to the environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA) and Part 792—Good Laboratory Practice Standards of this chapter apply to this test guideline. In addition, the following definitions apply to this guideline:

(1) "Acclimation" Physiological or behavioral adaptation of test animals to environmental conditions and basal diet associated with the test procedure.

(2) "LC_{50}" The empirically derived concentration of the test substance in the diet that is expected to result in mortality of 50 percent of a population of birds which is exposed exclusively to the treated diet under the conditions of the test.

(3) "Test Substance" The specific form of a chemical or mixture of chemicals that is used to develop the data.

(4) "Exposure period" The 5 day period during which test birds are offered a diet containing the test substance.

(5) "Post-exposure period" The portion of the test that begins with the test birds being returned from a treated diet to the basal diet. This period is typically 3 days in duration, but may be extended if birds continue to die or demonstrate other toxic effects.

(6) "Test period" The combination of the exposure period and the post-exposure period; or, the entire duration of the test.

(7) "Hatch" Eggs or young birds that are the same age and that are derived from the same adult breeding population, where the adults are of the same strain and stock.

(8) "Basal diet" The food or diet as it is prepared or received from the supplier, without the addition of any carrier, diluent, or test substance.

(c) *Test procedures*—(1) *Summary of test.* (i) After birds have been obtained, they should be acclimated for at least 7 days.

(ii) Test birds should be randomly assigned to the various treatment levels and controls.

(iii) Definitive test concentrations should be established, possibly requiring a range-finding test to be conducted first.

(iv) The test substance should be mixed thoroughly and evenly into the diet. Three treatment levels should be analyzed for test substance concentrations.

(v) Birds should be weighed at the beginning of the exposure period.

(vi) Birds should be observed regularly for mortality or abnormal behavior; any findings should be reported.

(vii) Food treated with the test substance should be replaced by untreated food (basal diet) after 5 days of exposure. Food consumption during the exposure period should be carefully estimated on a pen by pen basis.

(viii) Food consumption should be estimated for the post-exposure period and birds should be weighed at the end of 8 days. Additional weights and food consumption estimates should be determined if the test period is longer than the typical 8 days.

(ix) The mortality pattern should be examined, and a statistical analysis should be conducted. The LC_{50} slope, and confidence limits should be reported. A test for heterogeneity of data should be conducted.

(x) Treated or positive control birds should be sacrificed and disposed of properly. Negative control birds may be kept as breeding stock, but should not be used in any other tests.

(xi) The material to be tested should be analytically pure and the degree of purity should be reported along with the percentage of each impurity at levels specified in the test rule. If specifically required by a test rule for a particular substance or mixture, the technical grade should be tested. The test rule will specify the degree of purity or a range of compositions of the test substance.

(xii) A test is unacceptable if more than 10 percent of the control birds die during the test.

(2) [Reserved]

(3) *Range-finding test.* Unless the approximate toxicity of the test substance is known already, a range-finding test should be conducted to

determine the test substance concentrations to be used in the definitive test, under paragraph (d)(4)(iii) of this section for details on concentrations for definitive tests. Procedures for range-finding tests may vary, but generally, groups of a few birds are fed three to five widely-spaced concentrations for 5 days. A concentration series of 5, 50, 500, and 5,000 ppm is suggested. The results of the range-finding test then may be used to establish the definitive test concentrations.

(4) *Definitive test*—(i) *Controls.* (A) A concurrent control is required during every test. The control birds should be from the same hatch as the test groups. Control and test birds should be kept under the same experimental conditions. The test procedures should be the same for control and treated birds, except that no test substance should be added to the diets of control birds. If a carrier is used in preparation of the test diets, the same carrier should be added to the diets of control birds in the highest concentration used for test diets. The use of shared controls is acceptable for concurrent tests as long as the same carrier is used for all the tests.

(B) A test is not acceptable if more than 10 percent of the control birds die during the test period.

(C) A positive control (e.g., dieldrin standard) may be run, but is not required for each test. However, a quarterly or semi-annual laboratory standard (positive control) is recommended as a means of detecting possible interlaboratory or temporal variation. A laboratory standard is also recommended when there is any significant change in food, housing, or source of birds.

(ii) *Number of animals tested.* In the definitive test, a minimum of 10 birds should be used for each dietary concentration of the test substance. A minimum of 20 birds should be used for the negative or carrier control. Thirty or more control birds are preferable. If a positive control or laboratory standard is used, 10 or more birds should also be used for each concentration of the positive control. When a test substance is known or expected to result in high experimental variation, it may be appropriate or required by the test rule to use additional birds.

(iii) *Concentrations and dosage-mortality data.* A minimum of five concentrations of the test substance should be used in the definitive test. These concentrations should be spaced geometrically. The recommended spacing is for each concentration to be at least 60 percent of the next higher dose (less than 1.67 times the next lower dose). If concentrations are spaced more widely than is recommended, then at least three concentrations should result in mortality between, but not including, 0 percent and 100 percent. For any concentration spacing, at least one concentration should kill more than 50 percent (including 100 percent) and at least one concentration should kill less than 50 percent (including 0 percent) of the birds in a pen. For some test substances, it may be necessary to use more than five concentrations to achieve these results.

(iv) *Duration of test.* The definitive test should include 5 days of exposure to the test substance in the diet (exposure period) followed by at least 3 days of additional observation (post-exposure period) while the test birds are receiving an untreated diet. If any test birds die during the second or third day of the post-exposure period or if toxic signs are evident on the third day of the post-exposure period, the test period should be extended until 2 successive mortality-free days and 1 day free of toxic signs occur, or until 21 days after beginning the test, whichever comes first.

(v) *Observations of record.* (A) Throughout the test period, all signs of intoxication, other abnormal behavior, and mortality should be recorded and reported by dose level and by day. Signs of intoxication are those behaviors apparently due to the test chemical and may include a wide array of behaviors, such as labored respiration, leg weakness, hemorrhage, convulsions, ruffled feathers, etc. All signs of intoxication and any other abnormal behavior, such as excessive aggression, toe-picking etc. that may or may not be attributed to the test substance should be reported. Among survivors, remission of signs of intoxication and cessation of abnormal behavior should be recorded by dose level and by day. When differential signs of intoxication are observed within a dose level, an estimate of the number of birds exhibiting such signs should be recorded. Observation of test birds should be made, at a minimum, three times on the first day of the exposure period. Observations also should be made at least daily throughout the remainder of the test period; twice daily observations are recommended, where feasible.

(B) Average body weights of birds should be recorded and reported for each pen within each treatment and control group at the beginning of the exposure period and the end of the normal 3-day post-exposure period of each test. Body weights 72 hours before the exposure period are not required, but would provide valuable base-line data. Average food consumption should be measured in control pens and pens with the second lowest and second highest concentration levels either daily or every other day. Any significant amount of food spilled onto litter pans should be estimated and reported. For all other pens, average food consumption should be measured for both the exposure period and the normal 3-day post-exposure period. If the study is continued beyond 8 days, body weight and food consumption data should be recorded weekly.

(C) Gross pathology examinations are not required, but they may provide valuable information on target site, mode of action, etc.

(5) [Reserved]

(6) *Analytical measurements*—(i) *Statistical analysis.* (A) A statistical analysis should be conducted by transforming the dietary concentrations to logarithmic values and the mortality pattern to probits. Other acceptable methods that will result in a theoretically straight line through ±2 standard deviations from the LC_{50} value may be used. The LC_{50} value and slope of the transformed concentration-response curve should be determined for mortality at the end of test period. Probit analysis by calculations or graphical probit methods are preferred. Any standard method that is used should provide the slope of the transformed concentration-response curve as well as the LC_{50} value. A statistical test for goodness-of-fit (e.g., chi-square test) also should be performed.

(B) All methods used for statistical analysis should be described completely.

(ii) *Analysis for test substance concentrations.* (A) Samples of treated diets should be analyzed to confirm proper dietary concentration of the test substance. Analyses should be conducted at the beginning of the exposure period with samples from high, middle, and low concentrations. If not already available, data should be generated to indicate whether or not the test substance degrades or volatilizes. If the test substances is known or found to be volatile or labile to the extent that 25 percent or more loss occurs over a 5 day period, then a second series of analyses of the same concentrations previously analyzed should be conducted at the end of the exposure period.

(B) The assay method used to determine actual concentrations should be reported.

(iii) *Analysis of basal diet.* A nutrient analysis of the basal diet should be included in the test report. For commercially prepared basal diets, the list of ingredients supplied by the company is normally sufficient if it is detailed. The composition of any vitamin or other supplements should also be reported.

(d) *Test conditions*—(1) *Test Species*—(i) *Selection.* (A) Bobwhite, *Colinus virginianus* (L.), and mallard, *Anas platyrhynchos* L., are the test species. Birds may be reared in the laboratory or purchased from a breeder. If bobwhite are purchased, it is preferable that they be obtained as eggs which then are hatched and reared in the testing facility. During incubation, a temperature of 39 °C and relative humidity of 70 percent are recommended for bobwhite. It is feasible to purchase live young bobwhite chicks if they can be obtained locally; however, young bobwhite may suffer adverse effects if shipped by air or other commercial means. Young mallard ducklings normally can be shipped without undue adverse effects.

(B) All control and treatment birds used in a test should be from the same source and hatch. Birds should be obtained only from sources whose colonies have known breeding histories. Birds should be phenotypically indistinguishable (except for size) from wild stock. It is recommended that birds be obtained from flocks that have been outbred periodically with genetically wild stock in order to maintain a genetic composition that approximates the natural heterogeneity of the species.

(C) Birds used in the test should be in apparent good health. Deformed, abnormal, sick, or injured birds should not be used. Birds should not be used for a test if more than 5 percent of the total test population die during the 72 hours immediately preceding the exposure period. Purchased birds should be certified as disease free or as bred from disease free stocks. Birds should not have been selected in any way for genetic resistance to toxic substances. Birds should not have been used in a previous test, either in a treatment or control group.

(D) Test birds should be 10 to 17 days old at the beginning of the exposure period. All treatment and control birds in a test should be the same age ± one day. The exact age should be recorded and reported.

(E) Test birds should be acclimated to test facilities and basal diet for a minimum of 7 days. Acclimation to test pens may be either in the actual pens used in the test or in identical pens. Birds used in the test should be assigned randomly to treatment and control pens without respect to sex. Randomization may be done either at the initiation of the acclimation period or at the time when the birds are weighed at the beginning of the exposure period.

(F) During holding, acclimation, and testing, birds should be shielded from excessive noise, activity, or other disturbance. Birds should be handled only as much as is necessary to conform to test procedures.

(ii) *Diet.* (A) A standard commercial game bird (for bobwhite) or duck (for mallard) starter mash, or the nutritional equivalent, should be used for diet preparation. Antibiotics or other medication should not be used in the diet before or during the test. For bobwhite only, an antibiotic demonstrated to fully depurate in 72 hours may be added to the drinking water, if necessary, for birds up through 10 days of age; however, only clean unmedicated water should be offered during the 96 hours preceding the exposure period and during the test period. It may not be possible to obtain food that is completely free of pesticides, heavy metals, and other contaminants; however, diets should be analyzed periodically, and should be selected to be as free from contaminants as possible. A nutrient analysis and list of the ingredients in the diet should be included with the test report.

(B) The test substance should be mixed into the diet in a manner that will result in even distribution of the test substance throughout the diet. If possible, the test substance should be added to the diet without the use of a diluent. If a diluent is needed, the preferred diluent is distilled water; but water should not be used as a diluent for test substances known to hydrolyze readily. When a test substance is not water soluble, it may be dissolved in a reagent grade evaporative diluent (e.g., acetone, methylene chloride) and then mixed with the test diet. The diluent should be completely evaporated prior to feeding. Other acceptable diluents may be used, if necessary, and include table grade corn oil, propylene glycol, and gum arabic (acacia). If a diluent is used, it should not comprise more than 2 percent by weight of the treated diet, and an equivalent amount of diluent should be added to control diets for untreated birds.

(C) Diets can be mixed by commercial, mechanical food mixers. For many test substances, it is recommended that treated diets be mixed under a hood. Mashes and test substances should be mixed freshly just prior to the beginning of the test. For certain volatile or other test substances, the Test Rule may require preparation of fresh diets at frequent intervals. Analysis of the diet for test substance concentrations is required under paragraph (c)(6)(ii) of this section.

(D) Clean water should be available *ad libitum.* Water bottles or automatic watering devices are recommended. If water pans or bowls are used, water should be changed at least once a day.

(2) *Facilities.* (i) Tests should be conducted with birds being maintained in commercial brooder pens or pens of similar construction. Pens should be constructed of galvanized metal, stainless steel, or perfluorocarbon plastics. Materials that are toxic, may affect toxicity, or may adsorb test substances should not be used. Wire mesh should be used for floors and external walls; solid sheeting should be used for common walls and ceilings. Wire mesh for floors should be fine enough so as to not interfere with the normal movement of young birds. Pens for housing 10 young birds should have a floor area of at least 3,000 square centimeters (approximately 500 square inches) for bobwhite and 6,000 square centimeters (approximately 1,000 square inches) for mallards and should be at least 24 centimeters (approximately 9.5 inches) high. Pens should be disassembled (if feasible) and should be cleaned thoroughly between tests. Steam cleaning of cages is recommended. Cages may be brushed thoroughly, as an alternative method. The use of detergents or bleach is acceptable, but other chemical disinfectants such as quaternary ammonium compounds should not be used. When necessary to control disease vectors, hot or cold sterilization techniques are recommended, as long as such techniques will not leave chemical residues on the cages. For cold sterilization, ethylene oxide is recommended. Pens should not be cleaned during a test.

(ii) Pens should be kept indoors to control lighting, temperature, and other environmental variables. Pens should be heated, preferably by thermostatic control. A temperature gradient in the pen of approximately 35 °C to approximately 22 °C will allow young birds to seek a proper temperature. Temperature requirements for young birds typically decline over this range from birth through the first several weeks of life. Relative humidity is not as critical, but the test room should be maintained at a relative humidity of 45–70 percent. A photoperiod of 14 hours light and 10 hours dark is recommended. Other light/dark cycles should not be used, but continuous lighting is acceptable. Lighting may be either incandescent or fluorescent. Pens and lights should be positioned so that all pens will receive similar illumination. The facilities should be well ventilated.

(iii) Where feasible, it is recommended that pens not be stacked upon each other. If pens are stacked, only one test substance is allowed in any single stack. If a test substance volatilizes or otherwise forms aerosols or vapors in the air, no more than one test substance should be tested in a room in order to avoid cross-contamination. Pens should be randomly arranged, whether or not in a stack, with respect to dose levels and controls. Pens, such as stacked, unmodified, commercial pens with external feeders, that allow food to be spilled from one pen to a lower pen, should be avoided. Any modifications that prevent cross contamination of concentration levels are acceptable. For example, commercially available, 30 cm (one foot) long chick feeders may be placed inside the pens and be covered with 1.27 cm (0.5 inch) mesh hardware cloth over the food, for bobwhite. The same feeders covered with approximately 2.5 cm (one inch) mesh wire are appropriate for mallards. For either species, external feeders can be covered with the appropriate size wire mesh and a solid piece of metal extended from the bottom of the cage to a point exterior to the feeder. Spillage may occur, but the added metal will prevent food from spilling into another feeder.

(e) *Reporting.* (1) The test report should include the following information:

(i) Name of test, sponsor, test laboratory and location, principal investigator(s), and actual dates of beginning and end of test.

(ii) Name of species tested (including scientific name), age of birds (in days) at the beginning of the test, average body weights for birds in each pen at the beginning of the test, the end of the exposure period, and end of the test, and individual weights of all birds that die during the test.

(iii) Description of housing conditions, including type, size, and material of pen, pen temperatures, approximate test room humidity, photoperiod and lighting intensity.

(iv) Detailed description of the basal diet, including source, diluents (if used), and supplements (if used). A nutrient analysis of the diet should be included in the test report.

(v) Detailed description of the test substance including its chemical name(s), source, lot number, composition (identity of major ingredients and impurities), and known physical and chemical properties that are pertinent to the test (e.g., physical state, solubility, etc.).

(vi) The number of concentrations used, nominal and (where required) measured dietary concentration of test substance in each level, assay method used to determine actual concentrations, number of birds per concentration and for controls, and names of toxicants used for positive controls (if applicable).

(vii) Acclimation procedures and methods of assigning birds to test pens.

(viii) Frequency, duration, and methods of observation.

(ix) Description of signs of intoxication and other abnormal behavior, including time of onset, duration, severity (including death), and numbers affected in the different dietary concentrations and controls each day of the test period.

(x) Estimated food consumption per pen daily or every other day in the second highest and second lowest concentration and control pens. For other pens, food consumption should be estimated for the exposure period and for the post-exposure period.

(xi) Location of raw data storage.

(xii) Results of range finding tests (if conducted).

(xiii) The calculated EC_{50} value, 95 percent confidence limits, slope of the concentration-response curve, the results of the goodness-of-fit test (e.g., chi-square test), and a description of statistical methods used. The same statistics for positive controls (when used). The methods used for statistical analysis should be described completely.

(xiv) Anything unusual about the test, any deviation from these procedures, and any other relevant information.

(2) In addition to the above information required in every report, the following information should be available upon request:

(i) A general description of the support facilities.

(ii) A description of the Quality Control/Quality Assurance program, including the Average Quality Level for the program element performing the test, procedures used, and documentations that these levels have been achieved.

(iii) The names, qualifications, and experience of personnel working in the program element performing the test, including the study director, principal investigator, quality assurance officer, as well as other personnel involved in the study.

(iv) Standard operating procedures for all phases of the test and equipment involved in the test.

(v) Sources of all supplies and equipment involved in the test.

(vi) Originals or exact copies of all raw data generated in performing the test.

§ 797.2130 Bobwhite Reproduction Test.

(a) *Purpose.* This guideline is designed to develop data on the reproductive effects on the bobwhite of chemical substances and mixtures subject to chronic environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94-469, 90 Stat. 2003 15 U.S.C. 2601 *et seq.*). The Agency will use these and other data to assess the reproductive effects on birds that these chemicals may present to the environment.

(b) *Definitions.* (1) The definitions in section 3 of the Toxic Substances Control Act (TSCA) and Part 792—Good Laboratory Practice Standards of this chapter apply to this test guideline. In addition, the following definitions apply generally to this guideline:

(i) "Acclimation" Physiological and behavioral adaptation to environmental conditions (e.g., housing and diet) associated with the test procedure.

(ii) "Test substance" The specific form of a chemical or mixture of chemicals that is used to develop the data.

(iii) "Photoperiod" The light and dark periods in a 24 hour day. This is usually expressed in a form such as 17 hours light/7 hours dark or 17L/7D.

(iv) "Basal diet" The untreated form of the diet, such as the diet obtained from a commercial source.

(2) The definitions in this section refer specifically to the production and quality of eggs and the subsequent development of these eggs up to the point where young are 14 days old.

(i) "Eggs laid" This term refers to the total egg production during the test, which normally includes 10 weeks of laying. Values are expressed as numbers of eggs per pen per season (or test).

(ii) "Eggs cracked" Eggs determined to have cracked shells when inspected with a candling lamp. Fine cracks cannot be detected without using a candling lamp and if undetected will bias data by adversely affecting embryo development. Values are expressed as a percentage of eggs laid by all hens during the test.

(iii) "Eggs set" All eggs placed under incubation, i.e., total eggs minus cracked eggs and those selected for analysis of eggshell thickness. The number of eggs set, itself, is an artificial number, but it is essential for the statistical analysis of other development parameters.

(iv) "Viable embryos (fertility)" Eggs in which fertilization has occurred and embryonic development has begun. This is determined by candling the eggs 11 days after incubation has begun. It is difficult to distinguish between the absence of fertilization and early embryonic death. The distinction can be made by breaking out eggs that appear infertile and examining further. This distinction is especially important when a test compound induces early embryo mortality. Values are expressed as a percentage of eggs set.

(v) "Live 18-day embryos" Embryos that are developing normally after 18 days of incubation. This is determined by candling the eggs. Values are expressed as a percentage of viable embryos (fertile eggs).

(vi) "Hatchability" Embryos that mature, pip the shell, and liberate themselves from the eggs on day 23 or 24 of incubation. Values are expressed as percentage of viable embryos (fertile eggs).

(vii) "14-day old survivors" Birds that survive for 2 weeks following hatch. Values are expressed both as a percentage of hatched eggs and as the number per pen per season (test).

(viii) "Eggshell thickness" The thickness of the shell and the membrane of the egg at several points around the girth after the egg has been opened, washed out, and the shell and membrane dried for at least 48 hours at room temperature. Values are expressed as the average thickness of the several measured points in millimeters.

(c) *Test procedures*—(1) *Summary of the test.* (i) After birds have been obtained they should be observed for health and acclimated for at least 2 weeks.

(ii) Test birds should be randomly assigned to control and various treatment groups.

(iii) The test substance should be thoroughly and evenly mixed into the diet at concentrations specified in the test rule. All treatment levels should be analyzed for test substance concentrations at the beginning and midway through the test.

(iv) Birds should be weighed at the beginning of the test, at 14-day intervals until the onset of laying, and at termination of the test.

(v) Photoperiod should be carefully controlled on a shortday basis during the initial exposure phase, then increased to 16 to17 hours to induce egg laying.

(vi) Birds should be observed regularly for abnormal behavior or mortality throughout the test.

(vii) Eggs should be removed daily and stored until there is a sufficient quantity for incubation. All eggs should be candled for cracks and cracked eggs removed. Once every 2 weeks, all eggs produced that day should be analyzed for eggshell thickness. Incubated eggs should be candled on day 11 and day 18. Hatching should be completed by day 24.

(viii) Hatchlings should be maintained until they are 14 days old. Abnormal behavior or death should be reported. Chicks should be weighed on day 14.

(ix) A statistical analysis should be performed, preferably by analysis of variance or regression analysis.

(x) The report should include all conditions, procedures, and results. Data should be sufficiently detailed for an independent statistical analysis.

(xi) All treated birds should be sacrificed and disposed of properly. Control birds may be kept as breeding stock, but should not be used in any other tests. Control offspring may be reared and used in another test as adults.

(2) [Reserved]

(3) [Reserved]

(4) *Definitive test*—(i) *Test substance.* (A) The concentrations of test substance in the diet will be specified in the test rule. Generally, three treatment groups and a control group will be used. The higher two treatment concentrations will be multiples (often 5×, 10×, or 20×) of the lowest treatment level. The highest treatment levels usually will be below lethal levels, unless predicted environmental exposure levels are high enough to approximate lethal concentrations.

(B) The material to be tested should be analytically pure and the degree of purity should be reported along with the percentage of each impurity at levels specified in the test rule. If specifically required by a test rule for a particular substance or mixture, the technical grade should be tested. The test rule will specify the degree of purity or a range of compositions of the technical grade material.

(ii) *Controls.* A concurrent control is required during every test. The control birds should be from the same hatch as the test groups. Control and test birds should be kept under the same experimental conditions. The test procedures should be the same for control and treated birds, except that no test substance should be added to the diets of control birds. If a carrier or diluent is used in preparation of the test diets, the same carrier should be added to the diets of control birds in the highest concentration used for test diets. The use of shared controls is acceptable for concurrent tests as long as the same carrier is used for all the tests.

(iii) *Test groups and numbers of birds.* (A) Each of the three treatment groups and the control group should consist of a minimum of 12 replicate pens. Each pen should contain one male and one female, or alternatively one male and two females. The use of 20 replicate pens in the control group may yield a test with greater statistical power. Either arrangement is acceptable if productivity reaches the definitive values given in paragraph (c)(4)(xii) of this section. Because the behavioral interactions of birds in the two arrangements are likely to be different, testing facilities using an arrangement with which they are not familiar are advised to experiment first without test substances in order to determine the feasibility of obtaining acceptable productivity levels.

(B) All control and treatment birds should be randomly distributed to pens from the same population.

(iv) *Duration of test.* (A) The test consists of three phases following acclimation to test facilities. The initial phase begins with exposure of treatment groups to diets containing the test substance and is typically 6 to 8 weeks long. After the initial phase, the photoperiod is manipulated according to paragraph (c)(4)(v) of this section to bring the hens into laying condition. This second phase ends with the onset of egg-laying and is typically 2 to 4 weeks long. The final phase begins with the onset of laying and lasts for at least 8 weeks, preferably 10 weeks. A withdrawal study period may be added to the test phase if reduced reproduction is observed. The withdrawal period, if used, need not exceed 3 weeks.

(B) Exposure of adult birds to the test substance should be continuous throughout the test. Unless otherwise specified in the test rule, test birds should be exposed for at least 10 weeks prior to the onset of egg laying.

(v) *Preparation for reproduction (photoperiod).* (A) Lighting regimes (photoperiod) are critical to successful reproduction. Various photoperiod regimes have been demonstrated to give acceptable results. Any photoperiod regime that results in productivity that meets the definitive values given in paragraph (c)(4)(xii) of this section is acceptable as long as birds are exposed to treated diets a minimum of 10 weeks prior to the onset of laying. Regardless of the methods selected, lighting should be controlled carefully. It is important during the initial phase to not interrupt the dark period unless absolutely necessary.

(B) A suggested photoperiod regime would consist of maintaining birds under a photoperiod for 7 or 8 hours of light during the initial phase. At the end of the initial phase, the photoperiod may be increased to 16 to 17 hours of light per day. The photoperiod may be maintained at this level for the remainder of the study, although an increase each week of 15 minutes per day is acceptable.

(vi) *Observations of record on adult birds.* (A) Body weights should be recorded for each adult bird at the beginning of the treatment period, at 14-day intervals until the onset of egg laying, and at termination of treatment. Recording of body weights during egg laying is discouraged because of possible adverse effects on egg production. Food consumption should be measured and recorded by pen as often as body weights are measured prior to the onset of laying and at least bi-weekly throughout the rest of the study.

(B) Observations on adult birds should be made at least once a day. Any mortality or other signs of toxicity should be described and recorded by date or day of test. Gross pathological examinations should be conducted on all birds that die during the test period, and for all survivors at the end of the test. Analysis of two or more tissues (e.g., muscle, fat) for test substance residues is encouraged, but not required (unless specified in the Test Rule).

(vii) *Egg collection, storage, and incubation.* All eggs should be collected daily, marked according to the pen from which collected, and should be stored at 16 °C and 55 to 80 percent relative humidity. Storage in plastic bags may improve uniformity of hatching. Stored eggs should be turned daily. At weekly or bi-weekly intervals, eggs should be removed from storage and be candled to detect eggshell cracks. Except for eggs with cracked shells and those eggs removed for eggshell thickness measurements, all eggs should be set after candling for incubation in a commercial incubator. If incubators are not equipped to automatically turn eggs, they should be turned daily by hand. During the incubation period, eggs should be maintained at 37.5 °C and approximately 70 percent relative humidity. Eggs should be candled again on day 11 of incubation to determine fertility and early death of embryos. A final candling should be done on day 18 to measure embryo survival. On day 21, eggs should be removed to a separate incubator or hatcher. Hatching will normally be complete by the end of day 24.

(viii) *Chick maintenance.* By day 24 of incubation, the hatched bobwhite chicks should be removed from the hatcher or incubator. Chicks should be either housed according to the appropriate parental pen group or individually marked (such as by leg bands) as to parental group and housed together. Chicks should be maintained in commercial brooder pens or pens of similar construction. Pens should be constructed of galvanized metal or stainless steel. Temperature in the pens should be controlled, preferably by a thermostatically controlled device. A temperature gradient in the pen from approximately 35 °C to approximately 22 °C will allow young birds to seek a proper temperature. Temperature requirements for young birds typically decline over this range from birth through the first several weeks of life. Chicks should be provided a standard commercial game bird starter ration, or its nutritional equivalent. No test substance may be added to the diet of chicks. Chicks should be maintained until they are 14 days old.

(ix) *Observations of record on chicks.* The hatchability, percentage of normal hatchlings, percentage of 14-day old survivors, and number of 14-day old survivors per hen should be recorded and reported. Chicks should be observed daily from hatching until they are 14 days old. Mortality, signs of toxicity, and other clinical abnormalities should be recorded at least cumulatively through day 5 and recorded by age from days 5 through 14. Average body weights should be recorded for chicks at day 14.

(x) *Eggshell thickness.* Once every two weeks all eggs newly laid that day should be removed and measured for eggshell thickness. Eggs should be opened at the girth (the widest portion), the contents washed out (or used or saved for egg residue analysis), and the shell air dried for at least 48 hours. The thickness of the shell plus the dried membrane should be measured at a minimum of 3 points around the girth using a micrometer calibrated at least to 0.01 mm units.

(xi) *Typical observed values.* The values reported here represent those observed from a few testing facilities under their conditions. These values are not necessarily representative of those from all facilities, however, if a reproduction test does not meet or at least approach these values for control birds, then there is likely to be a problem with test procedures or conditions that should be investigated and corrected.

(A) *Eggs laid.* Normal values for bobwhite—28 to 38 eggs per hen per season.

(B) *Eggs cracked.* Normal values for bobwhite—0.6 to 2.0 percent of eggs laid.

(C) *Viable embryos (fertility).* (Normal fertility values for bobwhite—75 to 90 percent of eggs set.

(D) *Live 18-day embryos.* Normal values for bobwhite—97 to 99 percent of viable embryos.

(E) *Hatchability.* Normal values for bobwhite—50 to 90 percent of viable embryos (fertile eggs).

(F) *14-day-old survivors.* Normal values for bobwhite—75 to 90 percent of eggs hatched.

(G) *Eggshell thickness.* Normal average values for bobwhite—0.19 to 0.24 mm.

(xii) *Definitive test criteria.* (A) A test is unacceptable if bobwhite chick productivity in control groups does not average twelve 14-day old survivors per pen over a 10 week period.

(B) A test is unacceptable if the average eggshell thickness in control groups is less than 0.19 mm.

(C) A test is unacceptable if more than 10 percent of the adult control birds die during the test.

(5) [Reserved]

(6) *Analytical measurements*—(i) *Statistical analysis.* Experimental groups should be individually compared to the control group by analysis of variance. Other accepted statistical methods may be used as long as they are documented. In particular, regression analysis is highly desirable if the data and number of dose levels allow the use of this technique. Sample units are the individual pens within each treatment level or control. Analysis should include:

(A) Body weight of adults.

(B) Food consumption of adults.

(C) Percentage of hens laying eggs. This should always be determined when pens contain a single pair; if feasible, it should be determined when pens contain groups.

(D) Number of eggs laid per pen.

(E) Percentage of cracked eggs.

(F) Percent viable embryos of eggs set.

(G) Percent live 18-day embryos of viable embryos.

(H) Percent hatching of viable embryos.

(I) Percentage of hatchlings that are normal.

(J) Percent 14-day-old survivors of normal hatchlings.

(K) Number of 14-day-old survivors per hen.

(L) Body weights of 14-day-old survivors.

(M) Eggshell thickness.

(ii) *Test substance concentrations.* (A) Samples of treated diets should be analyzed to confirm proper dietary concentrations of the test substance. If samples cannot be analyzed immediately, they should be stored appropriately (e.g., frozen at a temperature of −15 °C or lower) until analysis can be performed. Analyses should be conducted on all test substance concentrations at the beginning of the test period and again 10 to 12 weeks later. If not otherwise available, data should be generated to indicate whether or not the test substance degrades or volatilizes. If the test substance is known or found to be volatile or labile to the extent that 25 percent or more loss occurs within one week, then test substance diets should be prepared (freshly or from frozen concentrate) at a frequency that will prevent more than 25 percent loss of test substance.

(B) The assay method used to determine actual concentrations should be reported according to paragraph (e)(1)(vi) of this section.

(iii) *Analysis of Basal Diet.* A nutrient analysis of the basal diet should be included with the test report. For commercially prepared basal diets, the list of ingredients supplied by the manufacturer is normally sufficient if it is detailed. The composition of any vitamin or other supplements should also be reported.

(d) *Test conditions*—(1) *Test species*—(i) *Selection.* (A) Bobwhite, *Colinus virginianus* (L.), is the test species. Test birds should be pen-reared. They may be reared in the laboratory or purchased from commercial breeders. Rearing stock and/or tests birds should be obtained only from sources that have met the requirements for "U.S. Pullorum-Typhoid Clean" classification under paragraph (f)(1) of this section. Birds should be obtained only from sources whose colonies have known breeding histories. If possible, a history of rearing practices for test birds should be obtained and made available upon request. This history should include lighting practices during rearing, disease record, drug and any other medication administered, and exact age. Test birds should be phenotypically indistinguishable (except for size) from wild stock. Conscientious breeders of such birds will periodically outbreed their flocks with genetically wild stock in order to maintain a genetic composition that approximates the heterogeneity of naturally occurring birds.

(B) All control and experimental birds used in a test should be from the same source and strain. If shipped, all birds should be examined following shipment for possible physical injury that may have occurred in transit. All birds should have a health observation period of at least 2 weeks prior to selection for treatment. Birds should be in apparent good health. Deformed, abnormal, sick, or injured birds should not be used. A population of birds should not be used if more than 3 percent of either sex die during the health observation period. Birds should not have been selected in any way for resistance to toxic substances. Birds should not have been used in a previous test, either in a control or treatment group. Offspring of birds used in a treatment group in a previous test should not be used, but offspring of birds used as a control in a previous test are acceptable.

(C) Tests birds should be approaching their first breeding season and should be at least 7 months old. All test birds should be the same age within 1 month. The age of test birds should be reported.

(D) Bobwhite should be acclimated to test facilities and untreated basal diet for at least 2 weeks. Acclimation may be in the actual pens used in the test or in identical pens. The acclimation period may coincide with the health observation period. Birds should be randomly assigned to treatment and control pens. However, when birds in a pen are incompatible, they may be rearranged within a control or treatment group at any time prior to initiating treatment.

(E) During holding, acclimation, and testing, birds should be shielded from excessive noise, activity, or other disturbance. Birds should be handled only as much as is necessary to conform to test procedures.

(ii) *Diet*—(A) *Adult birds.* (*1*) A standard commercial game bird breeder ration, or its nutritional equivalent, should be used for diet preparation. This ration or basal diet should be used for both control and treatment birds and should be constant throughout the duration of the study. Antibiotics or other medication should not be used in the diet or water of breeding birds. It may not be possible to obtain food that is completely free of pesticides, heavy metals, and other contaminants. However, diets should be analyzed periodically for these substances and should be selected to be as free from contaminants as possible. A nutrient analysis (quantitative list of ingredients) of the diet should be included with the test report.

(*2*) The test substance should be mixed into the diet in a manner that will ensure even distribution of the test substance throughout the diet. If possible, the test substance should be added to the diet without the use of a carrier or diluent. If a diluent is needed, the preferred diluent is distilled water; but water should not be used for test substances known to hydrolyze readily. When a test substance is not water soluble, it may be dissolved in a reagent grade evaporative diluent (e.g., acetone, methylene chloride) and then mixed with the test diet. The solvent should be completely evaporated prior to feeding. Other acceptable diluents may be used, if necessary, and include table grade corn oil, propylene glycol, and gum arabic (acacia). If a diluent is used, it should comprise no more than 2 percent by weight of the treated diet, and an equivalent amount of diluent should be added to control diets.

(*3*) Diets may be mixed by commercial or mechanical food mixers. Other means are acceptable as long as they result in even distribution of the test substance throughout the diet. Screening of the basal diet before mixing is suggested to remove large particles. For many tests substances, it is recommended that diets be mixed under a hood. Frequently, the test substance is added to an aliquot of the basal diet to form a premix concentrate. The premix concentrate should be stored so as to maintain the chemical concentration. For final preparation of test diets, the premix is mixed with additional basal diet to form the proper concentrations. The frequency with which final treated diets are prepared will depend upon the stability and other characteristics of the test substance. Unless otherwise specified in the test rule or determined by degradation or volatility studies, it is recommended that final diets be prepared weekly, either fresh or from a concentrate. For volatile or labile test substances, test diets should be mixed frequently enough so that the concentrations are not reduced from initial concentrations by more than 25 percent. Analysis of diets for test substance concentration is required as specified in paragraph (c)(6)(ii) of this section.

(*4*) Clean water should be available *ad libitum.* Water bottles or automatic watering devices are recommended. If water pans or bowls are used, water should be changed daily or more often.

(B) *Young birds.* Young birds produced during the test should be fed a commercial game bird starter ration, or its nutritional equivalent. No test substance should be added to the diets of young birds. No antibiotics or medication may be used in the diet. Bacitracin, or one of its forms, may be added to the drinking water of young birds, if necessary.

(2) *Facilities.* (i) Bobwhite should be housed in breeding pens or cages of adequate size conforming to good husbandry practices. Space requirements for bobwhite have not been well defined, but it is recommended that there be at least 5000 square centimeters (approximately 2.7 square feet) of floor space per bird. Documentation that reproductive

parameters and health of birds are not adversely affected should be provided for cages much smaller than this area. The preferred construction materials are stainless steel, galvanized sheeting, and wire mesh. For enclosed cages, floors and external walls may be wire mesh; ceilings and common walls should be solid sheeting. Wire mesh for floors should be fine enough so as to not interfere with normal movement of bobwhite. Open-topped pens may be constructed of the same materials for the side walls with open tops and wire mesh or concrete floors. Concrete floors should be covered with litter such as straw, wood shavings, or sawdust. Other construction materials, except wood, are acceptable if they can be kept clean. Wood may be used as vertical framing posts for the support of wire mesh or for horizontal framing along the top of the pen. Wood should not be used for floors or lower sides of pens unless it has been coated with a non-adsorbent material such as perfluorocarbon plastic (e.g., Teflon), or unless the wood is replaced between tests.

(ii) Pens should be disassembled (if feasible) and should be cleaned thoroughly between tests. Steam cleaning of enclosed cages is recommended. Enclosed cages may be brushed thoroughly, as an alternative method. For open-topped pens, the sides and vertical supports should be thoroughly brushed. Any used floor litter should be discarded. The floor composition will dictate methods used to clean the floor. If litter is used on the floor, it should be fresh and clean when birds are placed in the pen. The use of detergents or bleach is acceptable, but other chemical disinfectants (such as quaternary ammonium compounds) should not be used. When necessary to control disease vectors, hot or cold sterilization techniques are recommended, as long as such techniques will not leave chemical residues on the cages. For cold sterilization, ethylene oxide is recommended.

(iii) Pens should be kept indoors in order to better control lighting, temperature, humidity, and other factors. Outdoor pens may be used only during the normal breeding season. The photoperiod should be carefully controlled, preferably by automatic timers. A 15 to 30 minute transition period is desirable. The photoperiod regime is described under test procedures under paragraph (c)(4)(v) of this section. Lights should emit a spectrum simulating that of daylight. The use of shorter wave-length "cool-white" fluorescent lights that do not emit the daylight spectrum should be avoided. Illumination intensity should be about 6 foot-candles at the level of the birds.

(iv) Temperature and humidity should be controlled during the study. Recommended levels are 21 °C and 55 percent relative humidity. Temperature should be recorded at least weekly at the same time of day and should be reported. For tests conducted without temperature control, temperature minimums and maximums should be recorded daily. Continuous temperature monitoring is desirable. Temperature recordings should be made at a level of 2.5 to 4 cm above the floor of the cage. Recording of approximate humidity levels is also desirable. Good ventilation should be maintained. Suggested ventilation rates are 4 changes per hour in winter and 15 changes per hour in summer.

(v) If facilities are being used for the first time, it may be desirable to allow birds to breed in the facility prior to testing in order to ensure that controls will have acceptable productivity according to the requirements given in paragraph (c)(4)(xi) and (xii) of this section.

(e) *Reporting.* (1) The test report should include the following information:

(i) Name of test, sponsor, test laboratory and location, principal investigator(s), and actual dates of beginning and end of test.

(ii) Name of species tested (including scientific name), age of birds (in months) at the beginning of the test, source of birds, and body weights for adult birds throughout the test.

(iii) Description of housing conditions, including type, size, and material of pen, temperature, humidity, photoperiod and lighting intensity, and any changes during the test.

(iv) Detailed description of the basal diet, including source, composition, diluents (if used), and supplements (if used). A nutrient analysis of the basal diet should be included.

(v) Detailed description of the test substance including its chemical name(s), source, lot number, composition (identity of major ingredients and impurities), and known physical and chemical properties pertinent to the test (e.g., solubility, volatility, degradation rate, etc.).

(vi) The number of concentrations used, nominal and measured concentrations of test substance in each level, assay method used to determine actual concentrations, storage conditions and stability of treated diets, number of birds per pen and number of replicate pens per concentration and for controls.

(vii) Acclimation procedures and methods of assigning birds to test pens, including method of randomization, and any rearrangement due to incompatibility.

(viii) Frequency, duration, and methods of observation.

(ix) Description of any signs of intoxication, including time of onset, duration, severity (including death), and numbers affected, including accidental deaths or injuries.

(x) Food consumption per pen and any observations of repellancy or food palatability.

(xi) Method of marking all birds and eggs.

(xii) Details of autopsies.

(xiii) Egg and hatching data in summary and by pen per week in sufficient detail to allow an independent statistical analysis. Data should be presented for all of the parameters listed in paragraph (c)(6)(i) of this section. The number of eggs set should also be reported.

(xiv) Egg storage, incubation, and hatching temperatures, relative humidities, and turning frequencies.

(xv) Observations of health and weights of young at 14 days of age.

(xvi) Location of all raw data storage.

(xvii) Methods of statistical analysis and interpretation of results.

(xviii) Anything unusual about the test, any deviation from these procedures, and any other relevant information.

(2) In addition, the following information should be available upon request:

(i) A general description of the support facilities.

(ii) A description of the Quality Control/Quality Assurance program, including the Average Quality Level for the program element performing the test, procedures used, and documentations that these levels have been achieved.

(iii) The names, qualifications, and experience of personnel working in the program element performing the test, including the study director, principal investigator, quality assurance officer, as well as other personnel involved in the study.

(iv) Standard operating procedures for all phases of the test and equipment involved in the test.

(v) Sources of all supplies and equipment involved in the test.

(vi) Diagram of the test layout.

(vii) Originals or exact copies of all raw data generated in performing the test.

(viii) A detailed description, with references, of all statistical methods.

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) U.S. Department of Agriculture. "National Poultry Improvement Plan," Report No. 2. *Directory of Participants Handling Waterfowl, Exhibition Poultry, and Game Birds.* U.S.D.A., Science and Education Administration, Beltsville, MD 20705 (1979).

(2) [Reserved]

§ 797.2150 Mallard Reproduction Test.

(a) *Purpose.* This guideline is designed to develop data on the reproductive effects on the mallard of chemical substances and mixtures subject to chronic environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94-469, 90 Stat. 2003 15 U.S.C. 2601 *et seq.*). The Agency will use these and other data to assess the reproductive effects on birds that these chemicals may present in the environment.

(b) *Definitions.* (1) The definitions in section 3 of the Toxic Substances Control Act (TSCA) and Part 792—Good Laboratory Practice Standards of this chapter apply to this test guideline. In addition, the following definitions apply generally to this guideline:

(i) "Acclimation" Physiological and behavioral adaptation to environmental conditions (e.g., housing and diet) associated with the test procedure.

(ii) "Test substance" The specific form of a chemical or mixture of chemicals that is used to develop the data.

(iii) "Photoperiod" The light and dark periods in a 24 hour day. This is usually expressed in a form such as 17 hours light/7 hours dark or 17L/7D.

(iv) "Basal diet" The untreated form of the diet, such as the diet obtained from a commercial source.

(2) The definitions in this section refer specifically to the production and quality of eggs and the subsequent development of these eggs up to the point where young are 14 days old.

(i) "Eggs laid" this term refers to the total egg production during the test, which normally includes 10 weeks of laying. Values are expressed as numbers of eggs per pen per season (or test).

(ii) "Eggs cracked" Eggs determined to have cracked shells when inspected with a candling lamp. Fine cracks cannot be detected without using a candling lamp and if undetected will bias data by adversely affecting embryo development. Values are expressed as a percentage of eggs laid by all hens during the test.

(iii) "Eggs set" All eggs placed under incubation, i.e., total eggs minus cracked eggs and those selected for analysis of eggshell thickness. The number of eggs set, itself, is an artificial number, but it is essential for the statistical analysis of other development parameters.

(iv) "Viable embryos (fertility)" Eggs in which fertilization has occurred and embryonic development has begun. This is determined by candling the eggs 14 days after incubation has begun. It is difficult to distinguish between the absence of fertilization and early embryonic death. The distinction can be made by breaking out eggs that appear infertile and examining further. This distinction is especially important when a test compound induces early embryo mortality. Values are expressed as a percentage of eggs set.

(v) "Live 21-day embryos" Embryos that are developing normally after 21 days of incubation. This is determined by candling the eggs. Values are expressed as a percentage of viable embryos (fertile eggs).

(vi) "Hatchability" Embryos that mature, pip the shell, and liberate themselves from their eggs on day 25, 26, or 27 of incubation. Values are expressed as a percentage of viable embryos (fertile eggs).

(vii) "14-day old survivors" Birds that survive for 2 weeks following hatch. Values are expressed both as a percentage of hatched eggs and as the number per pen per season (test).

(viii) "Eggshell thickness" The thickness of the shell and the membrane of the egg at several points around the girth after the egg has been opened, washed out, and the shell and membrane dried for at least 48 hours at room temperature. Values are expressed as the average thickness of the several measured points in millimeters.

(c) *Test Procedures*—(1) *Summary of test.* (i) After birds have been obtained they should be observed for health and acclimated for at least 2 weeks.

(ii) Test birds should be randomly assigned to control and various treatment groups.

(iii) The test substance should be thoroughly and evenly mixed into the diet at concentrations specified in the test rule. All treatment levels should be analyzed for test substance concentrations at the beginning and midway through the test.

(iv) Birds should be weighed at the beginning of the test, at 14-day intervals until the onset of laying, and at termination of the test.

(v) Photoperiod should be carefully controlled on a shortday basis during the initial exposure phase, then increased to 16 to 17 hours to induce egg laying.

(vi) Birds should be observed regularly for abnormal behavior or mortality throughout the test.

(vii) Eggs should be removed daily and stored until there is a sufficient quantity for incubation. All eggs should be candled for cracks and cracked eggs removed. Once every 2 weeks, all eggs produced that day should be analyzed for eggshell thickness. Incubated eggs should be candled on day 14 and day 21. Hatching should be completed by day 27.

(viii) Hatchlings should be maintained in pens until they are 14 days old. Abnormal behavior or death should be reported. Ducklings should be weighed on day 14.

(ix) A statistical analysis should be performed, preferably by analysis of variance or regression analysis.

(x) The report should include all conditions, procedures, and results. Data should be sufficiently detailed for an independent statistical analysis.

(xi) All treated birds should be sacrificed and disposed of properly. Control birds may be kept as breeding stock, but should not be used in any other tests. Control offspring may be reared and used in another test as adults.

(2) [Reserved]

(3) [Reserved]

(4) *Definitive test*—(i) *Test substance.* (A) The concentrations of test substance in the diet will be specified in the test rule. Generally, three treatment groups and a control group will be used. The higher two treatment levels will be multiples (often 5×, 10×, or 20×) of the lowest treatment level. The highest treatment levels usually will be below lethal levels, unless predicted exposure levels are high enough to approximate lethal levels.

(B) The material to be tested should be analytically pure and the degree of purity should be reported along with the percentage of each impurity at levels specified in the test rule. If specifically required by a test rule for a particular substance or mixture, the technical grade should be tested. The test rule will specify the degree of purity or a range of compositions of the technical grade material.

(ii) *Controls.* A concurrent control is required during every test. The control birds should be from the same hatch as the test groups. Control and test birds should be kept under the same experimental conditions. The test procedures should be the same for control and treated birds, except that no test substance should be added to the diets of control birds. If a carrier is used in preparation of the test diets, the same carrier should be added to the diets of control birds in the highest concentration used for test diets. The use of shared controls is acceptable for concurrent tests as long as the same carrier is used for all the tests.

(iii) *Test groups and numbers of birds.* (A) Either one of two designs may be used for numbers of animals and pens. For one design, each of the three treatment groups and the control group should consist of a minimum of 8 replicate pens, with each pen containing one male and three females. For the alternative design, each group should consist of 12 replicate pens containing one male and one female per pen; the use of 20 replicate pens in the control group may yield a test with greater statistical power. Either design is acceptable as long as productivity reaches the definitive values given in paragraph (c)(4)(xii) of this section. Testing facilities using an experimental design with which they are not familiar are advised to experiment first without test substances in order to determine the feasibility of obtaining acceptable productivity levels.

(B) All control and treatment birds should be randomly distributed to pens from the same population.

(iv) *Duration of test.* (A) The test consists of three phases following acclimation to test facilities. the initial phase begins with exposure of treatment groups to diet containing the test substance and is typically 6 to 8 weeks long. After the initial phase, the photoperiod is manipulated according to paragraph (c)(4)(v) of this section to bring the hens into laying condition. This second phase ends with the onset of egglaying and is typically 2 to 4 weeks long. The final phase begins with the onset of laying and lasts for at least 8 weeks, preferably 10 weeks. A withdrawal study period may be added to the test phase if reduced reproduction is observed. The withdrawal period, if used, need not exceed 3 weeks.

(B) Exposure of adult birds to the test substance should be continuous throughout the test. Unless otherwise specified in the test rule, test birds should be exposed to the test substance for at least 10 weeks prior to the onset of egg laying.

(v) *Preparation for reproduction (photoperiod).* (A) Lighting regimes (photoperiod) are critical to successful reproduction. Various photoperiod regimes have been demonstrated to give acceptable results. Any photoperiod regime that results in productivity that meets the definitive values given in paragraph (c)(4)(xii) of this section is acceptable as long as birds are exposed to treated diets a minimum of 10 weeks prior to the onset of laying. Regardless of the method selected, lighting should be controlled carefully. It is important during the initial phase to not interrupt the dark period unless absolutely necessary.

(B) A suggested photoperiod regime would consist of maintaining birds under a photoperiod of 7 or 8 hours of light during the initial phase. At the end of the initial phase, the photoperiod may be increased to 16–17 hours of light per day. The photoperiod may be maintained at this level for the remainder of the study, although an increase each week of 15 minutes per day is acceptable.

(vi) *Observations of record on adult birds.* (A) Body weights should be recorded for each adult bird at the beginning of the treatment period, at 14-day intervals until the onset of egg laying, and at termination of treatment. Birds may be weighed during egg production phase of the study only if they are not unduly stressed by the procedure. Food consumption should be measured and recorded by pen at least as often as body weights are measured prior to the onset of laying and at least bi-weekly throughout the rest of the study.

(B) Observations on adult birds should be made at least once a day. Any mortality or other signs of toxicity should be described and recorded by date or day of test. Gross pathological examinations should be conducted on all birds that die during the test period, and for all survivors at the end of the test. Analysis for test substance residues of two or more tissues (e.g., muscle, fat) is encouraged, but not required unless specified in the individual Test Rule under Part 799 of this chapter.

(vii) *Egg collection, storage, and incubation.* All eggs should be collected daily, marked according to the pen from which collected, and should be stored at 16 °C and 55 to 80 percent relative humidity. Storage in plastic bags may improve uniformity of hatching. Stored eggs should be turned daily. At weekly or biweekly intervals, eggs should be removed from storage and be candled to detect eggshell cracks. Except for eggs with cracked shells and those eggs removed for eggshell thickness measurements all eggs should be set after candling for incubation in a commercial incubator. If incubators are not equipped to automatically turn eggs, they should be turned daily by hand. During the incubation period, eggs should be maintained at 37.5 °C and approximately 70 percent relative humidity. Eggs should be candled again on day 14 of incubation to determine fertility and early death of embryo. A final candling should be done on day 21 to measure embryo survival. On day 23, eggs should be removed to a separate incubator or hatcher. Hatching will normally be complete by the end of day 27.

(viii) *Duckling maintenance.* By day 27 of incubation, the hatched mallard ducklings should be removed from the hatcher or incubator. Ducklings should be either housed according to the appropriate parental pen group or individually marked (such as by leg bands) as to parental group and housed together. Ducklings should be maintained in commercial brooder pens or pens of similar construction. Pens should be constructed of galvanized metal or stainless steel. Temperature in the pens should be controlled, preferably by a thermostatic control device. A temperature gradient in the pen from approximately 35 °C to approximately 22 °C will allow young birds to seek a proper temperature. Temperature requirements for young birds typically decline over this range from birth through the first several weeks of life. Ducklings should be provided a standard commercial duck starter ration, or its nutritional equivalent. No test substance may be added to the diets of ducklings. Ducklings should be maintained until they are 14 days old.

(ix) *Observation of record on ducklings.* The hatchability, percentage of normal hatchlings, percentage of 14-day old survivors, and number of 14-day old survivors per hen should be recorded and reported. Ducklings should be observed daily from hatching until they are 14 days old. Mortality, signs of toxicity, and other clinical abnormalities should be recorded at least cumulatively through day 5 and recorded by age from days 5 through 14. Average body weights should be recorded for ducklings at day 14.

(x) *Eggshell thickness.* Once every two weeks all eggs newly laid that day should be removed and measured for eggshell thickness. Eggs should be opened at the girth (the widest portion), the contents washed out (or used or saved for egg residue analysis), and the shells air dried for at least 48 hours. The thickness of the shell plus the dried membrane should be measured at a minimum of 3 points around the girth using a micrometer calibrated at least to 0.01 mm units.

(xi) *Typical observed values.* The values reported here represent those observed from a few testing facilities under their conditions. These values are not necessarily representative of those from all facilities, however, if a reproduction test does not meet or at least approach these values for control birds, then there is likely to be a problem with test procedures or conditions that should be investigated and corrected. Typical values include:

(A) *Eggs laid.* Normal values for mallards—28 to 38 eggs per hen per season.

(B) *Eggs cracked.* Normal values for mallards—0.6 to 6 percent of eggs laid.

(C) *Viable embryos (fertility).* Normal fertility values for mallards—85 to 98 percent of eggs set.

(D) *Live 21-day embryos.* Normal values for mallards—97 to 99 percent of viable embryos.

(E) *Hatchability.* Normal values for mallards—50 to 90 percent of viable embryos (fertile eggs).

(F) *14-day-old survivors.* Normal values for mallards—94 to 99 percent of eggs hatched.

(G) *Eggshell thickness.* Normal average values for mallards—0.34 to 0.39 mm.

(xii) *Definitive test criteria.* (A) A test is unacceptable if mallard duckling productivity in control groups does not average fourteen 14-day old survivors per hen over a 10 week period.

(B) A test is unacceptable if the average eggshell thickness in control groups is less than 0.34 mm.

(C) A test is unacceptable if more than 10 percent of the adult control birds die during the test.

(5) [Reserved]

(6) *Analytical measurements*—(i) *Statistical analysis.* (A) Experimental groups should be individually compared to the control group by analysis of variance. Other accepted statistical methods may be used as long as they are documented and described. In particular, regression analysis is highly desirable if the data and number of dose levels allow the use of this technique. Sample units are the individual pens within each treatment level or control. Analysis should include:

(*1*) Body weights of adults.

(*2*) Food consumption of adults.

(*3*) Percentage of hens laying eggs. This should always be determined when pens contain a single pair; if feasible, it should be determined when pens contain groups.

(*4*) Number of eggs laid per pen.

(*5*) Percentage of cracked eggs.

(*6*) Percent viable embryos of eggs set.

(*7*) Percent live 21-day embryos of viable embryos.

(*8*) Percent hatching of viable-embryos.

(*9*) Percentage of hatchlings that are normal.

(*10*) Percent 14-day-old survivors of normal hatchlings.

(*11*) Number of 14-day-old survivors per hen.

(*12*) Body weights of 14-day-old survivors.

(*13*) Eggshell thickness.

(ii) *Analysis for test substance concentrations.* (A) Samples of treated diets should be analyzed to confirm proper dietary concentrations of the test substance. If samples cannot be analyzed immediately, they should be stored appropriately (e.g., frozen at a temperature of −15 °C or lower) until analysis can be performed. Analyses should be conducted on all test substance concentrations at the beginning of the test period and again 10 to 12 weeks later. If not otherwise available, data should be generated to indicate whether or not the test substance degrades or volatilizes. If the test substance is known or found to be volatile or labile to the extent that 25 percent or more loss occurs within one week, then test substance diets should be prepared (freshly or from frozen concentrate) at a frequency that will prevent more than 25 percent loss of test substance.

(B) The assay method used to determine actual concentrations should be reported according to paragraph (e)(1)(vi) of this section.

(C) Analysis of basal diet. A nutrient analysis of the basal diet should be included in the test report. For commercially prepared basal diets, the list of ingredients supplied by the manufacturer is normally sufficient, if it is detailed. The composition of any vitamin or other supplements should also be reported.

(d) *Test conditions*—(1) *Test species*—(i) *Selection.* (A) The mallard, *Anas platyrhynchos* L., is the test species. Test birds should be pen-reared. They may be reared in the laboratory or purchased from commercial breeders. Rearing stock and/or test birds should be obtained only from sources that have met the requirements for "U.S. Pullorum-Typhoid Clean" classification under paragraph (f)(1) of this section. Birds should be obtained only from sources whose colonies have known breeding histories. If possible, a history of rearing practices for test birds should be obtained and made available upon request. This history should include lighting practices during rearing, disease record, drug and any other medication administered, and exact age. Test birds should be phenotypically indistinguishable (except for size) from wild stock. Conscientious breeders of such birds will periodically outbreed their flocks with genetically wild stock in order to maintain a genetic composition that approximates the heterogeneity of naturally occurring birds.

(B) All control and experimental birds used in a test should be from the same source and strain. If shipped, all birds should be examined following shipment for possible physical injury that may have occurred in transit. All birds should have a health observation period of at least 2 weeks prior to selection for treatment. Birds should be in apparent good health. Deformed, abnormal, sick, or injured birds should not be used. A population of birds should not be used if more than 3 percent of either sex die during the health observation period. Birds should not have been selected in any way for resistance to toxic substances. Birds should not have been used in a previous test, either in a control or treatment group. Offspring of birds used in a treatment group in a previous test should not be used, but offspring of birds used as a control in a previous test are acceptable.

(C) Test birds should be approaching their first breeding season and should be at least 7 months old. All test birds should be the same age within 1 month. The age of test birds should be reported.

(D) Mallards should be acclimated to test facilities and untreated basal diet for at least 2 weeks. Acclimation may be in the actual pens used in the test or in identical pens. The acclimation period may coincide with the health observation period. Birds should be randomly assigned to treatment and control pens. However, when birds in a pen are incompatible, they may be rearranged within a control or treatment group at any time prior to initiating treatment.

(E) During holding, acclimation, and testing, birds should be shielded from excessive noise, activity, or other disturbance. Birds should be handled only as much as is necessary to conform to test procedures.

(ii) *Diet*—(A) *Adult birds.* (*1*) A standard commercial duck breeder ration, or its nutritional equivalent, should be used for diet preparation. This ration or basal diet should be used for both control and treatment birds and should be constant throughout the duration of the study. Antibiotics or other medication should not be used in the diet or water of breeding birds. It may not be possible to obtain food that is completely free of pesticides, heavy metals, and other contaminants. However, diets should be analyzed periodically for these substances and should be selected to be as free from contaminants as possible. A nutrient analysis (quantitative list of ingredients) of the diet should be included with the test report.

(*2*) The test substance should be mixed into the diet in a manner that will ensure even distribution of the test substance throughout the diet. If possible, the test substance should be added to the diet without the use of a carrier or diluent. If a diluent is needed, the preferred diluent is distilled water; but water should not be used for test substances known to hydrolyze readily. When a test substance is not water soluble, it may be dissolved in a reagent grade evaporative diluent (e.g., acetone, methylene chloride) and then mixed with the test diet. The solvent should be completely evaporated prior to feeding. Other acceptable diluents may be used, if necessary, and include table grade corn oil, propylene glycol, and gum arabic (acacia). If a diluent is used, it should comprise no more than 2 percent by weight of the treated diet, and an equivalent amount of diluent should be added to control diets.

(*3*) Diets may be mixed by commercial or mechanical food mixers. Other means are acceptable as long as they result in even distribution of the test substance throughout the diet. Screening of the basal diet before mixing is suggested to remove large particles. For many test substances, it is recommended that diets be mixed under a hood. Frequently, the test substance is added to an aliquot of the basal diet to form a premix concentrate. The premix concentrate should be stored so as to maintain the chemical concentration. For final preparation of test diets, the premix is mixed with additional basal diet to form the proper concentrations. The frequency with which final treated diets are prepared will depend upon the stability and other characteristics of the test substance. Unless otherwise specified in the test rule or determined by degradation or volatility studies, it is recommended that final diets be prepared weekly, either fresh or from a concentrate. For volatile or labile test substances, test diets should be mixed frequently enough so that the concentrations are not reduced from initial concentrations by more than 25 percent. Analysis of diets for test substance concentrations is required as specified in paragraph (c)(6)(ii) of this section.

(*4*) Clean water should be available *ad libitum*. Water bottles or automatic watering devices are recommended. If water pans or bowls are used, water should be changed daily or more often.

(B) *Young birds*. Young birds produced during the test should be fed a commercial duck starter ration, or its nutritional equivalent. No test substance should be added to the diets of young birds. No antibiotics or medication should be used in the diet.

(2) *Facilities*. (i) Mallards should be housed in breeding pens or cages of adequate size conforming to good husbandry practices. Space requirements for mallards have not been well defined, but it is recommended that there be at least 10,000 square centimeters (approximately 5.4 square feet) of floor space per bird. Documentation that reproductive parameters and health of birds are not adversely affected should be provided for cages much smaller than this area. The preferred construction materials are stainless steel, galvanized sheeting, and wire mesh. For enclosed cages, floors and external walls may be wire mesh; ceilings and common walls should be solid sheeting. Open-topped pens may be constructed of the same materials for the side walls with open tops and wire mesh or concrete floors. Concrete floors should be covered with litter such as straw, wood shavings, or sawdust. Other construction materials, except wood, are acceptable if they can be kept clean. Wood may be used as vertical framing posts for the support of wire mesh or for horizontal framing along the top of the pen. Wood should not be used for floors or lower sides of pens unless it has been coated with a non-adsorbent material such as perfluorocarbon plastic (e.g., Teflon) or unless the wood is replaced between tests.

(ii) Pens should be disassembled (if feasible) and should be cleaned thoroughly between tests. Steam cleaning of enclosed cages is recommended. Enclosed cages may be brushed thoroughly, as an alternative method. For open-topped pens, the sides and vertical supports should be thoroughly brushed. Any used floor litter should be discarded. The floor composition will dictate methods used to clean the floor. If litter is used on the floor, it should be fresh and clean when birds are placed in the pen. The use of detergents or bleach is acceptable, but other chemical disinfectants (such as quaternary ammonium compounds) should not be used. When necessary to control disease vectors, hot or cold sterilization techniques are recommended, as long as such techniques will not leave chemical residues on the cages. For cold sterilization, ethylene oxide is recommended.

(iii) Pens should be kept indoors in order to better control lighting, temperature, humidity, and other factors. Outdoor pens may be used only during the normal breeding season. The photoperiod should be carefully controlled, preferably by automatic timers. A 15 to 30 minute transition period is desirable. The photoperiod regime is described under paragraph (c)(4)(v) of this section. Lights should emit a spectrum simulating that of daylight. The use of shorter wave-length "cool-white" fluorescent lights that do not emit the daylight spectrum should be avoided. Illumination intensity should be about 6 foot-candles at the level of the birds.

(iv) Temperature and humidity should be controlled during the study. Recommended levels are 21 °C and 55 percent relative humidity. Temperature for indoor tests should be recorded at least weekly at the same time of day and should be reported. For tests conducted without temperature control, temperature minimums and maximums should be recorded daily. Continuous temperature monitoring is desirable. Temperature recording should be made at levels of 2.5 to 4 cm above the floor of the cage. Recording of approximate humidity levels is also desirable. Good ventilation should be maintained. Suggested ventilation rates are 4 changes per hour in winter and 15 changes per hour in the summer.

(v) If facilities are being used for the first time, it may be desirable to allow birds to breed in the facility prior to testing in order to ensure that controls will have acceptable productivity according to the requirements given in paragraph (c)(4) (xi) and (xii) of this section.

(e) *Reporting*. (1) The test report should include the following information:

(i) Name of test, sponsor, test laboratory and location, principal investigator(s), and actual dates of beginning and end of test.

(ii) Name of species tested (including scientific name), age of birds (in months) at the beginning of the test, source of birds, and body weights for adult birds throughout the test.

(iii) Description of housing conditions, including type, size, and material of pen, temperature, humidity, photoperiod and lighting intensity, and any changes during the test.

(iv) Detailed description of the basal diet, including source, composition, diluents (if used), and supplements (if used). A nutrient analysis of the basal diet should be included.

(v) Detailed description of the test substance including its chemical name(s), source, lot number, composition (identity of major ingredients and impurities), and known physical and chemical properties pertinent to the test (e.g., solubility, volatility, degradation rate, etc.).

(vi) The number of concentrations used, nominal and measured concentrations of test substance in each level, assay method used to determine actual concentrations, storage conditions and stability of treated diets, number of birds per pen and number of replicate pens per concentration and for controls.

(vii) Acclimation procedures and methods of assigning birds to test pens, including method of randomization, and any rearrangements due to incompatibility.

(viii) Frequency, duration, and methods of observation.

(ix) Description of any signs of intoxication, including time of onset, duration, severity (including death), and numbers affected, including accidental deaths or injuries.

(x) Food consumption per pen and any observations of repellency or food palatability.

(xi) Method of marking all birds and eggs.

(xii) Details of autopsies.

(xiii) Egg and hatching data in summary and by pen per week in sufficient detail to allow an independent statistical analysis. Data should be presented for all of the parameters listed in paragraph (c)(6)(i) of this section. The number of eggs set also should be reported.

(xiv) Egg storage, incubation, and hatching temperatures, relative humidities, and turning frequencies.

(xv) Observations of health and weights of young at 14 days of age.

(xvi) Location of all raw data storage.

(xvii) Methods of statistical analysis and interpretation of results.

(xviii) Anything unusual about the test, any deviation from these procedures, and any other relevant information.

(2) In addition, the following information should be available upon request:

(i) A general description of the support facilities.

(ii) A description of the Quality Control/Quality Assurance program, including the Average Quality Level for the program element performing the test, procedures used, and documentations that these levels have been achieved.

(iii) The names, qualifications, and experience of personnel working in the program element performing the test, including the study director, principal investigator, quality assurance officer, as well as other personnel involved in the study.

(iv) Standard operating procedures for all phases of the test and equipment involved in the test.

(v) Sources of all supplies and equipment involved in the test.

(vi) Diagram of the test layout.

(vii) Originals or exact copies of all raw data generated in performing the test.

(viii) A detailed description, with references, of all statistical methods.

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) U.S. Department of Agriculture. "National Poultry Improvement Plan," Report No. 2. *Directory of Participants Handling Waterfowl, Exhibition Poultry, and Game Birds.* U.S.D.A., Science and Education Administration, Beltsville, MD 20705 (1979).

(2) [Reserved]

§ 797.2175 Avian Acute Oral Toxicity Test.

(a) *Purpose.* The guideline in this section is designed to develop data on the acute oral toxicity to Northern bobwhite and Mallard for chemical substances and mixtures subject to acute environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94-469, 90 Stat. 2003 15 U.S.C. 2601 *et seq.*). The Agency will use these and other data to assess the acute hazard to birds.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA) and Part 792—Good Laboratory Practice Standards of this chapter apply to this test guideline. In addition, the following definitions apply to this guideline:

(1) "Acclimation" Physiological or behavioral adaptation of test animals to environmental conditions and basal diet associated with the test procedure.

(2) "LD_{50}" The empirically derived dose of the test substance that is expected to result in mortality of 50 percent of a population of birds which is treated with a single oral dose under the conditions of the test.

(3) "Test substance" The specific form of a chemical or mixture of chemicals that is used to develop the data.

(4) "Observation period" The portion of the test that begins after the test birds have been dosed and extends at least 14 days.

(5) "Hatch" Eggs or birds that are the same age and that are derived from the same adult breeding population, where the adults are of the same strain and stock.

(c) *Test procedures*—(1) *Summary of test.* (i) After birds have been obtained, they are acclimated for at least 14 days. The dosage levels for the definitive test are established, possibly requiring a range-finding test to be conducted first. Test birds are randomly assigned to the various dosage levels and controls. Birds are weighed and the test substance is administered as a single oral dose either by gavage or capsule. Birds are closely monitored for 60 to 120 minutes after doses are given and then observed regularly for mortality or other signs of intoxication throughout the observation period. Birds are weighed and feed consumption is estimated at least weekly. The mortality pattern is examined and subjected to the appropriate statistical analysis to derive the LD_{50}, confidence limits, and slope of the dose-response line. The complete mortality pattern, along with signs of intoxication and necropsy data, should be reported.

(ii) A test is unacceptable if more than 10 percent of the control birds die during the test.

(2) [Reserved]

(3) *Range-finding test.* Unless the approximate toxicity of the test substance is known already, a range-finding test should be conducted to determine the dosage levels of the test substance to be used in the definitive test. Refer to paragraph (c)(4)(iv) of this section for details on dosage levels for definitive tests. Procedures for range-finding tests may vary, but generally, groups of a few birds are administered three to five widely-spaced doses. A series of 2, 20, 200, and 2,000 mg/kg-body weight is suggested. If a test substance is expected to be of low toxicity, it may be of advantage to conduct a limit test at 2000 mg/kg first under paragraph (c)(4)(iv)(B) of this section. If mortality occurs at this level, then further range-finding at lower levels is suggested. The results of the range-finding test then may be used to establish the definitive test dosage levels.

(4) *Definitive test*—(i) *Administration of test substance.* (A) After acclimation under paragraph (d)(1)(i)(D) of this section, feed should be withheld from all test groups for a minimum of 15 hours prior to administration of the test substance. Dosing by gavage is preferred; where gavage is not feasible doses may be administered by gelatin capsule. Doses are to be based on the individual body weight of each bird; weights are typically determined at the

time of dosing, but may be taken, especially for capsules, within 24 hours prior to dosing. Dosing should be done in the early morning hours.

(B) If a carrier is used to administer the test substance, the preferred carrier is distilled or deionized water unless the test substance is known to hydrolyze readily. Other acceptable carriers include corn oil, propylene glycol, and gum acacia. Materials with known toxic or emetic properties should not be used. The dosing volume of test substance plus carrier in a test should be constant for all birds with respect to individual body weights and should not exceed 5 ml/kg-body weight. For those unusual test substances that might require a larger dosing volume (e.g., liquids with low purity), a dosing volume up to 8 ml/kg may be used; however, the test species should be bobwhite or else steps should be taken to ensure that mallards do not regurgitate the dose.

(ii) *Controls.* (A) A concurrent control is required during every test. Control birds should be from the same hatch as the test groups. Control and test birds should be kept under the same experimental conditions. The test procedures should be the same for control and treated birds, except that no test substance should be administered to the control birds. Control birds should receive a sham dose consisting of the same carrier or capsule as received by the test birds. The use of shared controls is acceptable for concurrent tests as long as the same carrier or capsule is used for all the tests.

(B) A test is not acceptable if more than 10 percent of the control birds die during the test period.

(C) A concurrent positive control with a substance of known toxicity is not required. However, a quarterly or semi-annual test with a laboratory standard (reference toxicant) is recommended as a means of detecting possible interlaboratory or temporal variation. A laboratory standard is also recommended when there is any significant change in food, housing, or source of birds.

(iii) *Number and sex of animals tested.* (A) In the definitive test, a minimum of ten birds should be used for each dosage level of the test substance and for the control. Birds may be of either sex or both sexes in any ratio. A 1:1 ratio is commonly used.

(B) Birds at a dosage level may be divided into two pens of five birds each. If this is done, dividing the groups by sex is encouraged.

(iv) *Concentrations and dosage-mortality data.* (A) A minimum of five dosage levels of the test substance should be used in the definitive test. These levels should be spaced geometrically. The recommended spacing is for each dosage level to be at least 60 percent of the next higher level (less than 1.67 times the next lower level). Ideally, dosage levels should be spaced so that at least three levels should result in mortality between, but not including, 0 percent and 100 percent and at least one level should kill more than 50 percent and at least one level should kill less than 50 percent of the birds in a group. For some test substances, it may be necessary to use more than five dosage levels to achieve these results.

(B) For test substances expected to have relatively low toxicity, a limit test may be conducted at 2000 mg/kg. The LD_{50} may be reported as greater than 2000 mg/kg if 10 birds are dosed at 2000 mg/kg, if no mortality occurs, and if test procedures, number of controls, and duration are the same, except for the number of dosage levels, as in the definitive test. Signs of intoxication, if any, should be reported. No further testing is required at lower dosage levels.

(v) *Duration of test.* The definitive test consists of the administration of the test substance followed by an observation period of at least 14 days. If mortality occurs during the last 3 days of the 14-day period, or if signs of intoxication are not clearly in remission, or if the test substance is expected to have delayed effects, then the observation period should be extended to at least 21 days or until mortality or signs of intoxication are not observed for 72 hours.

(vi) *Observations.* (A) Birds should be monitored closely for the first 60 to 120 minutes after dosing. Any regurgitation should be noted and reported. Additional observations of test birds should be made, at a minimum, 3 times on the day of dosing and at least daily throughout the remainder of the test period. Where feasible, twice daily observations are recommended.

(B) Throughout the test period, all signs of intoxication, other abnormal behavior, and mortality should be recorded and reported by dosage level and by day. Signs of intoxication are those behaviors apparently due to the test chemical and may include a wide array of behaviors, such as labored respiration, leg weakness, hemorrhage, convulsions, ruffled feathers, etc. All signs of intoxication and any other abnormal behavior, such as excessive aggression, toe-picking, etc. that may or may not be attributed to the test substance should be reported. Among survivors, remission of signs of intoxication and cessation of abnormal behavior should be recorded by dosage level and by day. An estimate of the number of birds exhibiting such signs should be recorded for each dosage level.

(C) Individual body weights of birds should be recorded and reported for control and treated birds at the time of calculating the dosage to be administered and weekly thereafter until the test is concluded. An extra weighing the third day after dosing may provide useful information, especially on anorexia. Body weights of birds a week prior to dosing are not required, but would provide valuable base-line data. Feed consumption should be recorded at least weekly throughout the test; valuable additional information can be obtained by monitoring food consumption daily, especially for the first few days following dosing.

(D) Gross pathology examinations should be conducted on at least two or three birds dying at each dosage level and on all control birds that die. Gross pathological examinations of survivors are optional, but may provide valuable information, especialy for lesions associated with sublethal effects.

(5) [Reserved]

(6) *Analytical measurements*—(i) *Statistical analysis.* (A) The data should be analyzed, preferably by graphical or computational methods of probit analysis. The LD_{50} value, 95 percent confidence limits, and slope of the transformed dose-response curve should be determined for mortality at the end of test. A test for heterogeneity of the data (e.g., chi square test) should be conducted. Other standard statistical methods are acceptable if they provide the slope of the dose-response line as well as the LD_{50} value.

(B) All methods used for statistical analysis should be described completely.

(ii) *Analysis of basal diet.* (A) A proximate analysis of the basal diet should be included in the test report. The analysis should include percentages by weight of protein, fat, fiber, ash, calcium, and phosphorus. In addition to these analyzed components, a list of expected amounts of vitamins, minerals, or other supplements also should be reported. Most commercial feed companies provide both the analysis and the list of supplements on the label.

(B) A contaminants analysis of the feed should be conducted periodically for heavy metals (e.g., arsenic, cadmium, lead, mercury, and selenium) and persistent pesticides, especially chlorinated insecticides. A broader pesticide screen to include, for example, diazinon, methyl parathion, and malathion may be useful.

(d) *Test conditions*—(1) *Test species*—(i) *Selection*. (A) Northern bobwhite, *Colinus virginianus* (L.), and mallard, *Anas platyrhynchos* L., are the test species. Birds may be reared in the laboratory or purchased from a breeder. All control and treatment birds used in a test should be from the same source and breeding population. Birds should be obtained only from sources whose colonies have known breeding histories. Birds should be phenotypically indistinguishable (except for size) from wild stock. It is recommended that birds be obtained from flocks that have been outbred periodically in order to maintain a genetic composition that approximates the natural heterogeneity of the species.

(B) Birds used in the test should be in apparent good health. Deformed, abnormal, sick, or injured birds should not be used. Birds should not be used for a test if more than 5 percent of the total test population die during the 14-day acclimation period. Birds purchased from a breeder should be certified as disease-free or as bred from disease-free stocks. Birds should not have been selected in any way for genetic resistance to toxic substances. Birds should not have been used in a previous test, either in a treatment or control group.

(C) Test birds should be young adults, not yet mated, at least 16 weeks old at the time of dosing. A less preferred alternative is for the use of first year birds that may have been mated, as long as the birds are brought completely out of production through reduced light cycles. All birds used in a test should be the same age ±1 week. It is recommended that weights be at least 180g for bobwhite and 900g for mallard. More consistent responses may be attainable if the range of body weights is no greater ± 10 percent of the mean body weight for the test population. The age should be recorded and reported.

(D) Test birds should be acclimated to test facilities and basal diet for a minimum of 14 days. Acclimation to test pens should be in the actual pens used in the test. Birds used in the test should be assigned randomly to treatment and control pens, except that assignment may be made to result in only one sex per pen if replicate pens are used for each dosage level, under paragraph (c)(4)(iii) of this section. Randomization should be done at the initiation of the acclimation period.

(E) During holding, acclimation, and testing, birds should be shielded from excessive noise, activity, or other disturbance. Birds should be handled only as much as is necessary to conform to test procedures.

(ii) *Diet*. (A) A standard commercial game bird (for bobwhite) or duck (for mallard) feed or the nutritional equivalent, should be used as the diet. Feed should not be used past its normal shelf life. Antibiotics or other medication should not be used in the diet during the acclimation period or the test. It may not be possible to obtain feed that is completely free of pesticides, heavy metals, and other contaminants; however, diets should be analyzed periodically, under paragraph (c)(6)(ii)(B) of this section, and selected to be as free from contaminants as possible. Extra precautions should be taken when fish meal or oil is a major ingredient, since fish are often contaminated with high levels of chlorinated hydrocarbons.

(B) Clean water should be available *ad libitum*. Only clean, unmedicated water should be offered during the acclimation and testing periods. Water bottles or automatic watering devices are recommended. If water pans or bowls are used, water should be changed at least once a day.

(2) *Facilities*. (i) Tests should be conducted indoors with birds being maintained in commercial breeder or holding pens or pens of similar construction. Pens should be constructed of galvanized metal, stainless steel, or perfluorocarbon plastics. Materials that are toxic, likely to influence toxicity, or sorb test substances should not be used. Wire mesh should be used for floors and external walls; solid sheeting should be used for common walls and ceilings. Wire mesh for floors should be fine enough so as to not interfere with the normal movement of birds yet coarse enough to allow fecal material to fall through. Pens should have a floor area of at least 500 square centimeters per bird (approximately 75 square inches) for bobwhite and 1,000 square centimeters per bird (approximately 150 square inches) for mallards and should be at least 24 centimeters (approximately 9.5 inches) high for bobwhite and 32 centimeters (approximately 12.5 inches) high for mallard. Between tests pens should be disassembled (if feasible) and should be cleaned thoroughly. Steam cleaning of cages is recommended. Cages may be hosed, brushed thoroughly and hosed again, as an alternative method. The use of detergents or bleach is acceptable, but other chemical disinfectants such as quaternary ammonium compounds should not be used. When necessary to control disease vectors, hot or cold sterilization techniques are recommended, as long as such techniques will not leave chemical residues on the cages. For cold sterilization, ethylene oxide is recommended. Pens should not be cleaned during a test.

(ii) Testing is done indoors to control lighting and other environmental variables. Temperatures for adult birds should be maintained at normal indoor temperatures, preferably between 15 °C and 27 °C (60 to 80 °F). Ventilation should be sufficient to supply 10 to 15 air changes per hour. The test room should be maintained at a relative humidity of 45 to70 percent. Higher humidities are appropriate for waterfowl. A photoperiod of 8 hours light and 16 hours dark is recommended in order to prevent birds from coming into reproductive condition. Lighting may be either incandescent of fluorescent. Pens and lights should be positioned so that all pens will receive approximately equal illumination.

(3) [Reserved]

(e) *Reporting*. (1) The report should include, but not necessarily be limited to, the following information:

(i) Name and address of the facility performing the study and the dates on which the study was initiated and was completed, terminated, or discontinued.

(ii) Objectives and procedures stated in the approved protocol, including any changes in the original protocol.

(iii) Statistical methods employed for analyzing the data.

(iv) The test and, if used, control substances identified by name, Chemical Abstracts Service (CAS) number or code number, source, lot or batch number, strength, purity, and composition or other appropriate characteristics.

(v) Stability of the test and, if used, control substances under the conditions of administration.

(vi) A description of the methods used, including:

(A) Description of housing conditions, including type, size, and material of pens, and the approximate test room temperature, humidity, ventilation rate, photoperiod, and lighting intensity.

(B) Detailed description of feed, including source, supplements (if used), and proximate analysis.

(C) Acclimation procedures and methods of assigning birds to test pens, and test pens to dose-levels.

(D) Frequency, duration, and methods of observations.

(vii) A description of the test system used, including the scientific name of the test species, number used, sex and reproductive history and condition, age (in weeks) at the beginning of the test, source, and procedures used for identification. Individual body weights (or means, extremes, and an estimate of variance) should be reported for the beginning of the test and weekly thereafter.

(viii) A description of the dosages, numbers of birds and replicates per dose, method and time of administration. The reported results should include:

(A) The results of range-finding tests, if conducted.

(B) For the definitive test, a description of signs of intoxication and other abnormal behavior, including time of onset, duration, severity (including death), and number affected at each dose level and control each day of the test.

(C) Feed consumption per pen at least weekly or as often as measured, if more frequently than weekly, along with an estimate of wastage.

(D) The results of gross pathological examinations.

(ix) A description of all circumstances that may have affected the quality or integrity of the data.

(x) The name of the sponsor, study director, principal investigator, names of other scientists or professionals, and the names of all supervisory personnel involved in the study.

(xi) A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis. Results of the analysis of data should include the calculated LD_{50} value, 95 percent confidence limits, slope of the transformed dose-response line, and the results of a goodness-of-fit test (e.g., chi-square test).

(xii) The signed and dated reports of each of the individual scientists or other professionals involved in the study, including each person who, at the request or direction of the testing facility or sponsor, conducted an analysis or evaluation of data or specimens from the study after data generation was completed.

(xiii) The locations where all specimens, raw data, and the final report are stored.

(xiv) The statement prepared and signed by the quality assurance unit.

§ 797.2750 Seed Germination/Root Elongation Toxicity Test.

(a) *Purpose.* The guideline in this section is intended for use in developing data on the acute toxicity of chemical substances and mixtures ("chemicals") subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (PUB. L. 94–469, 90 Stat. 2003, 15 U.S.C. 2601 *et seq.*). This guideline prescribes test procedures and conditions using seed of commercially important terrestrial plants to develop data on the phytotoxicity of chemicals. The United States Environmental Protection Agency (U.S. EPA) will use data from these tests in assessing the hazard of a chemical to the environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA) and the definitions in Part 792—Good Laboratory Practice Standards of this chapter apply to this test guideline. The following definitions also apply to this guideline:

(1) "ECX" means the experimentally derived chemical concentration that is calculated to effect X percent of the test criterion.

(2) "Embryo" means the young sporophytic plant before the start of germination.

(3) "Germination" means the resumption of active growth by an embryo. The primary root should attain a length of 5 mm for the seed to be counted as having germinated.

(4) "Hypocotyl" means that portion of the axis of an embryo or seedling situated between the cotyledons (seed leaves) and the radicle.

(5) "Radicle" means that portion of the plant embryo which develops into the primary root.

(6) "Test solution" means the test chemical and the dilution water in which the test chemical is dissolved or suspended.

(c) *Test procedures*—(1) *Summary of the test.* (i) Seed should be separated into appropriate size classes, and that size class containing the most seed used exclusively for the test. Fresh test solutions should be added to petri dishes that have been completely filled with either precleaned quartz sand, 200 micron glass beads, or other inert material. The seed should then be positioned on the substrate allowing adequate room for anticipated growth. It is recommended that the radicle end of the seed be aligned in the direction of this growth. Petri dish lids should be used to hold the seed in place, and the dishes sealed with tape. For those chemicals that are insoluble in water and that should be sorbed to the substrate, deionized or glass-distilled water should be added to the substrate prior to positioning the seed.

(ii) The dishes should be placed in a seed germinator or other growth facility at a slight angle to facilitate linear root growth. Seed should be incubated in the dark until at least 65 percent of the control seed have germinated and developed roots that are at least 20 mm long.

(iii) The number of seed that germinate should be counted, and root lengths measured. Concentration response curves, EC_{10}'s, and EC_{50}'s for seed germination and root elongation should be determined and reported for each of the species tested.

(2) *Chemical application.* (i) Test chemicals that are soluble in water should be dissolved in deionized or glass distilled water and added to the substrate in the petri dishes at the start of the test.

(ii) Test chemicals that are insoluble in water but which can be placed in aqueous suspension with a carrier should be suspended in deionized or glass-distilled water with the carrier and then added to the petri dishes. The carrier should be soluble in water, relatively nontoxic to plants, and should be used in the minimum amount required to dissolve or suspend the test chemical. There are no preferred carriers; however, acetone, gum arabic, polyethylene glycol, ethanol and others have extensively been used in testing herbicides, plant growth regulators, fungicides, and other chemicals that affect plants. Tests of the carrier effect should be included in the test experimental design and conducted simultaneously as controls.

(iii) Water-insoluble chemicals for which no nontoxic water-soluble carrier is available, should be dissolved in an appropriate volatile solvent. The solution and substrate should be placed in a rotary vacuum apparatus, and evaporated, leaving a uniform coating of test chemical on the substrate. A weighed portion of the substrate should be extracted with the same organic solvent and the chemical assayed before the containers are filled. Solvent controls should be included in the experimental design and tested simultaneously. Deionized or glass distilled water should be added to the treated substrate prior to positioning the seed on the substrate.

(3) *Range-Finding Test.* (i) A range-finding test should be conducted to establish (A) if definitive testing is necessary and (B) test solution concentrations for the definitive test.

(ii) The seed should be exposed to a chemical concentration series (e.g., 0.01, 0.1, 1.0, 10, 100, and 1,000 mg/l. The lowest concentration in the series, exclusive of controls, should be at the chemical's detection limit. The upper concentration, for water soluble compounds, should be the saturation concentration.

(iii) The test consists of one run for each of the recommended plant species or selected alternates. A minimum of 15 seed per species should be exposed to each chemical concentration and control. The test period may be ended when at least 65 percent of the control seed have germinated and developed roots that are at least 20 mm long. The exposure period may be shortened if data suitable to establish the test solution concentration series for the definitive test can be obtained in less time and if the definitive test is to be conducted. No replicates are required; and nominal concentrations of the chemical are acceptable unless definitive testing is not required as specified in paragraph (c)(3)(iv) of this section.

(iv) Definitive testing is not necessary if the highest chemical concentration tested results in less than a 50 percent inhibition of germination or reduction in root growth or if the lowest concentration tested (analytical detection limit) results in greater than a 50 percent inhibition of germination or reduction in growth.

(v) Graphical analysis of the range-finding data facilitates selection of chemical concentrations for the definitive test.

(4) *Definitive test.* (i) The purpose of the definitive test is to determine the concentration-response curves, the EC_{10}'s and EC_{50}'s for seed germination and root elongation for each species tested, with the minimum amount of testing beyond the range-finding test.

(ii) The seed of each species tested should be exposed to at least 6 concentrations of the chemical chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 2,4,8,16,32, and 64 mg/l). The concentration ranges should be selected to determine the concentration response curves between the EC_{10} and EC_{50} for both germination and root elongation. Test solutions or substrate extracts should be analyzed to determine chemical concentration prior to use. Selection of seed from the size class lot to be exposed to each test concentration should be unbiased.

(iii) At least three replicates, each with at least 10 seed per species should be tested for each concentration and control.

(iv) Every test should include controls consisting of the same dilution water, conditions, procedures and seed from the same lot used in the exposure group, except that none of the chemical is added. If a carrier (solvent) is needed to suspend or disperse the chemical, a separate carrier control should also be used.

(v) The test period may be ended when at least 65 percent of the control seed have germinated and developed roots that are at least 20 mm long. When both conditions are satisfied, the mean number of seed germinating and mean root length per treatment (and control) can be determined. If the test chemical concentration series does not bracket the EC_{10} through EC_{50}, for both germination and root elongation, the test should be repeated (at a higher or lower concentration series). Concentration response curves, EC_{10}'s and EC_{50}'s for germination and root elongation should be determined for each species tested and reported along with their 95 percent confidence limits.

(vi) Any abnormal seedling development or appearance such as lesions, enhanced root growth (measured), discoloration, swelling, loss of turgor, etc., should also be reported.

(vii) A randomized complete block design is recommended for the definitive test with blocks delineated within the seed germinator or growth chamber. If, for any reason, blocking is not feasible total randomization within chambers is acceptable.

(viii) Temperature in the germination facility should be recorded hourly. The pH of the test solutions should be recorded at the initiation of the definitive test.

(5) [Reserved]

(6) *Analytical measurements*—(i) *Test chemical.* Stock solutions should be diluted with glass distilled or deionized water to obtain the test solutions. Standard analytical methods, if available, should be used to establish concentrations of these solutions and should be validated before beginning the test. An analytical method is not acceptable if likely degradation products of the chemical, such as hydrolysis and oxidation products, give positive or negative interference. The pH of these solutions should also be measured prior to use.

(ii) *Numerical.* The number of seeds that germinate shall be counted and root lengths measured for each definitive test species. All root elongation measurements for a given species should be made sequentially before proceeding to the next species. Root length should be measured from the transition point between the hypocotyl and root to the tip of the root. Means and standard deviations should be calculated and plotted for each treatment and control. Appropriate statistical analyses should provide a goodness-of-fit determination for the concentration response curves.

(d) *Test conditions*—(1) *Test species.* (i) Test plants recommended for use include:

(A) *Lycopersicon esculentum* (tomato).

(B) *Cucumis sativus* (cucumber).

(C) *Lactuca sativa* (lettuce).

(D) *Glycine max* (soybean).

(E) *Brassica oleracea* (cabbage).

(F) *Avena sativa* (oat).

(G) *Lolium perenne* (perennial ryegrass).

(H) *Allium cepa* (common onion).

(I) *Daucus carota* (carrot).

(J) *Zea mays* (corn).

(ii) Other species of economic or ecological importance to the region of impact, may also be appropriate for testing. A minimum of 10 species should be tested.

(iii) Information on seed lot, the seed year or growing season collected, and germination percentage should be provided by the supplier of the seed. Only untreated seed (not treated with fungicides, repellents, etc.) taken from the same lot, and year or season of collection should be used in a given test. In addition, all seed of a species used in a test should be from the size class which contains the most seed. Damaged seed should be discarded. Standard seed dockage sieves should be used to size seed.

(2) *Facilities*—(i) *Apparatus.* (A) seed germinator, or other controlled environment chamber capable of maintaining a uniform testing temperature of 25 ± 1 °C is required. In addition, the facilities should include work areas for sizing, counting, and exposing seed for root measurement. If possible, these areas should be isolated from other activities. A fume hood may be needed when testing substances potentially hazardous to human health. Apparatus for distilling and deionizing water are needed unless reagent grade water is used. Refrigeration facilities to hold the seed in cold storage (5 °C) in moisture-proof containers at seed moisture contents of less than 10 percent are also needed.

(B) Disposal facilities should be adequate to accommodate spent glassware, sand, beads, and test solutions at the end of each run and any bench covering, lab clothing, or other contaminated materials.

(ii) *Containers and support media.* A minimum of 210 petri dishes and sufficient sand or glass beads, or other inert substrate to fill them are needed. Large (200 mm) glass petri dishes are recommended. Perlite, vermiculite, or native soils, should not be used as substrates.

(iii) *Cleaning and sterilization.* (A) All glassware and the substrate should be cleaned following standard good laboratory practice before each test. The substrate should be washed in half strength concentrated nitric acid and rinsed with a mild base followed by washes of glass-distilled or deionized water. The pH of the washed substrate should be near neutral. If the glass beads are to be reused, they should be heated to 100 °C for 8 to 12 hours prior to acid washing. A dichromate solution should not be used for cleaning beads or petri dishes. The sand and plastic petri dishes should not be reused.

(B) If fungal or other microbial contamination interferes with seed germination such that germination is less than 65 percent in the controls, glassware should be sterilized and/or the seed surface sterilized prior to use, e.g., the seed may be soaked for 10 minutes in a 10 percent sodium hypochlorite solution, then rinsed and soaked for 1 hour in glass-distilled water.

(3) *Test parameters.* Environmental conditions should be controlled to maintain incubation temperature at 25 ± 1 °C in complete darkness. If species other than the ten recommended for use are tested, incubation conditions may have to be adjusted to meet germination and root length criteria in the controls

(e) *Reporting.* The sponsor should submit to the USEPA all data developed during the test that are suggestive or predictive of phytotoxicity. In addition to the general reporting requirements prescribed in Part 792—Good Laboratory Practice Standards of this chapter, the following should be reported:

(1) Information on the source and history of the seed, germination percentage reported by the supplier, and the seed size class used for testing.

(2) The number of seed of each species per treatment, the number of replicates, carriers, incubation conditions, and seed sterilization procedures.

(3) The concentration of the chemical added to each treatment dish and its pH (pH is optional).

(4) If the range-finding test showed that the highest concentration of the chemical tested (not less than 1,000 mg/l) had no effect on the test species, report the results by species and concentration and a statement that the chemical is of minimum phytotoxic concern.

(5) If the range-finding test showed greater than 50 percent inhibition of germination or root elongation at a test concentration at the analytical detection limit, the results by species and concentration and a statement that the chemical is phytotoxic below the analytical detection limit.

(6) For each species included in the definitive test, means and standard deviations for germination and root length in each treatment. In addition, concentration response curves with 95 percent confidence limits delineated, goodness-of-fit determination, and EC_{10}'s and EC_{50}'s identified.

(7) Methods and data records of all chemical and numerical analyses including method validation and reagent blanks.

(8) The data records of the incubation temperature, germination counts, and root length measurements.

§ 797.2800 Early Seedling Growth Toxicity Test.

(a) *Purpose.* The guideline in this section is intended for use in developing data on the toxicity of chemical substances and mixtures ("chemicals") subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94–469, 90 Stat. 2003, 14 U.S.C. 2601 *et. seq.*). This guideline prescribes tests using commercially important terrestrial plants to develop data on the phytotoxicity of chemicals. The United States Environmental Protection Agency (U.S. EPA) will use data from these tests in assessing the hazard of a chemical to the environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA), and Part 792—Good Laboratory Practice Standards of this chapter apply to this test guideline. The following definitions also apply to this test guideline.

(1) "EC X" means the experimentally derived chemical concentration that is calculated to effect X percent of the test criterion.

(2) "Germination" means the resumption of active growth by an embryo.

(3) "Support media" means the quartz sand or glass beads used to support the plant.

(c) *Test Procedures*—(1) *Summary of the Test*—(i) *Root exposure.* In preparation for the test, seeds are planted in the potting containers (or in cotton or glass-wool plugs supported in hydroponic solution) and after germination seedlings are thinned by pinching the stem at the support medium surface to the 10 most uniform seedlings per pot. This marks the start of the test and the time of first application of test chemical. Seedlings emerging after this time are also pinched off at the surface. Potting mixtures of sand or glass beads are subirrigated with nutrient solution. Chemicals are applied to the plants via nutrient solution or are adsorbed to the support media. Plants are harvested after 14 days and analyzed for growth.

(ii) *Foliar exposure.* The foliar exposure test is identical to the root exposure test except that chemicals are applied to plants by either spraying or dusting the foliage or by exposing the plants to gas in a fumigation chamber.

(2) *Chemical application*—(i) *Root exposure.* (A) Chemicals that are soluble in water should be dissolved in the nutrient solution just prior to the beginning of the test Deionized or glass-distilled water should be used in making stock solutions of the test chemical. Sufficient quantities of each concentration should be made up as needed to minimize storage time and disposal volume.

(B) Chemicals that are insoluble in water, but which can be suspended in an aqueous solution by a carrier, should be added, with the carrier, to the nutrient solution. The carrier should be soluble in water, relatively nontoxic to plants, and should be used in the minimum amount required to dissolve or suspend the test chemical. There are no preferred carriers; however, acetone, gum arabic, polyethylene glycol, ethanol, and others have extensively been used in testing herbicides, plant growth regulators, fungicides, and other chemicals that affect plants. Carrier controls should be included in the experimental design of the test and tested simultaneously.

(C) Water-insoluble chemicals for which no nontoxic, water-soluble carrier is available, should be dissolved in an appropriate volatile solvent. The solution should be mixed with the sand or glass beads which are then placed in a rotary vacuum apparatus and evaporated leaving a uniform coating of chemical on the sand or beads. A weighed portion of beads should be extracted with the same organic solvent and the chemical assayed before the potting containers are filled. Solvent controls should be included in the experimental design and tested simultaneously.

(ii) *Foliar exposure.* (A) Water-soluble chemicals should be dissolved in deionized or glass-distilled water just prior to use. Sufficient quantities of each concentration should be made up as needed. These solutions should be applied daily (during the normal 5-day work week). Plants should be placed in an exhaust hood and the chemical applied to the foliage. A plastic sleeve may be fitted over the top of the pot, and the foliage sprayed with specific quantities of test solution at known concentrations. The plastic sleeve, confining the chemical to plant and pot, facilitates expression of chemical dosage to quantity per pot area (i.e., µg/m^2). Shoots of control plants should be sprayed in an identical manner with deionized or distilled water. Alternatively, a miniature compressed-air sprayer mounted on a pendulum and equipped to automatically spray a plant positioned directly beneath the center of its arc of swing may be used.

(B) Water-insoluble chemicals, existing as solids, may be prepared for testing by grinding or other reduction to particles of <200 um diameter. Each day (during the normal 5-day work week) plants should be placed in an exhaust hood, a plastic sleeve fitted over the top of the pot, and specific quantities of chemical sprinkled uniformly over the potted seedlings. Prior to chemical application, plants should be misted with water to promote foliar retention of the chemical. Control plants should also be misted with deionized or distilled water at each treatment date and dusted with an inert material of the same particle size. Applications are expressed as quantity per unit pot area (i.e., µg/m^2).

(C) Chemicals existing in gaseous form at normal ambient temperatures and pressures can be generated as needed or stored under pressure. The bottled gas may be 100 percent chemical or may be mixed with an inert carrier, such as nitrogen, to known concentrations. Chemicals of controlled or measured concentrations should be metered into the exposure chamber, uniformly mixed about the plants, and exhausted through an outlet port.

(3) *Range-finding test.* (i) A range-finding test should be conducted to establish (A) if definitive testing is necessary and (B) the concentrations of test substance used in the definitive test for each species.

(ii) The recommended procedure is to expose newly germinated seedlings to a series of widely spaced concentrations of test chemical and assess effect as growth reduction. Seeds (approximately 30) should be planted directly in containers filled to within 2.5 cm of the top with quartz sand or glass beads. If a hydroponic system is used, the seeds should be planted in plugs of cotton or glass wool supported at the top of the solution. When 50 percent of the seeds have germinated the seedlings should be thinned (by pinching) to the 10 most uniform per pot and exposed to a widely spaced concentration series (i.e., 0.01, 0.1, 1.0, 10, 100, 1,000 mg/l) of test chemical. The lowest concentration in the series, exclusive of controls, should be at the chemical's detection limit. The upper concentration, for water-soluble compounds, should be the saturation concentration. If the anticipated fate of the chemical is soil or soil water, and the mechanism of concern is root uptake, the chemical should be applied in nutrient solution to the root support media (or coated on sand or glass beads for non-water soluble chemicals). With a chemical whose anticipated mode of exposure to plants is surface deposition by atmospheric transport, or irrigation water, the appropriate testing method may be foliar application allowing subsequent movement into the rooting zone with watering. Effect is assessed as growth reduction.

(iii) Alternatively, the seed germination/root elongation test may be used to establish the appropriate concentration range for testing.

(iv) No replicates are required and nominal concentrations are acceptable unless definitive testing is not required.

(v) Definitive testing is not necessary if the highest chemical concentration tested results in less than a 50 percent reduction in growth or if the lowest concentration tested (analytical detection limit) results in greater than a 50 percent reduction in growth.

(4) *Definitive test.* (i) The purpose of the definitive test is to determine the concentration response curves and the EC_{10}'s and EC_{50}'s for each of the species tested with the minimum amount of testing beyond the range-finding test.

(ii) At least 5 concentrations of chemical, exclusive of controls, should be used in the definitive test. For each species tested the concentration range should be selected to define the concentration-response curve between the EC_{10} and EC_{90}'s. Test chemicals should be added to the hydroponic or nutrient solution or coated on the support media for the root exposure test; or sprayed, dusted, or gassed directly on the foliage in the foliage exposure tests.

(iii) Control pots should be included in the experimental design and should be used in each run. In addition, a carrier control should also be used for those chemicals that need to be solubilized.

(iv) If plants are to be grown hydroponically, seeds should be planted in plugs of cotton or glass wool supported in the tops of the containers. When sand or glass beads are used, the recommended planting procedure is to fill the potting containers to within 2.5 cm of the top and to sow seeds directly on the support media. After 50 percent of the seeds have germinated, the seedlings should be thinned to the 10 most uniform per pot.

(v) Alternative planting methods may be required when the chemical is highly volatile. An impervious barrier of polyethylene film, a modification of the double pot method, a glass plate, or other appropriate apparatus should be used to prevent volatilization from the root zone. Seeds should be germinated in the dark at 25 °C and seedlings with radicle lengths in the median range transplanted into the potting containers. The seedlings should be positioned such that their roots are exposed to the support media while the shoots pass through holes in the barrier. A ring of non-toxic, inert, pliable putty should be used to seal the holes around the stems. Control pots should be handled identically to the test pots except there is no exposure to the test chemical. This transplanting procedure, without the volatilization barrier, is also recommended when the test chemical is adsorbed to the support medium.

(vi) The test consists of one run for each of the recommended plant species or selected alternates. The duration of a run should be at least 14 days from the time that 50 percent of the seeds have germinated. For a particular chemical, a run is defined as exposure of the plant species to five concentrations of the chemical in a minimum of 3 replicate pots (10 plants per pot), with appropriate controls, followed by weight and height determinations and analysis.

(vii) All abnormalities (visible effects of the chemicals on plant growth and morphology including stunting of growth, discoloration, chlorosis and/or necrosis of the leaves, or morphological abnormalities) should be recorded. Observations of plants should be made daily (during the normal 5-day work week).

(viii) A randomized complete block design is recommended for this test with blocks delineated within the chambers or over greenhouse benches and randomization of treatment occurring within the blocks. If, because of very large pots, there exists inadequate space within chambers for blocking, total randomization within chambers is acceptable.

(ix) Irradiation measurements should be taken at the top of the plant canopy and the mean, plus a maximum and a minimum value, determined over the plant-growing area. These measurements should be taken daily and should be taken at least at the start and finish of the test. If the test is conducted in a greenhouse facility, hourly measurements of irradiation should be recorded and presented as daily total irradiance plus representative hourly curves for clear sky conditions and cloudy days.

(x) Temperature and humidity should be measured daily at the top of the plant canopy during each light and dark period.

(xi) Measurements of carbon dioxide concentration should be made at the top of the plant canopy (of chamber-grown plants) on a "continuous basis".

(5) [Reserved]

(6) *Analytical measurements*—(i) *Chemical.* Stock solutions should be diluted with glass distilled or deionized water to obtain the test solutions. Standard analytical methods, if available, should be used to establish concentrations of these solutions and should be validated before beginning the test. An analytical method is not acceptable if likely degradation products of the chemical, such as hydrolysis and oxidation products, give positive or negative interference. The pH of these solutions should also be measured prior to use.

(ii) *Numerical.* Mass and length of roots, shoots, and entire plants (root and shoot) should be measured for the definitive test. Means and standard deviations should be calculated and plotted for each treatment and control. Appropriate statistical analyses should provide a goodness-of-fit determination for the concentration-response curves.

(d) *Test conditions*—(1) *Test Species*—(i) *Selection.* (A) Test plants recommended for the definitive test include:

(*1*) *Lycopersicon esculentum* (tomato)
(*2*) *Cucumis sativus* (cucumber)
(*3*) *Lactuca sativa* (lettuce)
(*4*) *Glycine max* (soybean)
(*5*) *Brassica oleracea* (cabbage)
(*6*) *Avena sativa* (oat)
(*7*) *Lolium perenne* (perennial ryegrass)
(*8*) *Allium cepa* (common onion)
(*9*) *Daucus carota* (carrot)
(*10*) *Zea mays* (corn)

(B) Other species, of economic or ecologic importance to the region of impact, may also be appropriate and selected for testing.

(ii) *Seed selection.* Information on seed lot, the seed year or growing season collected and germination percentage should be provided by the source of the seed. Only untreated seed (not treated with fungicides, repellants, etc.) taken from the same lot, and year or season of collection should be used in a given test. In addition, all seed of a species used in a test should be of the same size class; and that size class which contains the most seed should be selected and used in a given test. Any seed which is damaged should be discarded.

(2) *Facilities*—(i) *Apparatus.* (A) Greenhouses or environmental chambers should provide adequate environmental controls to meet the carbon dioxide, humidity, irradiation, photoperiod, and temperature specifications. Chambers should be designed to prevent escape of internal air into the external environment other than through appropriate filtering material or media to prevent contamination of the external environment with the test chemical.

(B) Laboratory facilities for chemical determinations should include nonporous floor-covering, absorbent bench covering with non-porous backing, and adequate disposal facilities to accommodate plant nutrient, test and wash solutions containing test chemicals at the end of each run, and any bench covering, lab clothing, or other contaminated materials.

(ii) *Containers and support media.* For each run, 18 polyethylene pots sufficiently large to grow at least 10 plants up to 14 days, are required for each species. It is equally acceptable to use small, individual containers if plants are grown in hydroponic solution. An additional 3 pots will be needed if a carrier control is needed. Potting containers used in each experiment should be of equal size and volume and possess the same configuration. When sand or glass beads are used, the potting containers should be filled to within 2.5 cm of their tops. Perlite, vermiculite, native soils, etc., should not be used for root support.

(iii) *Cleaning and sterilization.* (A) Potting and receiving containers, nutrient storage containers, and root support medium should be cleaned before use. All equipment should be washed according to good standard laboratory procedures to remove any residues remaining from manufacturing or prior use. A dichromate solution should not be used for cleaning beads or pots.

(B) Rooting media other than glass beads should be discarded at the end of the experiment. Disposal should conform to existing regulations.

(iv) *Nutrient media.* Half-strength modified Hoagland nutrient solution should be utilized as nutrient media for this test. When sand or glass beads are used as a support media, the potting containers should be filled with nutrient solution and drained periodically. An automated system design is recommended.

(3) *Test parameters.* Environmental conditions should be maintained as specified in this paragraph.

(i) Carbon dioxide concentration at 350 ± 50 ppm.

(ii) Relative humidity should approach 70 ± 5 percent during light periods and 90 percent during dark periods.

(iii) Irradiation, measured at 1 meter from the source, at 350 ± 50 $\mu E/m^2$ sec at 400 to 700 nm.

(iv) Photoperiods of 16 hours light and 8 hours darkness.

(v) Day/night temperatures at 25°/20° ± 3°C.

(e) *Reporting.* Reporting requirements of Part 792—Good Laboratory Practice Standards of this chapter apply to this guideline. The following data should be reported for each of the species tested in tabular form:

(1) Concentration of chemical in nutrient solution and in the root support material when the chemical is soluble in water or solubilized with a carrier compound; or the concentration of carrier compound in nutrient solution when carrier is used; or the quantity of chemical per unit weight of root support material when it is coated on the material.

(2) The quantity of chemical, the concentration at which it was applied, and the number of applications for those chemicals applied to the foliage.

(3) Environmental conditions (day/night temperatures, relative humidity, light intensity, carbon dioxide concentration, and photoperiod).

(4) Mass of above ground (shoot) and below ground (root) portion of each plant and mass of each whole plant (dry weight at 70 °C).

(5) Length of shoot, root, and entire plant.

(6) Visible effects of chemical, if any, on the intact plants.

(7) Means and standard deviations for mass and length of roots, shoots, and entire plants in each treatment and control. In addition, concentration-response curves with 95 percent confidence limits delineated, goodness-of-fit determination, and EC_{10}'s and EC_{50}'s identified.

§ 797.2850 Plant Uptake and Translocation Test.

(a) *Purpose.* The guideline in this section is intended for use in developing data on the uptake and translocation of chemical substances and mixtures ("chemicals") by terrestrial plants subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94-469, 90 Stat. 2003, 14 U.S.C. 2601 *et seq.*). This guideline prescribes tests using commercially important terrestrial plants to develop data on the quantity of chemical substances incorporated in plant tissues and the potential for entry into food chains with resultant indirect human exposure. The United States Environmental Protection Agency (U.S. EPA) will use data from these tests in assessing the hazard of a chemical to the environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA), and Part 792—Good Laboratory Practice Standards of this chapter apply to this test guideline. The following definitions also apply to this guideline:

(1) "EC X" means the experimentally derived chemical concentration that is calculated to effect X percent of the test criterion.

(2) "Mass balance" means a quantitative accounting of the distributions of chemical in plant components, support medium, and test solutions. It also means a quantitative determination of uptake as the difference between the quantity of gas entering an exposure chamber, the quantity leaving the chamber, and the quantity adsorbed to the chamber walls.

(3) "Support media" means the sand or glass beads used to support the plant.

(4) "Translocation" means the transference or transport of chemical from the site of uptake to other plant components.

(c) *Test procedures*—(1) *Summary of the test*—(i) *Root exposure.* In preparation for the test, seeds are planted in the potting containers (or in cotton or glass-wool plugs supported in hydroponic solution) and, after germination, seedlings thinned, by pinching the stem at the support surface. Potting mixtures of sand or glass beads should be subirrigated with nutrient solution. Chemicals are applied to the plants via nutrient solution or adsorbed to the support media. Carrot, lettuce, onion, cabbage, and ryegrass may be harvested whenever there is adequate plant material for chemical analysis. Cucumber, corn, soybean, tomato, and oats should be grown until fruit or seed are mature.

(ii) *Foliar exposure.* The foliar exposure test is identical to the root exposure test except that chemicals are applied to plants by either spraying or dusting the foliage or exposing the plants to gas in a fumigation chamber. If plants are fumigated, either rates of uptake and surface adsorption should be calculated, or the plants may be harvested and analyzed for test chemical and residues.

(2) *Chemical Application*—(i) *Root exposure.* (A) Chemicals that are soluble in water should be dissolved in the nutrient solution just prior to the beginning of the test. Deionized or glass distilled water should be used in making stock solutions of the test chemical. Sufficient quantities of each concentration should be made up as needed to minimize storage time and disposal volume.

(B) Chemicals that are insoluble in water but which can be placed in aqueous suspension with a carrier should be added, with the carrier, to the nutrient solution. The carrier should be soluble in water, relatively nontoxic to plants, and should be used in the minimum amount required to dissolve or suspend the test chemical. There are no preferred carriers; however, acetone, gum arabic, polyethylene glycol, ethanol, and others have been used extensively in testing herbicides, plant growth regulators, fungicides, and other chemicals that affect plants. Carrier controls shoud be included in the experimental design and tested simultaneously.

(C) Water-insoluble chemicals for which no nontoxic, water-soluble carrier is available, should be dissolved in an appropriate volatile solvent. The solution should be mixed with the sand or glass beads which are then placed in a rotary vacuum apparatus and evaporated, leaving a uniform coating of chemical on the sand or beads. A weighed portion of beads should be extracted with the same organic solvent and the chemical assayed before the potting containers are filled. Solvent controls should be included in the experimental design and tested simultaneously.

(ii) *Foliar exposure.* (A) Water soluble chemicals should be dissolved in deionized or glass distilled water just prior to use. Sufficient quantities of each concentration should be made up as needed. These solutions should be applied at weekly intervals. Plants should be placed in an exhaust hood and the chemical applied to the foliage. A plastic sleeve may be fitted over the top of the pot, and the foliage sprayed with specific quantities of test solution at known concentrations. The plastic sleeve, confining the chemical to plant and pot, facilitates expression of chemical dosage as quantity per pot area (i.e., $\mu g/m^2$). Shoots of control plants should be sprayed in an identical manner with deionized or distilled water. Alternatively, a miniature compressed-air sprayer may be mounted on a pendulum and equipped to automatically spray a plant positioned directly beneath the center of its arc of swing. When radioisotope-labelled chemicals are applied, health and safety considerations prohibit spray application. Instead, specific quantities of labelled chemical should be applied directly to leaves in single drops.

(B) Water-insoluble chemicals, existing as solids, may be prepared for testing by grinding or other reduction to particles of <200 um diameter. These chemicals should be applied at weekly intervals. Plants should be placed in an exhaust hood, a plastic sleeve fitted over the top of the pot, and a specific quantity of chemical sprinkled uniformly over them. Prior to chemical application, plants should be misted with water to promote foliar retention of the chemical. Control plants also should be misted with deionized or distilled water at each treatment date and dusted with an inert material of the same particle size. Applications should be expressed as quantity per unit pot area (i.e., $\mu g/m^2$).

(C) Chemicals existing in gaseous form at normal ambient temperatures and pressures should be generated for use as needed or stored under pressure. The bottled gas may be 100 percent pure chemical or mixed with an inert carrier, such as nitrogen, to known concentrations. Chemicals of controlled or measured concentrations should be metered into the exposure chamber, uniformly mixed about the plants, and exhausted through the outlet port where the flow rate and concentration are again measured. Use of this systems design provides an alternate method of analysis if the quantity of chemical sorbed by plants is less than that required for chemical analysis. Plants should be fumigated whenever they have reached sufficient size for measurement of photosynthesis and transpiration rates, assuming equivalent detection sensitivity of carbon dioxide, water vapor, and chemical analyzers. The appropriate size is a function of the gas exchange system and constitutes an area of expert judgment.

(3) *Range-finding test.* (i) A range-finding test should be conducted to establish the chemical concentrations used in the uptake and translocation test.

(ii) Because of the different mechanisms involved in root and leaf uptake, and to more closely define the chemical concentrations to be used in the uptake test, the definitive early seedling growth test is recommended as the range-finding test. Seeds should be germinated directly in containers filled with sand or glass beads or in cotton or glass-wool plugs supported in hydroponic solution. When 50 percent of the seedlings have germinated, the seedlings should be thinned (by pinching) to the 10 most uniform per container and exposed to a concentration series of test chemical. The lowest concentration in the series, exclusive of controls, should be at or below the EC_{10} while the upper concentration should be at or above the EC_{90}. If the anticipated fate of the chemical is soil or soil-water, and the mechanism of concern is root uptake, the chemical should be applied in nutrient solution to the root support media (or coated on sand or glass beads for non-water soluble chemicals). With a chemical whose anticipated mode of exposure to plants is surface deposition by atmospheric transport or irrigation water, the appropriate testing method may be foliar application allowing subsequent movement into the rooting zone with watering. Effect is assessed as growth reduction. The concentration selected as the upper limit for the uptake and translocation test should be near the threshold of visible injury. Short exposure periods to gas in fumigation chambers are not expected to promote visible injury or gross reductions in growth but may alter stomatal resistance, transpiration, or photosynthesis. Absorption and adsorption rates may be calculated and gas concentrations for definitive testing selected based on the calculated sorption rates.

(iii) Alternatively, the seed germination/root elongation test or other appropriate phytotoxicity test may be used to establish the appropriate upper concentration for testing.

(4) *Definitive test.* (i) The purpose of the uptake and translocation test is to determine the propensity for a chemical's accumulation in plants or plant parts.

(ii) At least 3 concentrations of chemical, exclusive of controls, should be used in the uptake test. Recommended concentrations would be a descending geometric progression from the upper concentration tested (i.e. 100, 50, 25 mg/l). A minimum of 6 replicate pots per concentration, each containing from one to four seedlings, should be used. If techniques other than radioistopes are used to determine uptake, more replicates may be required to provide sufficient plant materials for analysis. Test chemicals should be added to the hydroponic or nutrient solution or coated on glass beads for the root uptake test; or sprayed, dusted, or gassed directly on the foliage in the foliage uptake tests. Only untreated seed (not treated with fungicides, repellants, etc.) taken from the same lot, and year or season of collection should be used in a given test.

(iii) Control pots should be included in the experimental design and should be used in each run. In addition, a carrier control should be used for those chemicals that need to be solubilized.

(iv) If plants are to be grown hydroponically, seeds should be planted in plugs of cotton or glass-wool supported in the tops of the containers. When sand or glass beads are used, the recommended planting procedure is to fill potting containers with sand or glass beads to within 2.5 cm of the top and to sow seeds directly. After germination, the seedlings should be thinned by pinching the stem at the support surface. From one to four seedlings per potting container are required depending on species tested, the size of the containers, and the size to which the plants will grow. When plants are grown hydroponically, one plant per pot will probably be the preferred method. The number of plants selected should provide sufficient biomass for analytical procedures. A greater number of plants may be required depending on species tested, duration of test, and analytical procedures. Too many plants in a container may actually reduce the growth and biomass.

(v) Alternate planting methods may be required when the chemical is highly volatile. An impervious barrier of polyethylene film, a modification of the double pot method, a glass plate, or other appropriate apparatus should be used to prevent volatilization from the root zone. Seeds should be germinated in the dark at 25 °C and seedlings with radicle length in the median range transplanted into the potting containers. The seedlings should be positioned such that their roots are exposed to the support media while the shoots pass through holes in the barrier. A ring of inert, non-phytotoxic, pliable putty should be used to seal the holes around the stems. Control pots should be handled identically except there is no exposure to the test chemical. This transplanting procedure, without the volatilization barrier, is also recommended when the test chemical is adsorbed to the support medium.

(vi) Hydroponic solutions should be aerated and sand or glass filled potting containers should be periodically filled with nutrient solution and drained to provide aeration. For root exposure tests, the test chemical should be added to the nutrient solution or directly to substrate. The entire test solution should be replaced weekly, or earlier if the concentration of chemical in the test or nutrient solution varies by more than 20 percent of that specified. The volume of solution added should be recorded.

(vii) The test consists of one run for each of two specified plant species. The duration of a run, for solid and liquid chemicals, should be equal to the length of time required for the particular test variety to achieve sufficient biomass for testing. The duration of a run for gaseous chemicals should be the length of time required to make the specified gas exchange measurements. For a particular chemical, a run is defined as exposure of the plant species to three concentrations of test chemical with a minimum of six replicate pots and appropriate controls. Exposure is followed by extraction and analysis for parent compound, metabolites, and bound residues in plant tissues, and in the whole plants for solids, liquids, and gasses or by calculating rates of absorption and adsorption of gasses.

(viii) Visible effects (stunting of growth, discloration, chlorosis and/or necrosis of the leaves, decreased moisture content, or morphological abnormalities, etc.) should be recorded.

(ix) A randomized complete block design is recommended for this test, with blocks delineated within the chambers or over greenhouse benches and randomization of treatments occurring within the blocks. If, because of very large pots and plants, there exists inadequate space within chambers for blocking, total randomization within chambers is acceptable. This design is also appropriate for the growth of plants to be used for foliar exposure with gas.

(x) Irradiation measurements should be taken at the top of the plant canopy and the mean, plus a maximum and a minimum value, determined over the plant-growing area. These measurements should be taken at the start of the test, at biweekly intervals during the test, and at test termination. If the test is conducted in a greenhouse facility, hourly measurements of irradiation should be recorded and presented as daily total irradiance plus representative hourly curves for clear sky conditions and cloudy days.

(xi) Temperature and humidity measurements should be measured daily at the top of the plant canopy during each light and dark period.

(xii) Measurements of carbon dioxide concentration should be made at the top of the plant canopy (of chamber-growth plants) on a "continuous" basis.

(xiii) The amount of water and nutrient solution depleted each week should be recorded, to observe changes in evapotranspiration rates which may indicate stress. Furthermore, these data will be used to compute chemical uptake per volume of water transpired for the uptake test.

(5) [Reserved]

(6) *Analytical measurements*—(i) *Solid or liquid test chemicals.* (A) Stock solutions should be diluted with glass distilled or deionized water to obtain the test solutions. Standard analytical methods, if available, should be used to establish concentrations of these solutions and should be validated before

beginning the test. An analytical method is not acceptable if likely degradation products of the chemical, such as hydrolysis and oxidation products, give positive or negative interference. The pH of these solutions should also be measured prior to use.

(B) The entire plant should be harvested, rinsed with a minimum amount of water (which is returned to the nutrient solution), and separated into its respective organs as follows: carrot—root peels, peeled roots, and tops; cucumber—fruit, vines plus leaves, and roots; corn—kernels, husk plus cob, stalk plus leaves, and roots; lettuce—tops and roots; onion—bulb and tops; ryegrass—tops and roots; soybean—grain, chaff plus tops, and roots; oats—grain, chaff plus tops, and roots; tomato—fruit, vines, and roots; cabbage—head and roots. Plants from two pots in each treatment may be pooled, giving 3 replicate sample pools per treatment. After the fresh weights of the plant organs are obtained, each pool of organs should be subsampled for percent moisture determinations by drying, at 70 °C for 24 hours in a forced-air drying oven, and weighing. Percent moisture determined from these subsamples is used to correct for dry weight of the fresh samples which should then be homogenized and extracted in organic and aqueous solvents. If radioisotopes are used, the amount of test chemical in each extract should be determined by liquid or solid scintillation depending on the type of radiation; otherwise, the amount of chemical should be determined by standard methods. At test completion, the root support material should be washed in organic and then aqueous solvent and analyzed for test chemical before discarding.

(C) A suggested extraction procedure appropriate for many organic chemicals is as follows: One gram of plant material should be homogenized with one gram of solvent-washed anhydrous sodium sulfate in 4 ml of hexane or acetonitrile. The homogenate should be filtered or centrifuged, the solid residue rinsed with an appropriate organic solvent, and the filtrate or supernatant combined with the rinse. The solid residue should be extracted by sequentially (*1*) homogenizing in water, (*2*) centrifuging and decanting the supernatant, (*3*) extracting of the pellet with 6N hydrochloric acid at 60°C for 10 hours, (*4*) subsequently digesting with 10N potassium hydroxide, and (*5*) combining supernatants. The resulting solution should be analyzed by liquid scintillation spectrometry or GLC methodology. The organic extract should be evaporated under vacuum to a sufficiently small volume for thin layer chromatography (TLC) and co-chromatographed on silica gel plates with known standards of the parent chemical. If radioisotopes were used, the chromatographs could be scanned for radioactive substances on a radiochromatogram scanner. Alternatively, zones may be removed from the plates, extracted, and the quantity of chemical from each zone determined by liquid scintillation spectrometry or GLC methodology. The unextractable chemical in the remaining residue may be determined by oxidizing the residue in a complete combustion oxidizer.

(ii) *Gaseous test chemicals.* (A) A gas exposure system yields requisite data for a direct calculation of uptake rates. At steady state, chemical uptake may be determined by a mass balance calculation. Correction for adsorption to surfaces of the exposure chamber should be made by operating the system without plants. Pots filled with hydroponic solution or support media should be included in the system adsorption calibration. Consequently, chemical analyses of plant tissues exposed to gaseous chemicals may not be required in order to demonstrate and quantitate uptake rates.

(B) Altered rates of net photosynthesis, transpiration, and stomatal conductance are anticipated as a result of chemical uptake. Rates of these physiological processes before, during, and after exposure to the gaseous chemical should be determined. Data required for these calculations are available as a consequence of maintaining the specified environmental conditions within the fumigation chamber.

(iii) *Numerical.* Mass of pooled plant organs and pooled whole plants should be measured for the uptake and translocation test and subjected to chemical analyses (above) to quantify free parent test chemical, its metabolites and soluble and bound residues. Mass balance of the test chemical and evapotranspiration rates of the plants are also determined. Means and standard deviations should be calculated and plotted for each of the above for every treatment and control. The data should also be subjected to an analysis of variance.

(d) *Test conditions*—(1) *Test species.* (i) Test plants recommended for the uptake test include:

(A) *Lycopersicon esculentum* (tomato)
(B) *Cucumis sativus* (cucumber)
(C) *Lactuca sativa* (lettuce)
(D) *Glycine max* (soybean)
(E) *Brassica oleracea* (cabbage)
(F) *Avena sativa* (oat)
(G) *Lolium perenne* (perennial ryegrass)
(H) *Allium cepa* (common onion)
(I) *Daucus carota* (carrot)
(J) *Zea mays* (corn)

(ii) Other species of economic or ecologic importance to the region of impact, may also be appropriate and selected for testing. Two species of potentially differing sensitivity should be selected such as a monocotyledonous and a dicotyledonous species. It is further suggested that the test plants selected should be of different growth forms, e.g., a root crop and a leaf crop.

(2) *Facilities*—(i) *Apparatus.* Greenhouses, environmental chambers, or growth rooms should provide adequate environmental control to meet the carbon dioxide, humidity, irradiation, photoperiod, and temperature specifications. Chambers should be designed to prevent escape of internal air into the external environment other than through appropriate filtering material or media to prevent contamination of the external environment with radioactive and/or test substances. Laboratory facilities for plant extractions and chemical determinations should include nonporous floor covering, absorbent bench covering with non-porous backing, and adequate disposal facilities to accommodate plant nutrient, test, and wash solutions containing radioisotope and/or test chemical at the end of each run, and any bench covering, lab clothing, or other contaminated materials.

(ii) *Containers and support media.* For testing purposes, at least 24 polyethylene pots sufficiently large to grow at least 5 plants up to 28 days or one to three plants to maturity are required. If plants are grown hydroponically, one plant per pot may be the preferred method. If a carrier control is needed, 30 pots are used. Potting containers used in each experiment should be of equal size and volume and possess the same configuration. When sand or glass beads are used the potting containers should be filled to within 2.5 cm of their tops with sand or glass beads. Perlite, vermiculite, native soils, etc., should not be used for root support. Potting containers should be covered with opaque polyethylene bags to exclude light and minimize volatilization of test chemical.

(iii) *Cleaning and sterilization.* Potting containers, nutrient storage containers, and root support medium should be cleaned before use. All equipment should be washed according to good standard laboratory procedures to remove any residues remaining from manufacturing or use. A dichromate solution should not be used for cleaning beads or pots. Rooting media other than glass beads should be discarded at the end of the experiment. Disposal should conform to existing regulations.

(iv) *Nutrient media.* Half-strength modified Hoagland nutrient solution should be utilized as nutrient media for this test. Hydroponic solution should be aerated and sand or glass beads potting containers should be filled with nutrient solution and drained periodically. An automated system design is recommended.

(3) *Test parameters.* Environmental conditions should be maintained as specified below:

(i) Carbon dioxide concentrations at 350 ± 50 ppm.

(ii) Relative humidity approaching 70 ± 5 percent during light periods and 90 percent during dark periods.

(iii) Irradiation, measured at 1 meter from the source, at $350 \pm 50\ \mu E/m^2$ sec at 400 to 700 nm.

(iv) Photoperiod of 16 hours light and 8 hours darkness for all species except soybean which should be provided with 11 hours light and 13 hours darkness prior to flowering.

(v) Day/night temperatures at $25°/20° \pm 3°C$.

(e) *Reporting.* Reporting requirements of Part 792—Good Laboratory Practice Standards of this chapter apply to this guideline. Concentrations should be expressed in appropriate weight units per grams of dry plant material and of water lost by evapotranspiration. Data should also include initial and final total concentration of the test chemical in the growth media. These data will be used to compute mass balance. The following should be reported for each of the species tested in tabular form:

(1) *Solid and liquid test chemicals.* (i) Concentration of chemical in nutrient solution and root support material when chemical is soluble in water or solubilized with a carrier compound, as well as the concentration of carrier compound in nutrient solution when carrier is used, or the quantity of chemical per unit weight of root support material when it is coated on the material.

(ii) The quantity of chemical, the concentration at which it was applied, and the number of applications for those chemicals applied to the foliage.

(iii) Environmental conditions (day/night temperatures, relative humidity, light intensity, carbon dioxide concentration, and photoperiod) and the occurrence and extent of any disruption of environmental control facilities.

(iv) Mass of each pool of plant organs and by summation, the mass of whole plants (dry weight after 24 hours at 70 °C).

(v) Concentration of free parent test chemical, metabolites and soluble residues, and bound residues in pooled plant organs and pooled whole plants.

(vi) Mass balance of chemical.

(vii) Mean evapotranspiration rate per plant.

(viii) Visible effects of chemical, if any, on the intact plants.

(ix) Analysis of variance, F-test, means, and standard deviation about the mean are calculated under paragraph (e)(1)(iv), (v), (vi), and (vii) of this section.

(2) *Gaseous test chemicals.* (i) Concentration of gaseous test chemical at inflow and outflow ports.

(ii) Environmental conditions within gas exposure system (air temperature, dew point temperature or water vapor pressure of incoming and outgoing air streams, light intensity, air speed within chamber, carbon dioxide concentration at inflow and outflow ports, gas flow rate into and out of exposure system).

(iii) Mass (dry weight after 24 hours at 70 °C) of leaves and stems and surface area (one side of leaves) in the exposure system.

(iv) Calculated measurements of photosynthesis, transpiration, and stomatal conductance before, during, and after exposure to test chemicals.

(v) Visible effects of chemical, if any, on the plants.

(vi) Analysis of variance, F-test, means, and standard deviation about the mean are calculated for each of the following: (A) Steady state rates of photosynthesis, transpiration, and chemical uptake before, during, and after fumigation.

(B) Stomatal conductance or leaf diffusion resistance before, during, and after fumigation.

(vii) If uptake is determined by direct chemical analysis of plant tissues, then the reporting requirements also include:

(A) Concentration of free parent test chemical, metabolites and soluble residues, and bound residues in pooled plant organs and pooled whole plants.

(B) Mass balance of the chemical.

(C) Analysis of variance, F-test, means and standard deviation about the mean under paragraph (e)(2)(vi) (A) and (B) of this section.

PART 798—HEALTH EFFECTS TESTING GUIDELINES

Subpart A—[Reserved]

Subpart B—General Toxicity Testing

Sec.
798.1100 Acute dermal toxicity.
798.1150 Acute inhalation toxicity.
798.1175 Acute oral toxicity.

Subpart C—Subchronic Exposure.

798.2250 Dermal toxicity.
798.2450 Inhalation toxicity.
798.2650 Oral toxicity.
798.2675 Oral toxicity with satellite reproduction and fertility study.

Subpart D—Chronic Exposure

798.3260 Chronic toxicity.
798.3300 Oncogenicity.
798.3320 Combined chronic toxicity/oncogenicity.

Subpart E—Specific Organ/Tissue Toxicity

798.4100 Dermal sensitization.
798.4350 Inhalation developmental toxicity study.
798.4420 Preliminary development toxicity screen.
798.4470 Primary dermal irritation.
798.4500 Primary eye irritation.
798.4700 Reproduction and fertility effects.
798.4900 Developmental toxicity study.

Subpart F—Genetic Toxicity

798.5100 Escheria coli WP2 and WP2 urvA reserve mutation assays.
798.5140 Gene mutations in aspergillus nidulans.
798.5200 Mouse visible specific locus test.
798.5250 Gene mutation in neurospora crassa.
798.5265 Salmonella typhimurium reverse mutation assay.
798.5275 Sex linked recessive lethal test in drosophila melanogaster.
798.5300 Detection of gene mutations in somatic cells in culture.
798.5375 In vitro mammalian cytogenetics.
798.5385 In vivo mammalian bone marrow cytogenetics tests: Chromosomal analysis.
798.5395 In vivo mammalian bone marrow cytogenetics tests: Micronucleus assay.
798.5450 Rodent dominant lethal assay.
798.5460 Rodent heritable translocation assays.
798.5500 Differential growth inhibition of repair proficient and repair deficient bacteria: "Bacterial DNA damage or repair tests."
798.5550 Unscheduled DNA synthesis in mammalian cells in culture.
798.5575 Mitotic gene conversion in Saccharomyces cervisiae.
798.5900 In vitro sister chromatid exchange assay.
798.5915 In vivo sister chromatid exchange assay.
798.5955 Heritable translocation test in drosophila melanogaster.

Subpart G—Neurotoxicity

798.6050 Functional observational battery.
798.6200 Motor activity.

Sec.
798.6400 Neuropathology.
798.6450 NTE neurotox assay.
798.6500 Schedule-controlled operant behavior.
798.6540 Acute delayed neurotoxicity of organophosphorus substances.
798.6560 Subchronic delayed neurotoxicity of organophosphorus substances.
798.6850 Peripheral nerve function.

Subpart H—Special Studies

798.7100 Metabolism.

Authority: 15 U.S.C. 2603.

Subpart A—[Reserved]

Subpart B—General Toxicity Testing

§ 798.1100 Acute dermal toxicity.

(a) *Purpose.* In the assessment and evaluation of the toxic characteristics of a substance, determination of acute dermal toxicity is usually an initial step. It provides information on health hazards likely to arise from short-term exposure by the dermal route. Data from an acute study may serve as a basis for classification and labeling. It is traditionally a step in establishing a dosage regimen in subchronic and other studies and may provide initial information on dermal absorption and the mode of toxic action of a substance. An evaluation of acute toxicity data should include the relationship, if any, between the animals' exposure to the test substance and the incidence and severity of all abnormalities, including behavioral and clinical abnormalities, the reversibility of observed abnormalities, gross lesions, body weight changes, effects on mortality, and any other toxic effects.

(b) *Definitions.* (1) Acute dermal toxicity is the adverse effects occurring within a short time of dermal application of a single dose of a substance or multiple doses given within 24 hours.

(2) Dose is the amount of test substance applied. Dose is expressed as weight of test substance (g, mg) per unit weight of test animal (e.g., mg/kg).

(3) Dose-response is the relationship between the dose and the proportion of a population sample showing a defined effect.

(c) *Approaches to the determination of acute toxicity.* (1) At present, the evaluation of chemicals for acute toxicity is necessary for the protection of public health and the environment. When animal testing is required for this purpose, this testing should be done in ways that minimize numbers of animals used and that take full account of their welfare.

(2) EPA recommends the following means to reduce the number of animals used to evaluate acute effects of chemical exposure while preserving its ability to make reasonable judgments about safety:

(i) Attempt the use of existing data on structurally related chemicals.

(ii) If data for calculating an LD_{50} are needed, perform an acute toxicity study whereby the value of the data derived from the investment of animal lives is enhanced. EPA does not encourage the use of animals solely for the calculation of an LD_{50}.

(iii) Use methods that minimize the numbers of animals in the test.

(3) The following provides an expanded discussion of these principles and their application to the evaluation of acute toxicity of chemicals.

(i) *Using data from structurally related chemicals.* In order to minimize the need for animal testing, the Agency encourages the review of existing acute toxicity information on chemical substances that are structurally related to the agent under investigation. In certain cases, one may be able to glean enough information from these surrogate chemicals to make preliminary safety evaluations that may obviate the need for further animal testing.

(ii) *"Limit" test.* When acute lethality data are desirable, EPA's test guideline encourages the use of methods that minimize the requirement for animals, sometimes by a factor of 90 percent as compared to the more traditional LD_{50} test. In the "limit" test, a single group of animals receives a large dose (2 g/kg body weight) of the agent by the dermal route. If no lethality is demonstrated, no further testing for acute dermal toxicity is pursued.

(iii) *Estimation of lethal dose.* For those substances demonstrating lethality in a "limit" test or for substances for which there are data on structurally related chemicals that indicate potential acute toxicity below 2 g/kg, the Agency can use estimates of the dose associated with some level of acute lethality that are derived from a study comprising three doses as described in this guideline. With such an approach, use of greater numbers of animals or increased numbers of dose levels are not necessary.

(iv) *Multiple endpoint evaluation.* The Agency stresses the simultaneous monitoring of several endpoints of toxicity in animals in a single acute study including sublethal effects as well as lethality. Dosed animals are observed for abnormal behavioral manifestations such as increased salivation or muscular incoordination, in addition to the recovery from these effects during the observation period. Both dead and surviving animals are autopsied to evaluate gross anatomical evidence of organ toxicity. In selected cases, additional testing may be justified to characterize better the kinds of abnormalities that have been found in the organs of the autopsied animals.

(4) These sound, scientific practices represent some of the means which maximize the utility of the data obtained from a limited number of test animals to achieve a balance between protecting humans and the environment, and the welfare and utilization of laboratory animals. When animal testing is, nonetheless, determined to be necessary to achieve this balance, the following test method incorporates the principles discussed in this section.

(d) *Principle of the test method.* When conducting acute toxicity testing, exposure by dermal application is recommended for chemicals where exposure of humans by the dermal route is likely. A single exposure and a 14-day observation period are used. The test substance is applied dermally in graduated doses to several groups of experimental animals, one dose being used per group. For the limit test, however, only one group is tested at a single (high) dose. Subsequent to exposure, systematic daily observations of effects and deaths are made. Based on the results of cage-side observations or gross necropsy, the tester may decide to initiate histopathological review of certain organs, and/or additional clinical laboratory tests. Animals that die during the test are necropsied, and at the conclusion of the observation period, the surviving animals are sacrificed and are necropsied.

(e) *Limit test.* If a test at a dose level of at least 2 g/kg body weight produces no compound-related mortality, then a study using three dose levels will not be necessary.

(f) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* The rat, rabbit, or guinea pig may be used. The albino rabbit is preferred because of its size, skin permeability, and extensive data base. Commonly used laboratory strains should be employed. If a species other than the three indicated above is used, the tester should provide justification and reasoning for its selection.

(ii) *Age.* Young adult animals should be used. The following weight ranges are suggested to provide animals of a size which facilitates the conduct of the test: rats, 200 to 300 g; rabbits 2.0 to 3.0 kg; guinea pigs 350 to 450 g.

(iii) *Sex.* (A) Equal numbers of animals of each sex with health intact skin should be used for each dose level.

(B) The females should be nulliparous and nonpregnant.

(iv) *Numbers per dose group.* At least 5 animals of each sex should be used at each dose level.

(2) *Control groups.* A concurrent untreated control is not necessary. A vehicle control group should be run concurrently except when historical data are available to determine the acute toxicity of the vehicle.

(3) *Dose levels and dose selection.* (i) Three dose levels should be used and spaced appropriately to produce test groups with a range of toxic effects and mortality rates. The data should be sufficient to produce a dose-response curve and permit an acceptable estimation of the median lethal dose. Range finding studies using single animals may help to estimate the positioning of the dose groups so that no more than three dose levels will be necessary.

(ii) *Vehicle.* (A) Where necessary, the test substance is dissolved or suspended in a suitable vehicle. It is recommended that wherever possible the use of an aqueous solution be considered first, followed by consideration of a solution in oil (e.g., corn oil) and then by possible solution in other vehicles. For non-aqueous vehicles the toxic characteristics of the vehicle should be known, and if not known should be determined before the test.

(B) When testing solids, which may be pulverized if appropriate, the test substance should be moistened sufficiently with water or, where necessary, a suitable vehicle to ensure good contact with skin. When a vehicle is used, the influence of the vehicle on penetration of skin by the test substance should be taken into account.

(4) *Exposure duration.* The test substance should be administered over a period not exceeding 24 hours.

(5) *Observation period.* The observation period should be at least 14 days. Although 14 days is recommended as a minimum period, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions, rate of onset and length of recovery period, and may thus be extended when considered necessary. The time at which signs of toxicity appear and disappear, their duration and the time to death are important, especially if there is a tendency for deaths to be delayed.

(6) *Preparation of animal skin.* (i) Shortly before testing, fur should be clipped from the dorsal area of the trunk of the test animals. Shaving may be employed, but is should be carried out approximately 24 hours before the test. Care must be taken to avoid abrading the skin, which would alter its permeability.

(ii) Not less than 10 percent of the body surface area should be clear for the application of the test substance. The weight of the animal should be taken into account when deciding on the area to be cleared and on the dimensions of any covering used.

(7) *Application of test substance.* (i) The test substance should be applied uniformly over an area which is approximately 10 percent of the total body surface area. With highly toxic substances the surface area covered may be less, but as much of the area should be covered with as thin and uniform a film as possible.

(ii) The test substance should be held in contact with the skin with a porous gauze dressing and nonirritating tape throughout a 24-hour exposure period. The test site should be further covered in a suitable manner to retain the gauze dressing and test substance and ensure that the animals cannot ingest the test substance. Restrainers may be used to prevent the ingestion of the test substance, but complete immobilization is not a recommended method.

(iii) At the end of the exposure period, residual test substance should be removed where practicable using water or an appropriate solvent.

(8) *Observation of animals.* (i) A careful clinical examination should be made at least once each day.

(ii) Additional observations should be made daily, especially in the early days of the study. Appropriate actions should be taken to minimize loss of animals to the study (e.g., necropsy or refrigeration of those animals found dead and isolation of weak or moribund animals).

(iii) Cage-side observations should include, at the least, evaluations of the skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Particular attention should be directed to observation of tremors, convulsions, lethargy, other signs of central nervous depression, salivation and diarrhea.

(iv) Individual weights of animals should be determined shortly before the test substance is applied, weekly thereafter, and at death. Changes in weights should be calculated and recorded when survival exceeds one day.

(v) The time of death should be recorded as precisely as possible.

(vi) At the end of the test, surviving animals should be weighed and sacrificed.

(9) *Gross pathology.* A gross necropsy should be performed on all animals under test. All gross pathology changes should be recorded.

(10) *Additional evaluations.* In animals surviving 24 hours or more, clinical chemistry tests or microscopic examination of organs showing evidence of gross pathology should be considered because they may yield additional useful information on the induced toxic effects.

(g) *Data and reporting*—(1) *Treatment of results.* Data should be summarized in tabular form, showing for each test group the number of animals at the start of the test, body weights, time of death of individual animals at different dose levels, number of animals displaying other signs of toxicity, description of toxic effects and necropsy findings.

(2) *Evaluation of results.* An evaluation of results should include the relationship, if any, between the dose of the test substance and the incidence, severity and reversibility of all abnormalities, including behavioral and clinical effects, gross lesions, body weight changes, effects on mortality, and any other toxicological effects.

(3) *Test report.* In addition to the reporting requirements as specified under 40 CFR Part 792, Subchapter J, the following specific information should be reported:

(i) Tabulation of response data by sex and dose level (i.e., number of animals exposed; number of animals showing signs of toxicity; number of animals dying).

(ii) Dose-response curves for mortality and other toxic effects (when permitted by the method of determination).

(iii) Description of toxic effects including their time of onset, duration, reversibility, and relationship to dose.

(iv) Time of death after dosing.

(v) Body weight data.

(vi) Gross pathology findings.

(vii) Histopathology findings and any additional clinical chemistry evaluations, if performed.

(h) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Bliss, C.I. "The determination of the dosage mortality curve from small numbers," *Quarterly Journal of Pharmacy and Pharmacology.* 11:192–216 (1938).

(2) Finney, D.G. "Chapter 3—Estimation of the median effective dose. Chapter 4—Maximum likelihood estimation." *Probit Analysis.* 3rd Ed. (London: Cambridge University Press 1971).

(3) Litchfield, J.T., Jr., Wilcoxon, F. "A simplified method of evaluating dose-effect experiments." *Journal of*

Pharmacology and Experimental Therapeutics. 96:99–115 (1949).

(4) Miller, L.C., Tainter. M.L. "Estimation of the ED_{50} and its error by means of logarithmic graph paper," *Proceedings of the Society for Experimental Biology and Medicine.* 57:261–264 (1944).

(5) National Academy of Sciences. "Principles and procedures for evaluating the toxicity of household substances." A report prepared by the Committee for the Revision of NAS Publication 1138, under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC (1977).

(6) Thompson, W.R. "Use of moving averages and interpolation to estimate median effective dose," *Bacteriological Review.* 11:115–145 (1947).

(7) Weil, C.S. "Tables for convenient calculation of median effective dose and instructions in their use," *Biometrics.* 8:249–263 (1952).

(8) World Health Organization. "Part I. Environmental Health Criteria 6," *Principles and Methods for Evaluating the Toxicity of Chemicals.* Geneva: World Health Organization (1978).

§ 798.1150 Acute inhalation toxicity.

(a) *Purpose.* In the assessment and evaluation of the toxic characteristics of a substance that may be inhaled, determination of acute toxicity is usually an initial step. It provides information on health hazards likely to arise from short-term exposure by the inhalation route. Data from an acute study may serve as a basis for classification and labeling. It is traditionally a step in establishing a dosage regimen in subchronic and other studies and may provide initial information on the mode of toxic action of a substance. An evaluation of acute toxicity data should include the relationship, if any, between the animals' exposure to the test substance and the incidence and severity of all abnormalities, including behavioral and clinical abnormalities, the reversibility of observed abnormalities, gross lesions, body weight changes, effects on mortality, and any other toxic effects.

(b) *Definitions.* (1) Acute inhalation toxicity is the adverse effects caused by a substance following a single uninterrupted exposure by inhalation over a short period of time (24 hours or less) to a substance capable of being inhaled.

(2) Aerodynamic diameter applies to the size of particles of aerosols. It is the diameter of a sphere of unit density which behaves aerodynamically as the particle of the test substance. It is used to compare particles of different size and densities and to predict where in the respiratory tract such particles may be deposited. This term is used in contrast to measured or geometric diameter which is representative of actual diameters which in themselves cannot be related to deposition within the respiratory tract.

(3) The geometric mean diameter or the median diameter is the calculated aerodynamic diameter which divides the particles of an aerosol in half based on the weight of the particles. Fifty percent of the particles by weight will be larger than the median diameter and 50 percent of the particles will be smaller than the median diameter. The median diameter and its geometric standard deviation is used to statistically describe the particle size distribution of any aerosol based on the weight and size of the particles.

(4) Inhalable diameter refers to that aerodynamic diameter of a particle which is considered to be inhalable for the organism. It is used to refer to particles which are capable of being inhaled and may be deposited anywhere within the respiratory tract from the trachea to the alveoli. For man, the inhalable diameter is considered as 15 micrometers or less.

(5) Dose response is the relationship between the dose (or concentration) and the proportion of a population sample showing a defined effect.

(c) *Approaches to the determination of acute toxicity.* (1) At present, the evaluation of chemicals for acute toxicity is necessary for the protection of public health and the environment. When animal testing is required for this purpose, this testing should be done in ways that minimize numbers of animals used and that take full account of their welfare.

(2) EPA recommends the following means to reduce the number of animals used to evaluate acute effects of chemicals exposure while preserving its ability to make reasonable judgments about safety:

(i) Attempt the use of existing data on structurally related chemicals.

(ii) If data for calculating an LC_{50} are needed, perform an acute toxicity study whereby the value of the data derived from the investment of animal lives is enhanced. EPA does not encourage the use of animals solely for the calculation of an LC_{50}.

(iii) Use methods that minimize the numbers of animals in the test.

(3) The following provides an expanded discussion of these principles and their application to the evaluation of acute toxicity of chemicals.

(i) *Using data from structurally related chemicals.* In order to minimize the need for animal testing, the Agency encourages the review of existing acute toxicity information on chemical substances that are structually related to the agent under investigation. In certain cases one may be able to glean enough information from these surrogate chemicals to make preliminary safety evaluations that may obviate the need for further animal testing.

(ii) *"Limit" test.* If a test at an exposure of 5 mg/l (actual concentration of respirable substances) for 4 hours or, where this is not possible due to physical or chemical properties of the test substance, the maximum attainable concentration, using the procedures described for this study, produces no compound-related mortality, then a full study using three dose levels will not be necessary.

(iii) *Estimation of lethal dose.* For those substances demonstrating lethality in a "limit" test or for substances for which there are data on structurally related chemicals that indicate potential acute toxicity below 5 mg/l, the Agency can use estimates of the dose associated with some level of acute lethality that are derived from a study comprising three doses as described in this guideline. With such an approach, use of greater numbers of animals or increased numbers of dose levels are not necessary.

(iv) *Multiple endpoint evaluation.* The Agency stresses the simultaneous monitoring of several endpoints of toxicity in animals in a single acute study including sublethal effects as well as lethality. Dosed animals are observed for abnormal behavioral manifestations such as increased salivation or muscular incoordination, in addition to the recovery from these effects during the observation period. Both dead and surviving animals are autopsied to evaluate gross anatomical evidence of organ toxicity. In selected cases, additional testing may be justified to characterize better the kinds of abnormalities that have been found in the organs of the autopsied animals.

(4) These sound, scientific practices represent some of the means which maximize the utility of the data obtained from a limited number of test animals to achieve a balance between protecting humans and the environment, and the welfare and utilization of laboratory animals. When animal testing is, nonetheless, determined to be necessary to achieve this balance, the following test method incorporates the principles discussed in this section.

(d) *Principle of the test method.* When conducting acute toxicity testing, exposure by inhalation is recommended for chemicals where exposure of humans by inhalation is likely. A single exposure and a 14-day observation period are used. The test substance is administered in graduated doses to several groups of experimental animals, one dose being used per group. For the limit test, however, only one group is tested at a single (high) dose. Subsequent to exposure, systematic daily observations of effects and deaths are made. Based on the results of cage-side observations or gross necropsy, the tester may decide to initiate histopathological review of certain organs, and/or additional clinical laboratory tests. Animals that die during the test are necropsied, and at the conclusion of the observation period, the surviving animals are sacrificed and are necropsied.

(e) *Limit test.* If a test at a dose level of at least 5 mg/l (actual concentration of respirable substances) for 4 hours or, where this is not possible due to physical or chemical properties of the test substance, the maximum attainable concentration, produces no compound-related mortality, then a full study using three dose levels will not be necessary.

(f) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* Although several mammalian test species may be used, the rat is the preferred species. Commonly used laboratory strains should be employed. If another mammalian species is employed, the tester should provide justification and reasoning for its selection.

(ii) *Age.* Young adult animals should be used. The weight variation of animals used in a test should not exceed ± 20 percent of the mean weight for each sex.

(iii) *Sex.* (A) Equal numbers of animals of each sex should be used for each dose level.

(B) The females should be nulliparous and nonpregnant.

(iv) *Numbers per dose group.* At least five animals of each sex should be used at each dose level.

(2) *Control groups.* A concurrent untreated control is not necessary. Where a vehicle is used to help generate an appropriate concentration of the substance in the atmosphere, a vehicle control group should be used when historical data are not available or adequate to determine the acute toxicity of the vehicle.

(3) *Dose levels and dose selection.* (i) Three exposure concentrations should be used and spaced appropriately to produce test groups with a range of toxic effects and mortality rates. The data should be sufficient to produce a dose-response curve and permit an acceptable estimation of the median lethal concentration. Range-finding studies using single animals may help to estimate the positioning of the test groups so that no more than three doses will be necessary.

(ii) Where necessary, a suitable vehicle may be added to the test substance to help generate an appropriate concentration of the test substance in the atmosphere. If a vehicle or diluent is needed, ideally it should not elicit important toxic effects itself or substantially alter the chemical or toxicological properties of the test substance.

(iii) In the case of potentially explosive test substances, care should be taken to avoid generating explosive concentrations.

(iv) To establish suitable exposure concentrations, a trial test is recommended.

(4) *Exposure duration.* The duration of exposure should be at least 4 hours after equilibration of the chamber concentrations.

(5) *Observation period.* The observation period should be at least 14 days. Although 14 days is recommended as a minimum period, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions, rate of onset and length of recovery period, and may thus be extended when considered necessary. The time at which signs of toxicity appear and disappear, their duration and the time of death are important, especially if there is a tendency for deaths to be delayed.

(6) *Inhalation exposure.* (i) The animals should be tested with inhalation equipment designed to sustain a dynamic air flow of 12 to 15 air changes per hour, ensure an adequate oxygen content of 19 percent and an evenly distributed exposure atmosphere. Where a chamber is used, its design should minimize crowding of the test animals and maximize their exposure to the test substance. This is best accomplished by individual caging. As a general rule to ensure stability of a chamber atmosphere, the total "volume" of the test animals should not exceed 5 percent of the volume of the test chamber. Alternatively, oro-nasal, head-only, or whole body individual chamber exposure may be used.

(ii) A suitable analytical concentration control system should be used. The rate of air flow should be adjusted to ensure that conditions throughout the equipment are essentially the same. Maintenance of a slight negative pressure inside the chamber will prevent leakage of the test substance into the surrounding area.

(iii) The temperature at which the test is performed should be maintained at 22 °C (± 2°). Ideally, the relative humidity should be maintained between 40 to 60 percent, but in certain instances (e.g., tests on aerosols, use of water vehicle) this may not be practicable.

(7) *Physical measurements.* Measurements or monitoring should be made of the following:

(i) The rate of air flow should be monitored continuously, but should be recorded at least every 30 minutes.

(ii) The actual concentrations of the test substance should be measured in the breathing zone. During the exposure period the actual concentration of the test substance should be held as constant as practicable. Continuous monitoring is desirable. Measurement of actual concentrations should be recorded near the beginning, middle, and end of the exposure period.

(iii) During the development of the generating system, particle size analysis should be performed to establish the stability of aerosol concentrations. During exposure, analysis should be made as often as necessary to determine the consistency of particle size distribution and homogeneity of the exposure stream.

(iv) Temperature and humidity should be monitored continuously but should be recorded at least every 30 minutes.

(8) *Food and water during exposure period.* Food should be withheld during exposure. Water may also be withheld in certain cases.

(9) *Observation of animals.* (i) A careful clinical examination should be made at least once each day.

(ii) Additional observations should be made daily, especially in the early days of the study. Appropriate actions should be taken to minimize loss of animals to the study (e.g., necropsy or refrigeration of those animals found dead and isolation of weak or moribund animals).

(iii) Cage-side observations should include, at the least, evaluations of the skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Particular attention should be directed to observation of tremors, convulsions, lethargy, other signs of central nervous system depression, salivation and diarrhea.

(iv) Individual weights of animals should be determined shortly before the test substance is administered, weekly thereafter, and at death. Changes in weights should be calculated and recorded when survival exceeds one day.

(v) The time of death should be recorded as precisely as possible.

(vi) At the end of the test, surviving animals should be weighed and sacrificed.

(10) *Gross pathology.* A gross necropsy should be performed on all animals under test, with particular reference to any changes in the respiratory tract. Where there are significant signs of toxicity indicating the possible involvement of other organs, these should be examined. All gross pathology changes should be recorded.

(11) *Additional evaluations.* In animals surviving 24 hours or more, clinical chemistry tests or microscopic examination of organs showing evidence of gross pathology should be considered because they may yield additional useful information on the nature of the induced toxic effects.

(g) *Data and reporting*—(1) *Treatment of results.* Data should be summarized in tabular form, showing for each test group the number of animals at the start of the test, body weights, time of death of individual animals at different dose levels, number of animals displaying other signs of toxicity, description of toxic effects and necropsy findings.

(2) *Evaluation of results.* An evaluation of results should include the relationship, if any, between the concentration of the test substance and the incidence, severity and reversibility of all abnormalities, including behavioral and clinical effects, gross lesions, body weight changes, effects on mortality, and any other toxicological effects.

(3) *Test report.* In addition to the reporting requirements as specified under 40 CFR Part 792, Subpart J the following specific information should be reported:

(i) *Test conditions.* (A) Description of exposure apparatus including design, type, dimensions, source of air, system for generating particulates and aerosols, method of conditioning air, treatment of exhaust air and the method of housing the animals in a test chamber.

(B) The equipment of measuring temperature, humidity, and particulate aerosol concentrations and size be described.

(ii) *Exposure data.* These should be tabulated and presented with mean values and a measure of variability (e.g., standard deviation) and should include:

(A) Airflow rates through the inhalation equipment.

(B) Temperature and humidity of air.

(C) Nominal concentration (total amount of test substance fed into the inhalation equipment divided by volume of air).

(D) Actual concentration in test breathing zone.

(E) Particle size distribution (e.g., median aerodynamic diameter of particles with standard deviation from the mean).

(iii) *Animal data.* (A) Tabulation of response data by sex and exposure level (i.e., number of animals exposed, number of animals showing signs of toxicity, number of animals dying).

(B) Dose-response curves for mortality and other toxic effects (when permitted by the method of determination).

(C) Description of toxic effects including their time of onset, duration, reversibility, and relationship to dose.

(D) Time of death during or following exposure.

(E) Body weight data.

(F) Gross pathology findings.

(G) Histopathology findings and any additional clinical chemistry evaluation, if performed.

(h) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Bliss, C.I. "The determination of the dosage mortality curve from small numbers," *Quarterly Journal of Pharmacy and Pharmacology.* 11:192–216 (1938).

(2) Finney, D.G. "Chapter 3—Estimation of the median effective dose, Chapter 4—Maximum likelihood estimation," Probit Analysis. 3rd Ed. (London: Cambridge University Press, 1971).

(3) Litchfield, J.T., Jr., Wilcoxon, F. "A simplified method of evaluating dose-effect experiments," *Journal of Pharmacology and Experimental Therapeutics.* 96:99–115 (1949).

(4) Miller, L.C., Tainter, M.L. "Estimation of the ED_{50} and its error by means of logarithmic graph paper," *Proceedings of the Society for Experimental Biology and Medicine.* 57:261–264 (1944).

(5) National Academy of Sciences. "Principles and procedures for evaluating the toxicity of household substances." A report prepared by the Committee for the Revision of NAS Publication 1138, under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC (1977).

(6) Smyth, H.F., Jr., Carpenter, C.P., Weil, C.S., Striegel, J.A. "Range finding toxicity data: List VI," *American Industrial Hygiene Association Journal.* 23:95 (1962).

(7) Thompson, W.R. "Use of moving averages and interpolation to estimate median effective dose," *Bacteriological Review.* 11:115–145 (1947).

(8) Weil, C.S. "Tables for convenient calculation of median effective dose and instructions in their use," *Biometrics.* 8:249–263 (1952).

(9) World Health Organization. "Part I. Environment Health Criteria 6," *Principles and Methods for Evaluating the Toxicity of Chemicals.* Geneva: World Health Organization (1979).

§ 798.1175 Acute oral toxicity.

(a) *Purpose.* In the assessment and evaluation of the toxic characteristics of a substance, determination of acute oral toxicity is usually an initial step. It provides information on health hazards likely to arise from short-term exposure by the oral route. Data from an acute study may serve as a basis for classification and labeling. It is traditionally a step in establishing a dosage regimen in subchronic and other studies and may provide initial information on the mode of toxic action of a substance. An evaluation of acute toxicity data should include the relationship, if any, between the animals' exposure to the test substance and the incidence and severity of all abnormalities, including behavioral and clinical abnormalities, the reversibility of observed abnormalities, gross lesions, body weight changes, effects on mortality, and any other toxic effects.

(b) *Definitions.* (1) Acute oral toxicity is the adverse effects occurring within a short time of oral administration of a single dose of a substance or multiple doses given within 24 hours.

(2) Dose is the amount of test substance administered. Dose is expressed as weight of test substance (g, mg) per unit weight of test animal (e.g. mg/kg).

(3) Dose-response is the relationship between the dose and the proportion of a population sample showing a defined effect.

(c) *Approaches to the determination of acute toxicity.* (1) At present, the evaluation of chemicals for acute toxicity is necessary for the protection of public health and the environment. When animal testing is required for this purpose, this testing should be done in ways that minimize numbers of animals used and that take full account of their welfare.

(2) EPA recommends the following means to reduce the number of animals used to evaluate acute effects of chemical exposure while preserving its ability to make reasonable judgments about safety:

(i) Attempt the use of existing data on structurally related chemicals.

(ii) If data for calculating an LD_{50} are needed, perform an acute toxicity study whereby the value of the data derived from the investment of animal lives is enhanced. EPA does not encourage the use of animals solely for the calculation of an LD$_{50}$.

(iii) Use methods that minimize the numbers of animals in the test.

(3) The following provides an expanded discussion of these principles and their application to the evaluation of acute toxicity of chemicals.

(i) *Using data from structurally related chemicals.* In order to minimize the need for animal testing, the Agency encourages the review of existing acute toxicity information on chemical substances that are structurally related to the agent under investigation. In certain cases one may be able to glean enough information from these surrogate chemicals to make preliminary safety evaluations that may obviate the need for further animal testing.

(ii) *"Limit" test.* When acute lethality data are desirable, EPA's test guideline encourages the use of methods that minimize the requirement for animals, sometimes by a factor of 90 percent as compared to the more traditional LD$_{50}$ test. In the "limit" test, a single group of animals is given a large dose (5 g/kg body weight) of the agent. If no lethality is demonstrated, no further testing for acute oral toxicity is pursued.

(iii) *Estimation of lethal dose.* For those substances demonstrating lethality in a "limit" test or for substances for which there are data on structurally related chemicals that indicate potential acute toxicity below 5 g/kg the Agency can use estimates of the dose associated with some level of acute lethality that are derived from a study comprised of three doses as described in this guideline. With such an approach, use of greater numbers of animals or increased numbers of dose levels are not necessary.

(iv) *Multiple endpoint evaluation.* The Agency stresses the simultaneous monitoring of several endpoints of toxicity in animals in a single acute study including sublethal effects as well as lethality. Dosed animals are observed for abnormal behavioral manifestations such as increased salivation or muscular incoordination, in addition to the recovery from these effects during the observation period. Both dead and surviving animals are autopsied to evaluate gross anatomical evidence of organ toxicity. In selected cases, additional testing may be justified to characterize better the kinds of abnormalities that have been found in the organs of the autopsied animals.

(4) These sound, scientific practices represent some of the means which maximize the utility of the data obtained from a limited number of test animals to achieve a balance between protecting humans and the environment, and the welfare and utilization of laboratory animals. When animal testing is, nonetheless, determined to be necessary to achieve this balance, the following test method incorporates the principles discussed in this section.

(d) *Principle of the test method.* When conducting acute toxicity testing, exposure by gavage is recommended for chemicals where exposure of humans by the oral route is likely. A single exposure and a 14-day observation period are used. The test substance is administered orally in graduated doses to several groups of experimental animals, one dose being used per group. For the limit test, however, only one group is tested at a single (high) dose. Subsequent to exposure, systematic daily observations of effects and deaths are made. Based on the results of cage-side observations or gross necropsy, the tester may decided to initiate histopathological review of certain organs, and/or additional clinical laboratory tests. Animals that die during the test are necropsied, and at the conclusion of the observation period, the surviving animals are sacrificed and are necropsied.

(e) *Limit test.* If a test at a dose level of at least 5 g/kg body weight produces no compound-related mortality, then a study using three dose levels will not be necessary.

(f) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* Although several mammalian test species may be used, the rat is the preferred species. Commonly used laboratory strains should be employed. If another species is used, the tester should provide justificaion and reasoning for its selection.

(ii) *Age.* Young adult animals should be used. The weight variation of animals used in a test should not exceed ± 20 percent of the mean weight for each sex.

(iii) *Sex.* (A) Equal numbers of animals of each sex should be used for each dose level.

(B) The females should be nulliparous and nonpregnant.

(iv) *Numbers per dose group.* At least five animals of each sex should be used at each dose level.

(2) *Control groups.* A concurrent untreated control is not necessary. A vehicle control group should be run concurrently except when historical data are available to determine the acute toxicity of the vehicle.

(3) *Dose levels and dose selection.* (i) Three dose levels should be used and spaced appropriately to produce test groups with a range of toxic effects and mortality rates. The data should be sufficient to produce a dose-response curve and permit an acceptable estimation of the median lethal dose. Range-finding studies using single animals may help to estimate the positioning of the dose groups so that no more than three dose levels will be necessary.

(ii) *Vehicle.* Where necessary, the test substance is dissolved or suspended in a suitable vehicle. It is recommended that wherever possible the use of an aqueous solution be considered first, followed by consideration of a solution in oil (e.g., corn oil) and then by possible solution in other vehicles. For nonaqueous vehicles the toxic characteristics of the vehicle should be known, and if not known should be determined before the test.

(iii) *Volume.* The maximum volume of liquid that can be administered at one time depends on the size of the test animal. In rodents, the volume should not exceed 1 ml/100 g body weight, except when an aqueous solution is used where 2 ml/100 g may be administered. Variability in test volume should be minimized by adjusting the concentration to ensure a constant volume at all dose levels.

(4) *Exposure duration.* The test substance should be administered over a period not exceeding 24 hours.

(5) *Observation period.* The observation period should be at least 14 days. Although 14 days is recommended as a mininum period, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions, rate of onset and length of recovery period, and may thus be extended when considered necessary. The time at which signs of toxicity appear and disappear, their duration, and the time to death are important, especially if there is a tendency for deaths to be delayed.

(6) *Exposure.* (i) The test substance should be administered in a single dose by gavage, using a stomach tube or suitable intubation cannula.

(ii) Animals should be fasted prior to test substance administration. For the rat, food should be withheld overnight; for other rodents with higher metabolic rates a shorter period of fasting is appropriate.

(iii) After the substance has been administered, food may be withheld for an additional 3 to 4 hours.

(iv) If a single dose is not possible, the dose may be given in smaller fractions over a period not exceeding 24 hours. Where a dose is administered in fractions, it may be necessary to provide the animals with food and water depending on the length of the dosing period.

(7) *Observation of animals.* (i) A careful clinical examination should be made at least once each day.

(ii) Additional observations should be made daily, especially in the early days of the study. Appropriate actions should be taken to minimize loss of animals to the study (e.g., necropsy or refrigeration of those animals found dead and isolation of weak or moribund animals).

(iii) Cage-side observations should include, at the least, evaluation of the skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Particular attention should be directed to observation of tremors, convulsions, lethargy, other signs of central nervous system depression, salivation, and diarrhea.

(iv) Individual weights of animals should be determined shortly before the test substance is administered, weekly thereafter, and at death. Changes in weights should be calculated and recorded when survival exceeds one day.

(v) The time of death should be recorded as precisely as possible.

(vi) At the end of the test, surviving animals should be weighed and sacrificed.

(8) *Gross pathology.* A gross necropsy should be performed on all animals under test. All gross pathology changes should be recorded.

(9) *Additional evaluations.* In animals surviving 24 hours or more, clinical chemistry tests or microscopic examination of organs showing evidence of gross pathology should be considered because they may yield additional useful information on the nature of the induced toxic effects.

(g) *Data and reporting*—(1) *Treatment of results.* Data should be summarized in tabular form, showing for each test group the number of animals at the start of the test, body weights, time of death of individual animals at different dose levels, number of animals displaying other signs of toxicity, description of toxic effects and necropsy findings.

(2) *Evaluation of results.* An evaluation of results should include the relationship, if any, between the dose of the test substance and the incidence, severity, and reversibility of all abnormalities, including behavioral and clinical effects, gross lesions, body weight changes, effects on mortality, and any other toxicological effects.

(3) *Test report.* In addition to the reporting requirements as specified under 40 CFR Part 792, Subpart J the following specific information should be reported.

(i) Tabulation of response data by sex and dose level (i.e., number of animals exposed; number of animals showing signs of toxicity; number of animals dying).

(ii) Dose-response curves for mortality and other toxic effects (when permitted by the method of determination).

(iii) Description of toxic effects, including their time of onset, duration, reversibility, and relationship to dose.

(iv) Time of death after dosing.

(v) Body weight data.

(vi) Gross pathology findings.

(vii) Histopathology findings and any additional clinical chemistry evaluations, if performed.

(h) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Balazs, T. "Measurement of acute toxicity," *Methods in Toxicology.* ed. G.E. Paget (Philadelphia: F.A. Davis Co., 1970), pp. 49–82.

(2) Bliss, C.I. "The determination of the dosage mortality curve from small numbers," *Quarterly Journal of Pharmacy and Pharmacology.* 11:192–216 (1938).

(3) Finney, D.G. "Chapter 3—Estimation of the median effective dose, Chapter 4—Maximum likelihood estimation," *Probit Analysis.* 3rd Edition. (London: Cambridge University Press, 1971).

(4) Hunter, W.J., Lingk, W., Recht, P. "Intercomparison study on the determination of single administration toxicity in rats," *Journal of the Association of Official Analytical Chemists.* 62(4):864–873 (1979).

(5) Litchfield, J.T., Jr., Wilcoxon, F. "A simplified method of evaluating dose-effect experiments," *Journal of Pharmacology and Experimental Therapeutics.* 96:99–115 (1949).

(6) Miller, L.C., Tainter, M.L. "Estimation of the ED_{50} and its error by means of logarithmic graph paper," *Proceedings of the Society for Experimental Biology and Medicine.* 57:261–264 (1944).

(7) National Academy of Sciences. "Principles and procedures for evaluating the toxicity of household substances," a report prepared by the Committee for the Revision of NAS Publication 1138, under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC (1977).

(8) Thompson, W.R. "Use of moving averages and interpolation to estimate median effective dose," *Bacteriological Review.* 11:115–145 (1947).

(9) Weil, C.S. "Tables for convenient calculation of median effective dose and instructions in their use," *Biometrics.* 8:249–263 (1952).

(10) World Health Organization. "Part I. Environmental Health Criteria 6," *Principles and Methods for Evaluating the Toxicity of Chemicals.* Geneva: World Health Organization (1978).

Subpart C—Subchronic Exposure

§ 798.2250 Dermal toxicity.

(a) *Purpose.* In the assessment and evaluation of the toxicharacteristics of a chemical, the determination of subchronic dermal toxicity may be carried out after initial information on toxicity has been obtained by acute testing. The subchronic dermal study has been designed to permit the determination of the no-observed-effect level and toxic effects associated with continuous or repeated exposure to a test substance for a period of 90 days. The test is not capable of determining those effects that have a long latency period for development (e.g., carcinogenicity and life shortening). It provides information on health hazards likely to arise from repeated exposure by the dermal route over a limited period of time. It will provide information on target organs, the possibilities of accumulation, and can be of use in selecting dose levels for chronic studies and for establishing safety criteria for human exposure.

(b) *Definitions.* (1) Subchronic dermal toxicity is the adverse effects occurring as a result of the repeated daily exposure of experimental animals to a chemical by dermal application for part (approximately 10 percent) of a life span.

(2) Dose in a dermal test is the amount of test substance applied to the skin (applied daily in subchronic tests). Dose is expressed as weight of the substance (g, mg) per unit weight of test animal (e.g., mg/kg).

(3) No-effect level/No-toxic-effect level/No-adverse-effect level/No-observed-effect level is the maximum dose used in a test which produces no observed adverse effects. A no-observed-effect level is expressed in terms of the weight of a test substance given daily per unit weight of test animal (mg/kg).

(4) Cumulative toxicity is the adverse effects of repeated doses occurring as a result of prolonged action on, or increased concentration of the administered test substance or its metabolites in susceptible tissues.

(c) *Principle of the test method.* The test substance is applied daily to the skin in graduated doses to several groups of experimental animals, one dose level per unit group, for a period of 90 days. During the period of application the animals are observed daily to detect signs of toxicity. Animals which die during the test are necropsied, and at the conclusion of the test the surviving animals are sacrificed and necropsied and appropriate histopathological examinations carried out.

(d) *Limit test.* If a test at one dose level of at least 1000 mg/kg body weight (expected human exposure may indicate the need for a higher dose level), using the procedures described for this study, produces no observable toxic effects and if toxicity would not be expected based upon data of structurally related compounds, then a full study using three dose levels might not be necessary.

(e) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* The rat, rabbit, or guinea pig may be used although the albino rabbit is preferred. The albino rabbit is preferred because of its size, skin permeability, and extensive data base. Commonly used laboratory strains should be employed. If another mammalian species is used, the tester should provide justification/reasoning for its selection.

(ii) *Age.* Young adult animals should be used. The following weight ranges at the start of the test are suggested in order to provide animals of a size which facilitates the conduct of the test: rats, 200 to 300 g; rabbits, 2.0 to 3.0 kg; guinea pigs, 350 to 450 g.

(iii) *Sex.* (A) Equal numbers of animals of each sex with healthy skin should be used at each dose level.

(B) The females should be nulliparous and nonpregnant.

(iv) *Numbers.* (A) At least 20 animals (10 females and 10 males) should be used at each dose level.

(B) If interim sacrifices are planned, the number should be increased by the number of animals scheduled to be sacrificed before completion of the study.

(2) *Control groups.* A concurrent control group is recommended. This group should be an untreated or sham treated control group or, if a vehicle is used in administering the test substance, a vehicle control group. If the toxic properties of the vehicle are not known or cannot be made available, both untreated and vehicle control groups are recommended.

(3) *Satellite group.* A satellite group of 20 animals (10 animals per sex) may be treated with the high dose level for 90 days and observed for reversibility, persistence, or delayed occurrence, of toxic effects for a posttreatment period of appropriate length, normally not less than 28 days.

(4) *Dose level and dose selection.* (i) In subchronic toxicity tests, it is desirable to have a dose-response relationship as well as a nonobserved-toxic-effect level. Therefore, at least three dose levels with a control and, where appropriate, a vehicle control (corresponding to the concentration of vehicle at the highest exposure level) should be used. Doses should be spaced appropriately to produce test groups with a range of toxic effects. The data should be sufficient to produce a dose-response curve.

(ii) The highest dose level should result in toxic effects but not produce severe skin irritation or an incidence of fatalities which would prevent a meaningful evaluation.

(iii) The lowest dose level should not produce any evidence of toxicity. Where there is a usable estimation of human exposure, the lowest dose level should exceed this.

(iv) Ideally, the intermediate dose level(s) should produce minimal observable toxic effects. If more than one intermediate dose is used, the dose levels should be spaced to produce a gradation of toxic effects.

(v) In the low and intermediate groups and in the controls the incidence of fatalities should be low, to permit a meaningful evaluation of the results.

(5) *Exposure conditions.* The animals are treated with test substance, ideally for at least 6 hours per day on a 7-day per week basis, for a period of 90 days. However, based primarily on practical considerations, application on a 5-day per week basis is considered to be acceptable.

(6) *Observation period.* (i) Duration of observation should be at least 90 days.

(ii) Animals in the satellite group scheduled for follow-up observations should be kept for a further 28 days without treatment to detect recovery from, or persistence of, toxic effects.

(7) *Preparation of animal skin.* (i) Shortly before testing, fur should be clipped from the dorsal area of the trunk of the test animals. Shaving may be employed, but it should be carried out approximately 24 hours before the test. Repeat clipping or shaving is usually needed at approximately weekly intervals. When clipping or shaving the fur, care should be taken to avoid abrading the skin, which could alter its permeability.

(ii) Not less than 10 percent of the body surface area should be clear for the application of the test substance. The weight of the animal should be taken into account when deciding on the area to be cleared and on the dimensions of any covering used.

(iii) When testing solids, which may be pulverized if appropriate, the test substance should be moistened sufficiently with water or, where necessary, a suitable vehicle to ensure good contact with the skin. When a vehicle is used, the influence of the vehicle on toxicity of and penetration of the skin by the test substance should be taken into account.

(8) *Application of the test substance.* (i) The test substance should be applied uniformly over an area which is approximately 10 percent of the total body surface area. With highly toxic substances, the surface area covered may be less, but as much of the area should be covered with as thin and uniform a film as possible.

(ii) During the exposure period, the test substance should be held in contact with the skin with a porous gauze dressing and nonirritating tape. The test site should be further covered in a suitable manner to retain the gauze dressing and test substance and ensure that the animals cannot ingest the test substance. Restrainers may be used to prevent the ingestion of the test substance, but complete immobilization is not a recommended method.

(9) *Observation of animals.* (i) Each animal should be handled and its physical condition appraised at least once each day.

(ii) Additional observations should be made daily with appropriate actions taken to minimize loss of animals to the study (e.g., necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals).

(iii) Signs of toxicity should be recorded as they are observed, including the time of onset, the degree, and duration.

(iv) Cage-side observations should include, but not be limited to, changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern.

(v) Animals should be weighed weekly. Food consumption should also be determined weekly if abnormal body weight changes are observed.

(vi) At the end of the study period, all survivors in the non-satellite treatment groups are sacrificed. Moribund animals should be removed and sacrificed when noticed.

(10) *Clinical examinations.* (i) The following examinations should be made on at least five animals of each sex in each group:

(A) Certain hematology determinations should be carried out at least three times during the test period: just prior to initiation of dosing (baseline data), after approximately 30 days on test and just prior to terminal sacrifice at the end of the test period. Hematology determinations which should be appropriate to all studies: hematocrit, hemoglobin concentration, erythrocyte count, total and differential leucocyte count, and a measure of clotting potential such as clotting time, prothrombin time, thromboplastin time, or platelet count.

(B) Certain clinical biochemistry determinations on blood should be carried out at least 3 times: just prior to initiation of dosing (baseline data), after approximately 30 days on test and just prior to terminal sacrifice at the end of the test period. Test areas which are considered appropriate to all studies: electrolyte balance, carbohydrate metabolism, and liver and kidney function. The selection of specific tests will be influenced by observations on the mode of action of the substance. Suggested determinations: calcium, phosphorus, chloride, sodium, potassium, fasting glucose (with the period of fasting appropriate to the species), serum glutamic-pyruvic transaminase (now known as serum alanine aminotransferase), serum glutamic oxaloacetic transaminase (now known as serum aspartate aminotransferase), ornithine decarboxylase, gamma glutamyl transpeptidase, urea nitrogen, albumen, blood creatinine, total bilirubin and total serum protein measurements. Other determinations which may be necessary for an adequate toxicological evaluation include: analyses of lipids, hormones, acid/base balance, methemoglobin and cholinesterase activity. Additional clinical biochemistry may be employed, where necessary, to extend the investigation of observed effects.

(ii) The following examinations should be made on at least five animals of each sex in each group:

(A) Ophthalmological examination, using an ophthalmoscope or equivalent suitable equipment, should be made prior to exposure to the test substance and at the termination of the study. If changes in the eyes are detected all animals should be examined.

(B) Urinalysis is not suggested on a routine basis, but only when there is an indication based on expected or observed toxicity.

(11) *Gross necropsy.* (i) All animals should be subjected to a full gross necropsy which includes examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents.

(ii) The liver, kidneys, adrenals, brain, and gonads should be weighed wet, as soon as possible after dissection, to avoid drying.

(iii) The following organs and tissues, or representative samples thereof, should be preserved in a suitable medium for possible future histopathological examination: normal and treated skin; all gross lesions; brain—including sections of medulla/pons, cerebellar cortex and cerebral cortex; pituitary; thyroid/parathyroid; thymus; trachea; lungs; heart; (sternum with bone marrow); salivary glands; liver; spleen; kidneys; adrenals; pancreas; gonads; uterus; accessory genital organs (epididymis, prostate and, if present, seminal vesicles); aorta; gall bladder (if present); esophagus; stomach; duodenum; jejunum; ileum; cecum; colon; rectum; urinary bladder; representative lymph node; (mammary gland); (thigh musculature); peripheral nerve; (eye); (femur—including articular surface); (spinal cord at three levels—cervical; midthoracic and lumbar); and (exorbital lachrymal glands).

(12) *Histopathology.* The following histopathology should be performed:

(i) Full histopathology on normal and treated skin and on organs and tissues, listed above, of all animals in the control and high dose groups.

(ii) All gross lesions in all animals.

(iii) Target organs in all animals.

(iv) The tissues mentioned in brackets (listed above)—if indicated by signs of toxicity or expected target organ involvement.

(v) Lungs of animals (rodents) in the low and intermediate dose groups should be subjected to histopathological examination for evidence of infection, since this provides a convenient assessment of the state of health of the animals.

(vi) When a satellite group is used, histopathology should be performed on tissues and organs identified as showing effects in other treated groups.

(f) *Data and reporting*—(1) *Treatment of results.* (i) Data should be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals showing lesions, the types of lesions, and the percentage of animals displaying each type of lesion.

(ii) All observed results, quantitative and incidental, should be evaluated by an appropriate statistical method. Any generally accepted statistical method may be used; the statistical methods should be selected during the design of the study.

(2) *Evaluation of results.* The findings of a subchronic dermal toxicity study should be evaluated in conjunction with the findings of preceding studies and considered in terms of the observed toxic effects and the necropsy and histopathological findings. The evaluation should include the relationship between the dose of the test substance and the presence or absence, the incidence and severity, of abnormalities, including behavioral and clinical abnormalities, gross lesions, identified target organs, body weight changes, effect on mortality and any other general or specific toxic effects. A properly conducted subchronic test should provide a satisfactory estimation of a no-effect level.

(3) *Test report.* In addition to the reporting requirements as specified in the EPA Good Laboratory Practice Standards under 40 CFR Part 792, Subpart J, the following specific information should be reported.

(i) *Group animal data.* Tabulation of toxic response data by species, strain, sex and exposure level for:

(A) Number of animals dying.

(B) Number of animals showing signs of toxicity.

(C) Number of animals exposed.

(ii) *Individual animal data.* (A) Time of death during the study or whether animals survived to termination.

(B) Time of observation of each abnormal sign and its subsequent course.

(C) Body weight data.

(D) Food consumption data when collected.

(E) Hematological tests employed and all results.

(F) Clinical biochemistry tests employed and all results.

(G) Necropsy findings.

(H) Detailed description of all histopathological findings.

(I) Statistical treatment of results where appropriate.

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Draize, J.H. "Dermal toxicity," *Appraisal of Chemicals in Food, Drugs and Cosmetics.* The Association of Food and Drug Officials of the United States (1959, 3rd printing 1975). pp. 46–59.

(2) Fitzhugh, O.G. "Subacute toxicity," *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics.* The Association of Food and Drug Officials of the United States (1959, 3rd printing 1975). pp. 26–35.

(3) National Academy of Sciences. "Principles and Procedures for Evaluating the Toxicity of Household

Substances," a report prepared by the Committee for the Revision of NAS Publication 1138, under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC (1977).

(4) World Health Organization. "Part I. Environmental Health Criteria 6,"*Principles and Methods for Evaluating the Toxicity of Chemicals.* (Geneva: World Health Organization, 1978).

§ 798.2450 Inhalation toxicity.

(a) *Purpose.* In the assessment and evaluation of the toxic characteristics of a gas, volatile substance, or aerosol/particulate, determination of subchronic inhalation toxicity may be carried out after initial information on toxicity has been obtained by acute testing. The subchronic inhalation study had been designed to permit the determination of the no-observed-effect level and toxic effects associated with continuous or repeated exposure to a test substance for a period of 90 days. The test is not capable of determining those effects that have a long latency period for development (e.g., carcinogenicity and life shortening). It provides information on health hazards likely to arise from repeated exposures by the inhalation route over a limited period of time. It will provide information on target organs, the possibilities of accumulation, and can be of use in selecting dose levels for chronic studies and for establishing safety criteria for human exposure. Hazards of inhaled substances are influenced by the inherent toxicity and by physical factors such as volatility and particle size.

(b) *Definitions.* (1) Subchronic inhalation toxicity is the adverse effects occurring as a result of the repeated daily exposure of experimental animals to a chemical by inhalation for part (approximately 10 percent) of a life span.

(2) Aerodynamic diameter applies to the size of particles of aerosols. It is the diameter of a sphere of unit density which behaves aerodynamically as the particle of the test substance. It is used to compare particles of different size and densities and to predict where in the respiratory tract such particles may be deposited. This term is used in contrast to measured or geometric diameter which is representative of actual diameters which in themselves cannot be related to deposition within the respiratory tract.

(3) The geometric mean diameter or the median diameter is the calculated aerodynamic diameter which divides the particles of an aerosol in half based on the weight of the particles. Fifty percent of the particles by weight will be larger than the median diameter and 50 percent of the particles will be smaller than the median diameter. The median diameter describes the particle size distribution of any aerosol based on the weight and size of the particles.

(4) Inhalable diameter refers to that aerodynamic diameter of a particle which is considered to be inhalable for the organism. It is used to refer to particles which are capable of being inhaled and may be deposited anywhere within the respiratory tract from the trachea to the alveoli. For man, inhalable diameter is considered as 15 micrometers or less.

(5) Dose refers to an exposure level. Exposure is expressed as weight or volume of test substance per volume of air (mg/l), or as parts per million (ppm).

(6) No-effect level/No-toxic-effect level/No-adverse-effect level/No-observed-effect level is the maximum dose used in a test which produces no observed adverse effects. A no-observed-effect level is expressed in terms of weight or volume of test substance given daily per unit volume of air (mg/l or ppm).

(7) Cumulative toxicity is the adverse effects of repeated doses occurring as a result of prolonged action on, or increased concentration of the administered substance or its metabolites in susceptible tissues.

(c) *Principle of the test method.* Several groups of experimental animals are exposed daily for a defined period to the test substance in graduated concentrations, one concentration being used per group, for a period of 90 days. During the period of administration, the animals are observed daily to detect signs of toxicity. Animals which die during the test are necropsied and at the conclusion of the test, surviving animals are sacrificed and necropsied and appropriate histopathological examinations carried out.

(d) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* A variety of rodent species may be used although the rat is the preferred species. Commonly used laboratory strains should be employed. If another mammalian species is used, the tester should provide justification/reasoning for its selection.

(ii) *Age.* Young adult animals should be used. At the commencement of the study the weight variation of animals should not exceed ±20 percent of the mean weight for each sex.

(iii) *Sex.* (A) Equal numbers of animals of each sex should be used at each dose level.

(B) Females should be nulliparous and nonpregnant.

(iv) *Numbers.* (A) At least 20 animals (10 females and 10 males) should be used for each test group.

(B) If interim sacrifices are planned, the number of animals should be increased by the number of animals scheduled to be sacrificed before the completion of the study.

(2) *Control groups.* A concurrent control group is recommended. This group should be an untreated or sham-treated control group. Except for treatment with the test substance, animals in the control group should be handled in a manner identical to the test group animals. Where a vehicle is used to help generate an appropriate concentration of the substance in the atmosphere, a vehicle control group should be used. If the toxic properties of the vehicle are not known or cannot be made available, both untreated and vehicle control groups are recommended.

(3) *Satellite group.* A satellite group of 20 animals (10 animals per sex) may be treated with the high concentration level for 90 days and observed for reversibility, persistence, or delayed occurrence of toxic effects for a post-treatment period of appropriate length, normally not less than 28 days.

(4) *Dose levels and dose selection.* (i) In subchronic toxicity tests, it is desirable to have a dose-response relationship as well as a no-observed-toxic-effect level. Therefore, at least three dose levels with a control and, where appropriate, a vehicle control (corresponding to the concentration of vehicle at the highest exposure level) should be used. Doses should be spaced appropriately to produce test groups with a range of toxic effects. The data should be sufficient to produce a dose-response curve.

(ii) The highest concentration should result in toxic effects but not produce an incidence of fatalities which would prevent a meaningful evaluation.

(iii) The lowest concentration should not produce any evidence of toxicity. Where there is a usable estimation of human exposure the lowest concentration should exceed this.

(iv) Ideally, the intermediate dose level(s) should produce minimal observable toxic effects. If more than one intermediate dose level is used, the concentrations should be spaced to produce a gradation of toxic effects.

(v) In the low and intermediate groups and in the controls the incidence of fatalities should be low, to permit a meaningful evaluation of the results.

(vi) In the case of potentially explosive test substances, care should be taken to avoid generating explosive concentrations.

(5) *Exposure conditions.* The animals are exposed to the test substance, ideally for 6 hours per day on a 7-day per week basis, for a period of 90 days. However, based primarily on practical considerations, exposure on a 5-day per week basis is considered to be acceptable.

(6) *Observation period.* (i) Duration of observation should be for at least 90 days.

(ii) Animals in a satellite group scheduled for follow-up observations should be kept for an additional 28 days without treatment to detect recovery from, or persistence of, toxic effects.

(7) *Inhalation exposure.* (i) The animals should be tested in inhalation equipment designed to sustain a dynamic air flow of 12 to 15 air changes per hour and ensure an adequate oxygen content of 19 percent and an evenly distributed exposure atmosphere. Where a chamber is used, its design should minimize crowding of the test animals and maximize their exposure to the test substance. This is best accomplished by individual caging. As a general rule, to ensure stability of a chamber atmosphere, the total "volume" of the test animals should not exceed 5 percent of the volume of the test chamber. Oro-nasal or head-only exposure may be used if it is desirable to avoid concurrent exposure by the dermal or oral routes.

(ii) A dynamic inhalation system with a suitable analytical concentration control system should be used. The rate of air flow should be adjusted to ensure that conditions throughout the equipment exposure chamber are essentially the same. Maintenance of slight negative pressure inside the chamber will prevent leakage of the test substance into the surrounding areas.

(iii) The temperature at which the test is performed should be maintained at 22 °C (±2°). Ideally, the relative humidity should be maintained between 40 to 60 percent, but in certain instances (e.g., tests of aerosols, use of water vehicle) this may not be practicable.

(8) *Physical measurements.* Measurements or monitoring should be made of the following:

(i) The rate of air flow should be monitored continuously but should be recorded at least every 30 minutes.

(ii) The actual concentrations of the test substance should be measured in the breathing zone. During the exposure period the actual concentrations of the test substance should be held as constant as practicable, monitored continuously and recorded at least at the beginning, at an intermediate time and at the end of the exposure period.

(iii) During the development of the generating system, particle size analysis should be performed to establish the stability of aerosol concentrations. During exposure, analysis should be conducted as often as necessary to determine the consistency of particle size distribution.

(iv) Temperature and humidity should be monitored continuously but should be recorded at least every 30 minutes.

(9) *Food and water during exposure period.* Food should be withheld during exposure. Water may also be withheld in certain cases.

(10) *Observation of animals.* (i) Each animal should be handled and its physical condition appraised at least once each day.

(ii) Additional observations should be made daily with appropriate actions taken to minimize loss of animals to the study (e.g., necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals).

(iii) Signs of toxicity should be recorded as they are observed including the time of onset, the degree, and duration.

(iv) Cage-side observations should include, but not be limited to, changes in the skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern.

(v) Animals should be weighed weekly. Food consumption should also be determined weekly if abnormal body weight changes are observed.

(vi) At the end of the study period all survivors in the nonsatellite treatment groups should be sacrificed. Moribund animals should be removed and sacrificed when noticed.

(11) *Clinical examinations.* (i) The following examinations should be made on at least five animals of each sex in each group:

(A) Certain hematology determinations should be carried out at least three times during the test period: just prior to initiation of dosing (base line data), after approximately 30 days on test, and just prior to terminal sacrifice at the end of the test period. Hematology determinations which should be appropriate to all studies: hematocrit, hemoglobin concentration, erythrocyte count, total and differential leucocyte count, and a measure of clotting potential such as clotting time, prothrombin time, thromboplastin time, or platelet count.

(B) Certain clinical biochemistry determinations on blood should be carried out at least three times: just prior to initiation of dosing (base line data), after approximately 30 days on test and just prior to terminal sacrifice at the end of the test period. Clinical biochemistry test areas which are considered appropriate to all studies: electrolyte balance, carbohydrate metabolism, and liver and kidney function. The selection of specific tests will be influenced by observations on the mode of action of the substance. Suggested determinations: calcium, phosphorus, chloride, sodium, potassium, fasting glucose (with period of fasting appropriate to the species), serum glutamic-pyruvic transaminase, (now known as serum alanine aminotransferase), serum glutamic-oxaloacetic transaminase (now known as serum aspartate aminotransferase), ornithine decarboxylase, gamma glutamyl transpeptidase, urea nitrogen, albumen, blood creatinine, total bilirubin, and total serum protein measurements. Other determinations which may be necessary for an adequate toxicological evaluation include: analyses of lipids, hormones, acid/base balance, methemoglobin, and cholinesterase activity. Additional clinical biochemistry may be employed, where necessary, to extend the investigation of observed effects.

(ii) The following examinations should be made on at least five animals of each sex in each group:

(A) Ophthalmological examination, using an ophthalmoscope or equivalent suitable equipment, should be made prior to exposure to the test substance and at the termination of the study. If changes in the eyes are detected, all animals should be examined.

(B) Urinalysis is not recommended on a routine basis, but only when there is an indication based on expected or observed toxicity.

(12) *Gross pathology.* (i) All animals should be subjected to a full gross necropsy which includes examination of the external surface of the body, all orifices and the cranial, thoracic and abdominal cavities and their contents.

(ii) At least the liver, kidneys, adrenals, brain, and gonads should be weighed wet, as soon as possible after dissection to avoid drying.

(iii) The following organs and tissues, or representative samples thereof, should be preserved in a suitable medium for possible future histopathological examination: all gross lesions; lungs—which should be removed intact, weighed, and treated with a suitable fixative to ensure that lung structure is maintained (perfusion with the fixative is considered to be an effective procedure]; nasopharyngeal tissues; brain—including sections of medulla/pons cerebellar cortex and cerebral cortex; pituitary; thyroid/parathyroid; thymus; trachea; heart; sternum with bone marrow; salivary glands; liver; spleen; kidneys; adrenals; pancreas; gonads; uterus; accessory genital organs (epididymis, prostrate, and, if present, seminal vesicles); aorta; (skin); gall bladder (if present); esophagus; stomach; duodenum; jejunum; ileum; cecum; colon; rectum; urinary bladder; representative lymph node; (mammary gland); (thigh musculature); peripheral nerve; (eyes); (femur-cervical, midthoracic, and lumbar); and (exorbital lachrymal glands).

(13) *Histopathology.* The following histopathology should be performed:

(i) Full histopathology on the respiratory tract and other organs and tissues, listed above, of all animals in the control and high dose groups.

(ii) All gross lesions in all animals.

(iii) Target organs in all animals.

(iv) The tissues mentioned in brackets (listed above) if indicated by signs of toxicity or target organ involvement.

(v) Lungs of animals in the low and intermediate dose groups should also be subjected to histopathological examination, primarily for evidence of infection since this provides a covenient assessment of the state of health of the animals.

(vi) When a satellite group is used, histopathology should be performed on tissues and organs identified as showing effects in other treated groups.

(e) *Data and reporting*—(1) *Treatment of results.* (i) Data should be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals showing lesions, the types of lesions, and the percentage of animals displaying each type of lesion.

(ii) All observed results, quantitative and incidental, should be evaluated by an appropriate statistical method. Any generally accepted statistical method may be used; the statistical methods should be selected during the design of the study.

(2) *Evaluation of results.* The findings of the subchronic inhalation toxicity study should be evaluated in conjunction with the findings of preceding studies and considered in terms of the observed toxic effects and the necropsy and histopathological findings. The evaluation will include the relationship between the concentration of the test substance and duration of exposure, and the presence or absence, the incidence and severity, of abnormalities, including behavioral and clinical abnormalities, gross lesions, identified target organs, body weight changes, effects on mortality and any other general or specific toxic effects. A properly conducted subchronic test should provide a satisfactory estimation of a no-effect level.

(3) *Test report.* In addition to the reporting requirements as specified under 40 CFR Part 792, Subpart J, the following specific information should be reported:

(i) *Test conditions.* (A) Description of exposure apparatus, including design, type, dimensions, source of air, system for generating particulates and aerosols, method of conditioning air, treatment of exhaust air, and the method of housing animals in a test chamber.

(B) The equipment for measuring temperature, humidity, and particulate aerosol concentrations and size should be described.

(ii) *Exposure data.* These should be tabulated and presented with mean values and measure of variability (e.g., standard deviation) and should include:

(A) Airflow rates through the inhalation equipment.

(B) Temperature and humidity of air.

(C) Nominal concentration (total amount of test substance fed into the inhalation equipment divided by volume of air).

(D) Actual concentration in test breathing zone.

(E) Particle size distribution (e.g., median aerodynamic diameter of particles with standard deviation from the mean).

(iii) *Group animal data.* Tabulation of toxic response data by species, strain, sex, and exposure level for:

(A) Number of animals dying.

(B) Number of animals showing signs of toxicity.

(C) Number of animals exposed.

(iv) *Individual animal data.* (A) Time of death during the study or whether animals survived to termination.

(B) Time of observation of each abnormal sign and its subsequent course.

(C) Body weight data.

(D) Food consumption data when collected.

(E) Hematological tests employed and all results.

(F) Clinical biochemistry tests employed and all results.

(G) Necropsy findings.

(H) Detailed description of all histopathological findings.

(I) Statistical treatment of results where appropriate.

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Cage, J.C. "Experimental Inhalation Toxicology," *Methods in Toxicology.* Ed. G.E. Paget. (Philadelphia: F.A. Davis Co. 1970, pp. 258–277.

(2) Casarett, L.J., Doull, J. "Chapter 9." *Toxicology: The Basic Science of Poisons* (New York: Macmillan Publishing Co. Inc. 1975).

(3) MacFarland, H.N. "Respiratory Toxicology," *Essays in Toxicology.* Ed. W.J. Hayes. Vol. 7 (New York: Academic Press, 1976) pp. 121–154.

(4) National Academy of Sciences. "Principles and Procedures for Evaluating the Toxicity of Household Substances," a report prepared by the Committee for the Revision of NAS Publication 1138, under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC (1977).

(5) World Health Organization. "Part I. Environmental Health Criteria 6," *Principles and Methods for Evaluating the Toxicity of Chemicals.* (Geneva: World Health Organization, 1978).

§ 798.2650 Oral toxicity.

(a) *Purpose.* In the assessment and evaluation of the toxic characteristics of a chemical, the determination of subchronic oral toxicity may be carried out after initial information on toxicity has been obtained by acute testing. The subchronic oral study has been designed to permit the determination of the no-observed-effect level and toxic effects associated with continuous or repeated exposure to a test substance for a period of 90 days. The test is not capable of determining those effects that have a long latency period for development (e.g., carcinogenicity and life shortening). It provides information on health hazards likely to arise from repeated exposure by the oral route over a limited period of time. It will provide information on target organs, the possibilities of accumulation, and can be of use in selecting dose levels for chronic studies and for establishing safety criteria for human exposure.

(b) *Definitions.* (1) Subchronic oral toxicity is the adverse effects occurring as a result of the repeated daily exposure of experimental animals to a chemical by the oral route for a part (approximately 10 percent for rats) of a life span.

(2) Dose is the amount of test substance administered. Dose is expressed as weight of test substance (g, mg) per unit weight of test animal (e.g., mg/kg), or as weight of test substance per unit weight of food or drinking water.

(3) No-effect level/No-toxic-effect level/No-adverse-effect level/No-observed-effect level is the maximum dose used in a test which produces no observed adverse effects. A no-observed-effect level is expressed in terms of the weight of a substance given daily per unit weight of test animal (mg/kg). When administered to animals in food or drinking water the no-observed-effect level is expressed as mg/kg of food or mg/ml of water.

(4) Cumulative toxicity is the adverse effects of repeated doses occurring as a result of prolonged action on, or increased concentration of the administered substance or its metabolites in susceptible tissue.

(c) *Principle of the test method.* The test substance is administered orally in graduated daily doses to several groups of experimental animals, one dose level per group, for a period of 90 days. During the period of administration the animals are observed daily to detect signs of toxicity. Animals which die during the period of administration are necropsied. At the conclusion of the test all animals are necropsied and histopathological examinations carried out.

(d) *Limit test.* If a test at one dose level of at least 1,000 mg/kg body weight (expected human exposure may indicate the need for a higher dose level), using the procedures described for this study, produces no observable toxic effects and if toxicity would not be expected based upon data of structurally related compounds, then a full study using three dose levels might not be necessary.

(e) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* A variety of rodent species may be used, although the rat is the preferred species. Commonly used laboratory strains should be employed. The commonly used non-rodent species is the dog, preferably of a defined breed; the beagle is frequently used. If other mammalian species are used, the tester should provide justification/reasoning for their selection.

(ii) *Age*—(A) *General.* Young adult animals should be employed. At the commencement of the study the weight variation of animals used should not exceed ± 20 percent of the mean weight for each sex.

(B) *Rodents.* Dosing should begin as soon as possible after weaning, ideally before the rats are 6, and in any case, not more than 8 weeks old.

(C) *Non-rodent.* In the case of the dog, dosing should commence after acclimatization, preferably at 4 to 6 months and not later than 9 months of age.

(iii) *Sex.* (A) Equal numbers of animals of each sex should be used at each dose level.

(B) The females should be nulliparous and nonpregnant.

(iv) *Numbers*—(A) *Rodents.* At least 20 animals (10 females and 10 males) should be used at each dose level.

(B) *Non-rodents.* At least eight animals (four females and four males) should be used at each dose level.

(C) If interim sacrifices are planned, the number should be increased by the number of animals scheduled to be sacrificed before the completion of the study.

(2) *Control groups.* A concurrent control group is recommended. This group should be an untreated or sham treated control group or, if a vehicle is used in administering the test substance, a vehicle control group. If the toxic properties of the vehicle are not known or cannot be made available, both untreated and vehicle control groups are recommended.

(3) *Satellite group.* (Rodent) A satellite group of 20 animals (10 animals per sex) may be treated with the high dose level for 90 days and observed for reversibility, persistence, or delayed occurrence of toxic effects for a post-treatment period of appropriate length, normally not less than 28 days.

(4) *Dose levels and dose selection.* (i) In subchronic toxicity tests, it is desirable to have a dose response relationship as well as no-observed-toxic-effect level. Therefore, at least three dose levels with a control and, where appropriate, a vehicle control (corresponding to the concentration of vehicle at the highest exposure level) should be used. Doses should be spaced appropriately to produce test groups with a range to toxic effects. The data should be sufficient to produce a dose response curve.

(ii) The highest dose level in rodents should result in toxic effects but not produce an incidence of fatalities which would prevent a meaningful evaluation; for non-rodents there should be no fatalities.

(iii) The lowest dose level should not produce any evidence of toxicity. Where there is a usable estimation of human exposure the lowest dose level should exceed this.

(iv) Ideally, the intermediate dose level(s) should produce minimal observable toxic effects. If more than one intermediate dose is used, the dose levels should be spaced to produce a gradation of toxic effects.

(v) For rodents, the incidence of fatalities in low and intermediate dose groups and in the controls should be low, to permit a meaningful evaluation of the results; for non-rodents, there should be no fatalities.

(5) *Exposure conditions.* The animals are dosed with the test substance ideally on a 7-day per week basis over a period of 90 days. However, based primarily on practical considerations, dosing in gavage or capsule studies on a 5-day per week basis is considered to be acceptable.

(6) *Observation period.* (i) Duration of observation should be for at least 90 days.

(ii) Animals in the satellite group scheduled for follow-up observations should be kept for a further 28 days without treatment to detect recovery from, or persistence of, toxic effects.

(7) *Administration of the test substance.* (i) The test substance may be administered in the diet or in capsules. In addition, for rodents it may also be administered by gavage or in the drinking water.

(ii) All animals should be dosed by the same method during the entire experimental period.

(iii) Where necessary, the test substance is dissolved or suspended in a suitable vehicle. If a vehicle or diluent is needed, ideally it should not elicit important toxic effects itself nor substantially alter the chemical or toxicological properties of the test substance. It is recommended that wherever possible the usage of an aqueous solution be considered first, followed by consideration of a solution of oil and then by possible solution in other vehicles.

(iv) For substances of low toxicity, it is important to ensure that when administered in the diet the quantities of the test substance involved do not interfere with normal nutrition. When the test substance is administered in the diet either a constant dietary concentration (ppm) or a constant dose level in terms of the animals' body weight may be used; the alternative used should be specified.

(v) For a substance administered by gavage or capsule, the dose should be given at similar times each day, and adjusted at intervals (weekly or bi-weekly) to maintain a constant dose level in terms of animal body weight.

(8) *Observation of animals.* (i) Each animal should be handled and its physical condition appraised at least once each day.

(ii) Additional observations should be made daily with appropriate actions taken to minimize loss of animals to the study (e.g., necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals).

(iii) Signs of toxicity should be recorded as they are observed including the time of onset, degree and duration.

(iv) Cage-side observations should include, but not be limited to, changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern.

(v) Measurements should be made weekly of food consumption or water consumption when the test substance is administered in the food or drinking water, respectively.

(vi) Animals should be weighed weekly.

(vii) At the end of the 90-day period all survivors in the nonsatellite treatment groups are sacrificed. Moribund animals should be removed and sacrificed when noticed.

(9) *Clinical examinations.* (i) The following examinations should be made on at least five animals of each sex in each group for rodents and all animals when non-rodents are used as test animals.

(A) Certain hematology determinations should be carried out at least three times during the test period: just prior to initiation of dosing (baseline data), after approximately 30 days on test and just prior to terminal sacrifice at the end of the test period. Hematology determinations which should be appropriate to all studies: hematocrit, hemoglobin concentration, erythrocyte count, total and differential leucocyte count, and a measure of clotting potential such as clotting time, prothrombin time, thromboplastin time, or platelet count.

(B) Certain clinical biochemistry determinations should be carried out at least three times during the test period: just prior to initiation of dosing (baseline data), after approximately 30 days on test and just prior to terminal sacrifice at the end of the test period. Clinical biochemical test areas which are considered appropriate to all studies: electrolyte balance, carbohydrate metabolism, and liver and kidney function. The selection of specific tests will be influenced by observations on the mode of action of the substance. Suggested determinations: calcium, phosphorus, chloride, sodium, potassium, fasting glucose (with period of fasting appropriate to the species/breed), serum glutamic-pyruvic transaminase (now known as serum alanine aminotransferase), serum glutamic oxaloacetic transaminase (now known as serum aspartate aminotransferase), ornithine decarboxylase, gamma glutamyl transpeptidase, urea nitrogen, albumen, blood creatinine, total bilirubin and total serum protein measurements. Other determinations which may be necessary for an adequate toxicological evaluation include analyses of lipids, hormones, acid/base balance, methemoglobin and cholinesterase activity. Additional clinical biochemistry may be employed where necessary to extend the investigation of observed effects. Non-rodents should be fasted for a period (not more than 24 hours) before taking blood samples.

(ii) The following examinations should be made on at least five animals of each sex in each group for rodents and all animals on test for non-rodents.

(A) Ophthalmological examination, using an ophthalmoscope or equivalent suitable equipment, should be made prior to the administration of the test substance and at the termination of the study. If changes in the eyes are detected all animals should be examined.

(B) Urinalysis is not recommended on a routine basis, but only when there is an indication based on expected or observed toxicity.

(10) *Gross necropsy.* (i) All animals should be subjected to a full gross necropsy which includes examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents.

(ii) At least the liver, kidneys, adrenals, and gonads should be weighed wet, as soon as possible after dissection to avoid drying. In addition, for the rodent, the brain; for the non-rodent, the thyroid with parathyroids also should be weighed wet.

(iii) The following organs and tissues, or representative samples thereof, should be preserved in a suitable medium for possible future histopathological examination: all gross lesions; brain-including sections of medulla/pons, cerebellar cortex and cerebral cortex; pituitary; thyroid/parathyroid; thymus; lungs; trachea; heart; sternum with bone marrow; salivary glands; liver; spleen; kidneys/adrenals; pancreas; gonads; uterus; accessory genital organs (epididymis, prostate, and, if present, seminal vesicles); aorta; (skin), (non-rat gall bladder); esophagus; stomach; duodenum; jejunum; ileum; cecum; colon; rectum; urinary bladder; representative lymph node; (mammary gland), (thigh musculature), peripheral nerve; (eyes); (femur-including articular surface); (spinal cord at three levels—cervical, midthoracic and lumbar); and, (rodent—exorbital lachrymal glands).

(11) *Histopathology.* The following histopathology should be performed:

(i) Full histopathology on the organs and tissues, listed above, of all rodents in the control and high dose groups, all non-rodents, and all rodents that died or were killed during the study.

(ii) All gross lesions in all animals.

(iii) Target organs in all animals.

(iv) The tissues mentioned in brackets (listed above) if indicated by signs of toxicity of target organ involvement.

(v) Lungs, liver and kidneys of all animals. Special attention to examination of the lungs of rodents should be made for evidence of infection since this provides a convenient assessment of the state of health of the animals.

(vi) When a satellite group is used (rodents), histopathology should be performed on tissues and organs identified as showing effects in the treated groups.

(f) *Data and reporting*—(1) *Treatment of results.* (i) Data should be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals showing lesions, the types of lesions and the percentage of animals displaying each type of lesion.

(ii) All observed results, quantitative and incidental, should be evaluated by an appropriate statistical method. Any generally accepted statistical methods may be used; the statistical methods should be selected during the design of the study.

(2) *Evaluation of the study results.* (i) The findings of a subchronic oral toxicity study should be evaluated in conjunction with the findings of preceding studies and considered in terms of the toxic effects and the necropsy and histopathological findings. The evaluation will include the relationship between the dose of the test substance and the presence or absence, the incidence and severity, of abnormalities, including behavioral and clinical abnormalities, gross lesions, identified target organs, body weight changes, effects on mortality and any other general or specific toxic effects. A properly conducted subchronic test should provide a satisfactory estimation of a no-effect level.

(ii) In any study which demonstrates an absence of toxic effects, further investigation to establish absorption and bioavailability of the test substance should be considered.

(3) *Test report.* In addition to the reporting requirements as specified under 40 CFR Part 792, Subpart J the following specific information should be reported:

(i) *Group animal data.* Tabulation of toxic response data by species, strain, sex and exposure level for:

(A) Number of animals dying.

(B) Number of animals showing signs of toxicity.

(C) Number of animal exposed.

(ii) *Individual animal data.* (A) Time of death during the study or whether animals survived to termination.

(B) Time of observation of each abnormal sign and its subsequent course.

(C) Body weight data.

(D) Food consumption data when collected.

(E) Hematological tests employed and all results.

(F) Clinical biochemistry tests employed and all results.

(G) Necropsy findings.

(H) Detailed description of all histopathological findings.

(I) Statistical treatment of results where appropriate.

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Boyd, E.M. "Chapter 14—Pilot Studies, 15—Uniposal Clinical Parameters, 16—Uniposal Autopsy Parameters." *Predictive Toxicometrics.* (Baltimore: Williams and Wilkins, 1972).

(2) Fitzhugh, O.G. "Subacute Toxicity," *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics.* The Association of Food and Drug Officials of the United States (1959, 3rd Printing 1975) pp. 26–35.

(3) Food Safety Council. "Subchronic Toxicity Studies," *Proposed System for Food Safety Assessment.* (Columbia: Food Safety Council, 1978) pp. 83–96.

(4) National Academy of Sciences. "Principles and Procedures for Evaluating the Toxicity of Household Substances," a report prepared by the Committee for the Revision of NAS Publication 1138, under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC (1977).

(5) World Health Organization. "Part I. Environmental Health Criteria 6," *Principles and Methods for Evaluating the Toxicity of Chemicals.* (Geneva: World Health Organization, 1978).

§ 798.2675 Oral toxicity with satellite reproduction and fertility study.

(a) *Purpose.* (1) In the assessment and evaluation of the toxic characteristics of a chemical, the determination of subchronic oral toxicity may be carried out after initial information on toxicity has been obtained by acute testing. The subchronic oral study has been designed to permit the determination of the no-observed-effect level and toxic effects associated with continuous or repeated exposure to a test substance for a period of 90 days. The test is not capable of determining those effects that have a long latency period for development (e.g., carcinogenicity and life shortening). It provides information on health hazards likely to arise from repeated exposure by the oral route over a limited period of time. It will provide information on target organs, and on the possibilities of accumulation, and can be of use in selecting dose levels for chronic studies and for establishing safety criteria for human exposure.

(2) The satellite reproduction and fertility study is designed to provide general information concerning the effects of a test substance on gonadal function, conception, parturition, and the growth and development of the offspring. The study may also provide information about the effects of the test substance on neonatal morbidity, mortality, and preliminary data on teratogenesis and serve as a guide for subsequent tests.

(b) *Definitions.* (1) Subchronic oral toxicity is the adverse effects occurring as a result of the repeated daily exposure of experimental animals to a chemical by the oral route for a part (approximately 10 percent for rats) of a life span.

(2) Dose is the amount of test substance administered. Dose is expressed as weight of test substance (g, mg) per unit weight of test animal (e.g., mg/kg), or as weight of test substance per unit weight of food or drinking water.

(3) No-effect level/No-toxic-effect level/No-adverse-effect level/No-observed effect level is the maximum dose used in a test which produces no observed adverse effects. A no-observed effect level is expressed in terms of the weight of a substance given daily per unit weight of test animal (mg/kg). When administered to animals in food or drinking water the no-observed-effect level is expressed as mg/kg of food or mg/ml of water.

(4) Cumulative toxicity is the adverse effects of repeated doses occurring as a result of prolonged action on, or increased concentration of the administered substance or its metabolites in susceptible tissue.

(c) *Principle of the test method.* (1) The test substance is administered orally in graduated daily doses to several groups of experimental animals, one dose level per group, for a period of 90 days for the subchronic study. During the period of administration the animals are observed daily to detect signs of toxicity. Animals which die during the period of administration are necropsied. At the conclusion of the test all surviving animals are necropsied and histopathological examinations carried out.

(2) Starting with the 10th week of dosing, males will be mated with females selected for the reproduction and fertility study. The test substance will continue to be administered throughout the period of mating until day 90 for all males and those females not selected for reproduction; for the mated females, dosing will continue throughout the resulting period of gestation and lactation until weaning of their offspring.

(d) *Limit test.* If a test at one dose level of at least 1,000 mg/kg body weight (expected human exposure may indicate the need for a higher dose level), using the procedures described for this study, produces no observable toxic effects and if toxicity would not be expected based upon data of structurally related compounds, then a full study using three dose levels might not be necessary.

(e) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* The rat is the preferred species. If another mammalian species is used, the tester should provide justification/reasoning for its selection. Strains with low fecundity should not be used.

(ii) *Age.* Animals should be about 5 to 8 weeks old at the start of dosing.

(iii) *Sex.* (A) Both males and females should be studied.

(B) The females should be nulliparous and non-pregnant.

(iv) *Number of animals.* Each test and control group should contain at least 10 males and at least 30 females (at least 10 for subchronic study and a sufficient amount of additional females to yield at least 20 pregnancies for the reproduction and fertility study).

(2) *Control groups.* (i) A concurrent control group should be used. This group should be an untreated or sham treated control group or if a vehicle is used in administering the test substance, a vehicle control group.

(ii) If a vehicle is used in administering the test substance, the control group should receive the vehicle in the highest volume used.

(iii) If a vehicle or other additive is used to facilitate dosing, it should not interfere with absorption of the test substance or produce toxic effects.

(3) *Dose levels and dose selection.* (i) In subchronic toxicity tests, it is desirable to have a dose response relationship as well as no-observed-toxic-effect level. Therefore, at least three dose levels with a control and, where appropriate, a vehicle control

(corresponding to the concentration of vehicle at the highest exposure level) should be used. Doses should be spaced appropriately to produce test groups with a range of toxic effects. The data should be sufficient to produce a dose response curve.

(ii) The highest dose level in rodents should result in toxic effects but not produce an incidence of fatalities which would prevent a meaningful evaluation.

(iii) The lowest dose level should not produce any evidence of toxicity. Where there is a usable estimation of human exposure the lowest dose level should exceed this.

(iv) Ideally, the intermediate dose level(s) should produce minimal observable toxic effects. If more than one intermediate dose is used, the dose levels should be spaced to produce a gradation of toxic effects.

(4) *Exposure conditions.* The animals are dosed with the test substance ideally on a 7-day per week basis over a period of 90 days. However, based primarily on practical considerations, dosing in gavage or capsule studies on a 5-day per week basis is considered to be acceptable, except during the periods of mating, gestation, and lactation during which time females selected for the reproduction and fertility study should be dosed daily.

(i) Dosing, mating, delivery, and sacrifice schedule.

(A) Dosing of the males and females should begin when they are 5 to 8 weeks old. For both sexes, dosing should be continued for at least 10 weeks before the mating period.

(B) Dosing of males should continue through the mating period and until day 90 of the subchronic study.

(C) Dosing of females not selected for the reproduction and fertility study should continue until day 90 of the subchronic study.

(D) Daily dosing (that is, on a 7-day per week basis) of the females selected for the reproduction and fertility study should begin with and continue through the mating period, pregnancy, and to the weaning of the offspring.

(ii) All animals are sacrificed as scheduled.

(A) All males and those females not selected for reproduction and fertility study should be sacrificed at the end of the 90-day subchronic study.

(B) The females selected for the reproduction and fertility study should be sacrificed upon weaning of their offspring.

(C) Offspring should be sacrificed when weaned.

(5) *Observation period.* (i) Duration of observation should be for at least 90 days for the subchronic study.

(ii) For the satellite reproduction and fertility study, observation of dams and their pups should continue until the weaning of the offspring.

(6) *Administration of the test substance.* (i) Test substance may be administered in the diet or in capsules. In addition, for rodents it may also be administered by gavage or in the drinking water.

(ii) All animals should be dosed by the same method during the entire experimental period.

(iii) Where necessary, the test substance is dissolved or suspended in a suitable vehicle. If a vehicle or diluent is needed, ideally it should not elicit important toxic effects itself nor substantially alter the chemical or toxicological properties of the test substance. It is recommended that wherever possible the usage of an aqueous solution be considered first, followed by consideration of a solution of oil and then by possible solution in other vehicles.

(iv) For substances of low toxicity, it is important to ensure that when administered in the diet the quantities of the test substance involved do not interfere with normal nutrition. When the test substance is administered in the diet either a constant dietary concentration (ppm) or a constant dose level in terms of the animals' body weight may be used; the alternative used should be specified.

(v) For a substance administered by gavage or capsule, the dose should be given at similar times each day and adjusted to maintain a constant dose level in terms of animal body weight. During pregnancy the dosage should be based on the body weight at day 0 and 6 of pregnancy.

(7) *Mating procedure*—(i) *Parental.* (A) For each mating, either a 1:1 (one male to one female) or a 1:2 (one male to two females) method may be used. Females should be placed with males from the same dose level until pregnancy occurs or one week has elapsed. If mating has not occurred after one week, the female should be placed with a different male. Paired matings should be clearly identified.

(B) Those pairs that fail to mate should be evaluated to determine the cause of the apparent infertility. This may involve such procedures as additional opportunities to mate with proven fertile males or females, histological examination of the reproductive organs, and examination of the estrus or spermatogenic cycles.

(C) Each day, the females should be examined for presence of sperm or vaginal plugs. Day 0 of pregnancy is defined as the day vaginal plugs or sperm are found.

(ii) *Special housing.* After evidence of copulation, pregnant animals should be caged separately in delivery or maternity cages and provided with nesting materials.

(iii) *Standardization of litter sizes.* (A) On day 4 after birth, the size of each litter should be adjusted by eliminating extra pups by random selection to yield, as nearly as possible, four males and four females per litter.

(B) Whenever the number of male or female pups prevents having four of each sex per litter, partial adjustment (for example, five males and three females) is permitted. Adjustments are not appropriate for litters of less than eight pups.

(C) Elimination of runts only is not appropriate.

(8) *Observation of animals.* (i) Each animal should be handled and its physical condition appraised at least once each day.

(ii) Additional observations should be made daily with appropriate actions taken to minimize loss of animals to the study (e.g., necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals).

(iii) Signs of toxicity should be recorded as they are observed including the time of onset, degree and duration.

(iv) Cage-side observations should include, but not be limited to, changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern.

(v) Measurements should be made weekly of food consumption or water consumption when the test substance is administered in the food or drinking water, respectively.

(vi) Animals should be weighed weekly.

(vii) At the end of the 90-day period all survivors in the non-satellite treatment groups are sacrificed. Moribund animals should be removed and sacrificed when noticed.

(viii) The duration of gestation should be calculated from day 0 of pregnancy. Signs of difficult or prolonged parturition should be recorded.

(ix) Each litter should be examined as soon as possible after delivery for the number of pups, stillbirths, live births, sex, and the presence of gross anomalies. Dead pups and pups sacrificed at day 4 should be preserved and studied for possible defects. Live pups should be counted and litters weighed, by weighing each individual pup at birth, or soon thereafter, and on days 4, 7, 14, and 21 after parturition.

(x) Physical or behavioral abnormalities observed in the dams or offspring should be recorded.

(xi) Males and females in subchronic and satellite studies should be weighed on the first day of dosing and weekly thereafter. Litters should be weighed at birth, or soon thereafter, and on days 4, 7, 14, and 21. In all cases, litter weights should be calculated from the weights of the individual pups.

(9) *Clinical examinations.* (i) The following examinations should be made on at least five animals of each sex in each group from the subchronic study.

(A) Certain hematology determinations should be carried out at least three times during the test period: just prior to initiation of dosing (baseline data), after approximately 30 days on test and just prior to terminal sacrifice at the end of the test period. Hematology determinations which should be appropriate to all studies: hematocrit, hemoglobin concentration, erythrocyte count, total and differential leucocyte count, and a measure of clotting potential such as clotting time, prothrombin time, thromboplastin time, or platelet count.

(B) Certain clinical biochemistry determinations should be carried out at least three times during the test period: just prior to initiation of dosing (baseline data), after approximately 30 days on test and just prior to terminal sacrifice at the end of the test period. Clinical biochemical test areas which are considered appropriate to all studies: electrolyte balance, carbohydrate metabolism, and liver and kidney function. The selection of specific tests will be influenced by observations on the mode of action of the substance. Suggested determinations: calcium, phosphorus, chloride, sodium, potassium, fasting glucose (with period of fasting appropriate to the species/breed), serum glutamic-pyruvic transaminase (now known as serum alanine aminotransferase), serum glutamic oxaloacetic transaminase (now known as serum aspartate aminotransferase), ornithine decarboxylase, gamma glutamyl transpeptidase, urea nitrogen, albumen, blood creatinine, total bilirubin and total serum protein measurements. Other determinations which may be necessary for an adequate toxicological evaluation include analyses of lipids, hormones, acid/base balance, methemoglobin and cholinesterase activity. Additional clinical biochemistry may be employed where necessary to extend the investigation of observed effects.

(ii) The following examinations should be made on at least five animals of each sex in each group.

(A) Ophthalmological examination, using an ophthalmoscope or equivalent suitable equipment, should be made prior to the administration of the test substance and at the termination of the study. If changes in the eyes are detected all animals should be examined.

(B) Urinalysis is not recommended on a routine basis, but only when there is an indication based on expected or observed toxicity.

(10) *Gross necropsy.* (i) All animals (except offspring) should be subjected to a full gross necropsy which includes examination of the external surface of the body, all orifices, and the cranial, thoracic and abdominal cavities and their contents.

(ii) Special attention should be directed to the organs of the reproductive system.

(iii) At least the liver, kidneys, adrenals, brain and gonads should be weighed wet, as soon as possible after dissection to avoid drying.

(iv) The following organs and tissues, or representative samples thereof, should be preserved in a suitable medium for possible future histopathological examination: all gross lesions; brain-including sections of medulla/pons, cerebellar cortex and cerebral cortex; pituitary; thyroid/parathyroid; thymus; lungs; trachea; heart; sternum with bone marrow; salivary glands; liver; spleen; kidneys/adrenals; pancreas; vagina; uterus; cervix; ovaries; testes; epididymides; prostate; seminal vesicles; aorta; (skin); esophagus; stomach; duodenum; jejunum; ileum; cecum; colon; rectum; urinary bladder; representative lymph node; (mammary gland); (thigh musculature); peripheral nerve; (eyes); (femur including articular surface); (spinal cord at three levels—cervical, midthoracic and lumbar); and, (exorbital lachrymal glands).

(v) Dead or moribund pups should be examined for defects.

(11) *Histopathology.* The following histopathology should be performed:

(i) Full histopathology on the organs and tissues, listed above, of all animals (except offspring) in the control and high dose groups, and all animals (except offspring) that died or were killed during the study.

(ii) All gross lesions in all animals.

(iii) Target organs in all animals.

(iv) The tissues mentioned in brackets (listed above) if indicated by signs of toxicity or target organ involvement.

(v) Lungs, liver and kidneys of all animals. Special attention to examination of the lungs of rodents should be made for evidence of infection since this provides a convenient assessment of the state of health of the animals.

(vi) As suggested under mating procedures, reproductive organs of animals suspected of infertility may be subjected to microscopic examination.

(f) *Data and reporting*—(1) *Treatment of results.* (i) For the subchronic study, data should be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals showing lesions, the types of lesions and the percentage of animals displaying each type of lesion.

(ii) For the reproduction and fertility study, data should be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals pregnant, the types of change and the percentage of animals displaying each type of change.

(iii) All observed results, quantitative and incidental, should be evaluated by an appropriate statistical method. Any generally accepted statistical methods may be used; the statistical methods should be selected during the design of the study.

(2) *Evaluation of the study results.* (i) The findings of a subchronic oral toxicity study should be evaluated in conjunction with the findings of preceding studies and considered in terms of the toxic effects and the necropsy and histopathological findings. The evaluation will include the relationship between the dose of the test substance and the presence or absence, the incidence and severity, of abnormalities, including behavioral and clinical abnormalities, gross lesions, identified target organs, body weight changes, effects on mortality and any other general or specific toxic effects. A properly conducted test should provide a satisfactory estimation of a no-effect level.

(ii) In any study which demonstrates an absence of toxic effects, further investigation to establish absorption and bioavailability of the test substance should be considered.

(3) *Test report.* In addition to the reporting requirements as specified under 40 CFR Part 792, Subpart J the following specific information should be reported:

(i) Tabulation of toxic response data by species, strain, sex, and exposure level for:

(A) Number of animals dying.

(B) Number of animals showing signs of toxicity.

(C) Number of animals exposed.

(ii) *Individual animal data.* (A) Time of death during the study or whether animals survived to termination.

(B) Time of observation of each abnormal sign and its subsequent course.

(C) Body weight data.

(D) Food consumption data when collected.

(E) Hematological tests employed and all results.

(F) Clinical biochemistry tests employed and all results.

(G) Necropsy findings.

(H) Detailed description of all histopathological findings.

(I) Statistical treatment of results where appropriate.

(iii) *Additional information from reproduction and fertility satellite study.* (A) Toxic response data by sex and dose, including fertility, gestation, viability and lactation indices, and length of gestation.

(B) Species and strain.

(C) Toxic or other effects on reproduction, offspring, or postnatal growth.

(D) Time of observation of each abnormal sign and its subsequent course.

(E) Body weight data for parental animals and offspring.

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Boyd, E.M. "Chapter 14—Pilot Studies, 15—Uniposal Clinical Parameters, 16—Uniposal Autopsy Parameters," *Predictive Toxicometrics.* (Baltimore: Williams and Wilkins, 1972).

(2) Clermont, Y., Perry, B. "Quantitative Study of the Cell Population of the Seminiferous Tubules in Immature Rats," *American Journal of Anatomy.* 100:241–267 (1957).

(3) Fitzhugh, O.C. Third Printing: 1975. "Subacute Toxicity," *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics.* The Association of Food and Drug Officials of the United States (1959), pp. 26–35.

(4) Food Safety Council. "Subchronic Toxicity Studies," *Proposed System for Food Safety Assessment.* (Columbia: Food Safety Council, 1978) pp. 83–96.

(5) Goldenthal, E.I. *Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use.* Drug Review Branch, Division of Toxicological Evaluation, Bureau of Science, Food and Drug Administration, Washington, DC (1966).

(6) Hasegawa, T., Hayashi, M., Ebling, F.J.G., Henderson, I.W. *Fertility and Sterility.* (New York: American Elsevier Publishing Co., Inc., 1973).

(7) National Academy of Sciences. "Principles and Procedures for Evaluating the Toxicity of Household Substances," a report prepared by the Committee for the Revision of NAS Publication 1138, under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC (1977).

(8) Oakberg, E.F. "Duration of Spermatogenesis in the Mouse and Timing of Stages of the Cycle of the Seminiferous Epithelium," *American Journal of Anatomy.* 9:507–516 (1956).

(9) Roosen-Runge, E.C. "The Process of Spermatogenesis in Mammals," *Biological Review.* 37:343–377 (1962).

(10) World Health Organization. "Part I. Environmental Health Criteria 6," *Principles and Methods for Evaluating the Toxicity of Chemicals.* (Geneva: World Health Organization, 1978).

Subpart D—Chronic Exposure

§ 798.3260 Chronic toxicity.

(a) *Purpose.* The objective of a chronic toxicity study is to determine the effects of a substance in a mammalian species following prolonged and repeated exposure. Under the conditions of the chronic toxicity test, effects which require a long latency period or which are cumulative should become manifest. The application of this guideline should generate data on which to identify the majority of chronic effects and shall serve to define long term dose-response relationships. The design and conduct of chronic toxicity tests should allow for the detection of general toxic effects, including neurological, physiological, biochemical, and hematological effects and exposure-related morphological (pathology) effects.

(b) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* Testing should be performed with two mammalian species, one a rodent and another a non-rodent. The rat is the preferred rodent species and the dog is the preferred non-rodent species. Commonly used laboratory strains should be employed. If other mammalian species are used, the tester should provide justification/reasoning for their selection.

(ii) *Age.* (A) Dosing of rats should begin as soon as possible after weaning, ideally before the rats are 6, but in no case more than 8 weeks old.

(B) Dosing of dogs should begin between 4 and 6 months of age and in no case later than 9 months of age.

(C) At commencement of the study the weight variation of animals used should not exceed ±20 percent of the mean weight for each sex.

(iii) *Sex.* (A) Equal numbers of animals of each sex should be used at each dose level.

(B) The females should be nulliparous and non-pregnant.

(iv) *Numbers.* (A) For rodents, at least 40 animals (20 females and 20 males) and for non-rodents (dogs) at least 8 animals (4 females and 4 males) should be used at each dose level.

(B) If interim sacrifices are planned the number should be increased by the number of animals scheduled to be sacrificed during the course of the study.

(C) The number of animals at the termination of the study must be adequate for a meaningful and valid statistical evaluation of chronic effects.

(2) *Control groups.* (i) A concurrent control group is suggested. This group should be an untreated or sham treated control group or, if a vehicle is used in administering the test substance, a vehicle control group. If the toxic properties of the vehicle are not known or cannot be made available, both untreated and vehicle control groups are strongly suggested.

(ii) In special circumstances such as in inhalation studies involving aerosols or the use of an emulsifier of uncharacterized biological activity in oral studies, a concurrent negative control group should be utilized. The negative control group should be treated in the same manner as all other test animals except that this control group should not be exposed to either the test substance or any vehicle.

(3) *Dose levels and dose selections.* (i) In chronic toxicity tests, it is necessary to have a dose-response relationship as well as a no-observed-toxic-effect level. Therefore, at least three dose levels should be used in addition to the concurrent control group. Dose levels should be spaced to produce a gradation of effects.

(ii) The high dose level in rodents should elicit some signs of toxicity without causing excessive lethality; for non-rodents, there should be signs of toxicity but there should be no fatalities.

(iii) The lowest dose level should not produce any evidence of toxicity. Where there is a usable estimation of human exposure the lowest dose level should exceed this even though this dose level may result in some signs of toxicity.

(iv) Ideally, the intermediate dose level(s) should produce minimal observable toxic effects. If more than one intermediate dose is used, the dose level should be spaced to produce a gradation of toxic effects.

(v) For rodents, the incidence of fatalities in low and intermediate dose groups and in the controls should be low to permit a meaningful evaluation of the results. For non-rodents, there should be no fatalities.

(4) *Exposure conditions.* The animals are dosed with the test substance ideally on a 7-day per week basis over a period of at least 12 months. However, based primarily on practical considerations, dosing on a 5-day per week basis is considered to be acceptable.

(5) *Observation period.* Duration of observation should be for at least 12 months, and may be concurrent with or subsequent to dosing. If there is a post-exposure observation period, an interim sacrifice should be performed on no fewer than half of the animals of each sex at each dose level immediately upon termination of exposure.

(6) *Administration of the test substance.* The three main routes of administration are oral, dermal, and inhalation. The choice of the route of administration depends upon the physical and chemical characteristics of the test substance and the form typifying exposure in humans.

(i) *Oral studies.* (A) The animals should receive the test substance in their diet, dissolved in drinking water, or given by gavage or capsule for a period of at least 12 months.

(B) If the test substance is administered in the drinking water, or mixed in the diet, exposure is continuous.

(C) For a diet mixture, the highest concentration should not exceed 5 percent.

(ii) *Dermal studies.* (A) The animals are treated by topical application with the test substance, ideally for at least 6 hours per day.

(B) Fur should be clipped from the dorsal area of the trunk of the test animals. Care must be taken to avoid abrading the skin which could alter its permeability.

(C) The test substance should be applied uniformly over a shaved area which is approximately 10 percent of the total body surface area. With highly toxic substances, the surface area covered may be less, but as much of the area should be covered with as thin and uniform a film as possible.

(D) During the exposure period, the test substance may be held if necessary, in contact with the skin with a porous gauze dressing and non-irritating tape. The test site should be further covered in a suitable manner to retain the gauze dressing and test substance and ensure that the animals cannot ingest the test substance.

(iii) *Inhalation studies.* (A) The animals should be tested with inhalation equipment designed to sustain a dynamic air flow of 12 to 15 air changes per hour, ensure an adequate oxygen content of 19 percent and an evenly distributed exposure atmosphere. Where a chamber is used, its design should minimize crowding of the test animals and maximize their exposure to the test substance. This is best accomplished by individual caging. As a general rule to ensure stability of a chamber atmosphere, the total "volume" of the test animals should not exceed 5 percent of the volume of the test chamber. Alternatively, oro-nasal, head-only or whole body individual chamber exposure may be used.

(B) The temperature at which the test is performed should be maintained at 22°C (±2°). Ideally, the relative humidity should be maintained between 40 to 60 percent, but in certain instances (e.g., tests of aerosols, use of water vehicle) this may not be practicable.

(C) Food and water should be withheld during each daily 6 hour exposure period.

(D) A dynamic inhalation system with a suitable analytical concentration control system should be used. The rate of air flow should be adjusted to ensure that conditions throughout the equipment are essentially the same. Maintenance of slight negative pressure inside the chamber will prevent leakage of the test substance into the surrounding areas.

(7) *Observation of animals.* (i) Each animal should be handled and its physical condition appraised at least once each day.

(ii) Additional observations should be made daily with appropriate actions taken to minimize loss of animals to the study (e.g., necropsy or refrigeration of those animals found dead and isolation or sacrific of weak or moribund animals).

(iii) Clinical signs of toxicity including suspected tumors and mortality should be recorded as they are observed, including the time of onset, the degree and duration.

(iv) Cage-side observations should include, but not be limited to, changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern.

(v) Body weights should be recorded individually for all animals once a week during the first 13 weeks of the test period and at least once every 4 weeks thereafter unless signs of clinical toxicity suggest more frequent weighings to facilitate monitoring of health status.

(vi) When the test substance is administered in the food or drinking water, measurements of food or water consumption, respectively, should be determined weekly during the first 13 weeks of the study and then at approximately monthly intervals unless health status or body weight changes dictate otherwise.

(vii) At the end of the study period all survivors should be sacrificed. Moribund animals should be removed and sacrificed when noticed.

(8) *Physical measurements.* For inhalation studies, measurements or monitoring should be made of the following:

(i) The rate of air flow should be monitored continuously, but should be recorded at intervals of at least once every 30 minutes.

(ii) During each exposure period the actual concentrations of the test substance should be held as constant as practicable, monitored continuously and measured at least three times during the test period: at the beginning, at an intermediate time and at the end of the period.

(iii) During the development of the generating system, particle size analysis should be performed to establish the stability of aerosol concentrations. During exposure, analysis should be conducted as often as necessary to determine the consistency of particle size distribution and homogeneity of the exposure stream.

(iv) Temperature and humidity should be monitored continuously, but should be recorded at intervals of at least once every 30 minutes.

(9) *Clinical examinations.* The following examinations should be made on at least 10 rats of each sex per dose and on all non-rodents.

(i) Certain hematology determinations (e.g., hemoglobin content, packed cell volume, total red blood cells, total white blood cells, platelets, or other measures of clotting potential) should be performed at termination and should be performed at 3 months, 6 months and at approximately 6 month intervals thereafter (for studies extending beyond 12 months) on blood samples collected from all non-rodents and from 10 rats per sex of all groups. These collections should be from the same animals at each interval. If clinical observations suggest a deterioration in health of the animals during the study, a differential blood count of the affected animals should be performed. A differential blood count should be performed on samples from those animals in the highest dosage group and the controls. Differential blood counts should be performed for the next lower group(s) if there is a major discrepancy between the highest group and the controls. If hematological effects were noted in the subchronic test, hematological testing should be performed at 3, 6, 12, 18, and 24 months for a two year study and at 3, 6, and 12 months for a 1-year study.

(ii) Certain clinical biochemistry determinations on blood should be carried out at least three times during the test period: just prior to initiation of dosing (base line data), near the middle and at the end of the test period. Blood samples should be drawn for clinical chemistry measurements from all non-rodents and at least ten rodents per sex of all groups; if possible, from the same rodents at each time interval. Test areas which are considered appropriate to all studies: electrolyte balance, carbohydrate metabolism and liver and kidney function. The selection of specific tests will be influenced by observations on the mode of action of the substance and signs of clinical toxicity. Suggested chemical determinations: calcium, phosphorus, chloride, sodium, potassium, fasting glucose (with period of fasting appropriate to the species), serum glutamic-pyruvic transaminase (now known as serum alanine aminotransferase), serum glutamic oxaloacetic transaminase (now known as serum aspartate aminotransferase), ornithine decarboxylase, gamma glutamyl transpeptidase, blood urea nitrogen, albumen, blood creatinine, creatinine phosphokinase, total cholesterol, total bilirubin and total serum protein measurements. Other determinations which may be necessary for an adequate toxicological evaluation include analyses of lipids, hormones, acid/base balance, methemoglobin and cholinesterase activity. Additional clinical biochemistry may be employed where necessary to extend the investigation of observed effects.

(iii) Urine samples from rodents at the same intervals as the hematological examinations under paragraph (b)(9)(i) of this section should be collected for analysis. The following determinations should be made from either individual animals or on a pooled sample/sex/group for rodents: appearance (volume and specific gravity), protein, glucose, ketones, bilirubin, occult blood (semi-quantitatively); and microscopy of sediment (semi-quantitatively).

(iv) Ophthalmological examination, using an ophthalmoscope or equivalent suitable equipment, should be made prior to the administration of the test substance and at the termination of the study. If changes in eyes are detected all animals should be examined.

(10) *Gross necropsy.* (i) A complete gross examination should be performed on all animals, including those which died during the experiment or were killed in moribund conditions.

(ii) The liver, kidneys, adrenals, brain and gonads should be weighed wet, as soon as possible after dissection to avoid drying. For these organs, at least 10 rodents per sex per group and all non-rodents should be weighed.

(iii) The following organs and tissues, or representative samples thereof, should be preserved in a suitable medium for possible future histopathological examination: all gross lesions and tumors; brain—including sections of medulla/pons, cerebellar cortex, and cerebral cortex; pituitary; thyroid/parathyroid; thymus; lungs; trachea; heart; sternum and/or femur with bone marrow; salivary glands; liver; spleen; kidneys; adrenals; esophagus; stomach; duodenum; jejunum; ileum; cecum; colon; rectum; urinary bladder; representative lymph nodes; pancreas; gonads; uterus; accessory genital organs (epididymis, prostate, and, if present, seminal vesicles; female mammary gland; aorta; gall bladder (if present); skin; musculature; peripheral nerve; spinal cord at three levels—cervical, midthoracic, and lumbar; and eyes. In inhalation studies, the entire respiratory tract, including nose, pharynx, larynx, and paranasal sinuses should be examined and preserved. In dermal studies, skin from sites of skin painting should be examined and preserved.

(iv) Inflation of lungs and urinary bladder with a fixative is the optimal method for preservation of these tissues. The proper inflation and fixation of the lungs in inhalation studies is considered essential for appropriate and valid histopathological examination.

(v) If other clinical examinations are carried out, the information obtained from these procedures should be available before microscopic examination, since they may provide significant guidance to the pathologist.

(11) *Histopathology.* (i) The following histopathology should be performed:

(A) Full histopathology on the organs and tissues, listed above, of all non-rodents, of all rodents in the control and high dose groups and of all rodents that died or were killed during the study.

(B) All gross lesions in all animals.

(C) Target organs in all animals.

(D) Lungs, liver and kidneys of all animals. Special attention to examination of the lungs of rodents should be made for evidence of infection since this provides an assessment of the state of health of the animals.

(ii) If excessive early deaths or other problems occur in the high dose group compromising the significance of the data, the next dose level should be examined for complete histopathology.

(iii) In case the results of an experiment give evidence of substantial alteration of the animals' normal longevity or the induction of effects that might affect a toxic response, the next lower dose level should be examined fully, as described under paragraph (b)(11)(i) of this section.

(iv) An attempt should be made to correlate gross observations with microscopic findings.

(c) *Data and reporting*—(1) *Treatment of results.* (i) Data should be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals showing lesions, the types of lesions and the percentage of animals displaying each type of lesion.

(ii) All observed results, quantitative and incidental, should be evaluated by an appropriate statistical method. Any generally accepted statistical methods may be used; the statistical methods should be selected during the design of the study.

(2) *Evaluation of study results.* (i) The findings of a chronic toxicity study should be evaluated in conjunction with the findings of preceding studies and considered in terms of the toxic effects, the necropsy and histopathological findings. The evaluation will include the relationship between the dose of the test substance and the presence, incidence and severity of abnormalities (including behavioral and clinical abnormalities), gross lesions, identified target organs, body weight changes, effects on mortality and any other general or specific toxic effects.

(ii) In any study which demonstrates an absence of toxic effects, further investigation to establish absorption and bioavailability of the test substance should be considered.

(3) *Test report.* (i) In addition to the reporting requirements as specified under 40 CFR Part 792 Subpart J, the following specific information should be reported:

(A) *Group animal data.* Tabulation of toxic response data by species, strain, sex and exposure level for:

(*1*) Number of animals dying.

(*2*) Number of animals showing signs of toxicity.

(*3*) Number of animals exposed.

(B) *Individual animal data.* (*1*) Time of death during the study or whether animals survived to termination.

(*2*) Time of observation of each abnormal sign and its subsequent course.

(*3*) Body weight data.

(*4*) Food and water consumption data, when collected.

(*5*) Results of ophthalmological examination, when performed.

(*6*) Hematological tests employed and all results.

(*7*) Clinical biochemistry tests employed and all results.

(*8*) Necropsy findings.

(*9*) Detailed description of all histopathological findings.

(*10*) Statistical treatment of results, where appropriate.

(ii) In addition, for inhalation studies the following should be reported:

(A) *Test conditions.* (*1*) Description of exposure apparatus including design, type, dimensions, source of air, system for generating particulates and aerosols, method of conditioning air, treatment of exhaust air and the method of housing the animals in a test chamber.

(*2*) The equipment for measuring temperature, humidity, and particulate aerosol concentrations and size should be described.

(B) *Exposure data.* These should be tabulated and presented with mean values and a measure of variability (e.g., standard deviation) and should include:

(*1*) Airflow rates through the inhalation equipment.

(*2*) Temperature and humidity of air.

(*3*) Nominal concentration (total amount of test substance fed into the inhalation equipment divided by volume of air).

(*4*) Actual concentration in test breathing zone.

(*5*) Particle size distribution (e.g., median aerodynamic diameter of particles with standard deviation from the mean).

(d) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Benitz, K.F. "Measurement of Chronic Toxicity," *Methods of Toxicology*. Ed. G.E. Paget. (Oxford: Blackwell Scientific Publications, 1970) pp. 82–131.

(2) D'Aguanno, W. "Drug Safety Evaluation—Pre-Clinical Considerations," *Industrial Pharmacology: Neuroleptics*. Vol. I, Ed. S. Fielding and H. Lal. (Mt. Kisco: Futura Publishing Co. 1974) pp. 317–332.

(3) Fitzhugh, O.G. Third Printing: 1975. "Chronic Oral Toxicity," *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics*. The Association of Food and Drug Officials of the United States (1959, 3rd Printing 1975) pp. 36–45.

(4) Goldenthal, E.I., D'Aguanno, W. "Evaluation of Drugs," *Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics*. The Association of Food and Drug Officials of the United States (1959, 3rd Printing 1975) pp. 60–67.

(5) National Academy of Sciences. "Principles and Procedures for Evaluating the Toxicity of Household Substances," a report prepared by the Committee for the Revision of NAS Publication 1138, under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC (1977).

(6) National Center for Toxicological Research. "Appendix B," *Report of Chronic Studies Task Force Committee, April 13–21, 1972*. (Rockville: National Center for Toxicological Research, 1972).

(7) Page, N.P. "Chronic Toxicity and Carcinogenicity Guidelines," *Journal of Environmental Pathology and Toxicology*, 1:161–182 (1977).

(8) Schwartz, E. "Toxicology of Neuroleptic Agents," *Industrial Pharmacology: Neuroleptics* Ed. S. Fielding and H. Lal. (Mt. Kisco, Futura Publishing Co., 1974) pp. 203–221.

(9) United States Pharmaceutical Manufacturers Association. *Guidelines for the Assessment of Drug and Medical Device Safety in Animals*. (1977).

(10) World Health Organization. "Guidelines for Evaluation of Drugs for Use in Man," *WHO Technical Report Series No. 563*. (Geneva: World Health Organization, 1975).

(11) World Health Organization. "Part I. Environmental Health Criteria 6," *Principles and Methods for Evaluating the Toxicity of Chemicals*. (Geneva: World Health Organization, 1978).

(12) World Health Organization. "Principles for Pre-Clinical Testing of Drug Safety," *WHO Technical Report Series No. 341*. (Geneva: World Health Organization, 1966).

§ 798.3300 Oncogenicity.

(a) *Purpose.* The objective of a long-term oncogenicity study is to observe test animals for a major portion of their life span for the development of neoplastic lesions during or after exposure to various doses of a test substance by an appropriate route of administration.

(b) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* It is recommended that a compound of unknown activity should be tested on two mammalian species. Rats and mice are the species of choice because of their relatively short life spans, the limited cost of their maintenance, their widespread use in pharmacological and toxicological studies, their susceptibility to tumor induction, and the availability of inbred or sufficiently characterized strains. Commonly used laboratory strains should be employed. If other species are used, the tester should provide justification/reasoning for their selection.

(ii) *Age.* (A) Dosing of rodents should begin as soon as possible after weaning, ideally before the animals are 6 weeks old, but in no case more than 8 weeks old.

(B) At commencement of the study, the weight variation of animals used should not exceed ±20 percent of the mean weight for each sex.

(C) Studies using prenatal or neonatal animals may be recommended under special conditions.

(iii) *Sex.* (A) Equal numbers of animals of each sex should be used at each dose level.

(B) The females should be nulliparous and non-pregnant.

(iv) *Numbers.* (A) For rodents, at least 100 animals (50 females and 50 males) should be used at each dose level and concurrent control.

(B) If interim sacrifices are planned the number should be increased by the number of animals scheduled to be sacrificed during the course of the study.

(C) The number of animals at the termination of the study should be adequate for a meaningful and valid statistical evaluation of long term exposure. For a valid interpretation of negative results, it is essential that survival in all groups does not fall below 50 percent at the time of termination.

(2) *Control groups.* (i) A concurrent control group is recommended. This group should be an untreated or sham treated control group or, if a vehicle is used in administering the test substance, a vehicle control group. If the toxic properties of the vehicle are not known or cannot be made available, both untreated and vehicle control groups are recommended.

(ii) In special circumstances such as in inhalation studies involving aerosols or the use of an emulsifier of uncharacterized biological activity in oral studies, a concurrent negative control group should be utilized. The negative control group should be treated in the same manner as all other test animals except that this control group should not be exposed to either the test substance or any vehicle.

(iii) The use of historical control data (i.e., the incidence of tumors and other suspect lesions normally occurring under the same laboratory conditions and in the same strain of animals employed in the test) is desirable for assessing the significance of changes observed in exposed animals.

(3) *Dose levels and dose selection.* (i) For risk assessment purposes, at least three dose levels should be used, in

addition to the concurrent control group. Dose levels should be spaced to produce a gradation of chronic effects.

(ii) The high dose level should elicit signs of minimal toxicity without substantially altering the normal life span.

(iii) The lowest dose should not interfere with normal growth, development and longevity of the animal; and it should not otherwise cause any indication of toxicity. In general, this should not be lower than ten percent of the high dose.

(iv) The intermediate dose(s) should be established in a mid-range between the high and low doses, depending upon the toxicokinetic properties of the chemical, if known.

(v) The selection of these dose levels should be based on existing data, preferably on the results of subchronic studies.

(4) *Exposure conditions.* The animals are dosed with the test substance ideally on a 7 day per week basis over a period of at least 24 months for rats, and 18 months for mice. However, based primarily on practical considerations, dosing on a 5 day per week basis is considered to be acceptable.

(5) *Observations period.* It is necessary that the duration of an oncogenicity test comprise the majority of the normal life span of the strain of animals to be used. This time period should not be less than 24 months for rats and 18 months for mice, and ordinarily not longer than 30 months for rats and 24 months for mice. For longer time periods, and where any other species are used, consultation with the Agency in regard to the duration of the test is advised.

(6) *Administration of the test substance.* The three main routes of administration are oral, dermal, and inhalation. The choice of the route of administration depends upon the physical and chemical characteristics of the test substance and the form typifying exposure in humans.

(i) *Oral studies.* (A) The animals should receive the test substance in their diet, dissolved in drinking water, or given by gavage or capsule for a period of at least 24 months for rats and 18 months for mice.

(B) If the test substance is administered in the drinking water, or mixed in the diet, exposure should be continuous.

(C) For a diet mixture, the highest concentration should not exceed 5 percent.

(ii) *Dermal studies.* (A) The animals are treated by topical application with the test substance, ideally for at least 6 hours per day.

(B) Fur should be clipped from the dorsal area of the trunk of the test animals. Care should be taken to avoid abrading the skin which could alter its permeability.

(C) The test substance should be applied uniformly over a shaved area which is approximately 10 percent of the total body surface area. With highly toxic substances, the surface area covered may be less, but as much of the area should be covered with as thin and uniform a film as possible.

(D) During the exposure period, the test substance may be held, if necessary, in contact with the skin with a porous gauze dressing and non-irritating tape. The test site should be further covered in a suitable manner to retain the gauze dressing and test substance and ensure that the animals cannot ingest the test substance.

(iii) *Inhalation studies.* (A) The animals should be tested with inhalation equipment designed to sustain a dynamic air flow of 12 to 15 air changes per hour, ensure an adequate oxygen content of 19 percent and an evenly distributed exposure atmosphere. Where a chamber is used, its design should minimize crowding of the test animals and maximize their exposure to the test substance. This is best accomplished by individual caging. As a general rule to ensure stability of a chamber atmosphere, the total "volume" of the test animals should not exceed 5 percent of the volume of the test chamber. Alternatively, oro-nasal, head-only, or whole body individual chamber exposure may be used.

(B) The temperature at which the test is performed should be maintained at 22 °C (±2°). Ideally, the relative humidity should be maintained between 40 to 60 percent, but in certain instances (e.g. tests of aerosols, use of water vehicle) this may not be practicable.

(C) Food and water should be withheld during each daily 6-hour exposure period.

(D) A dynamic inhalation system with a suitable analytical concentration control system should be used. The rate of air flow should be adjusted to ensure that conditions throughout the equipment are essentially the same. Maintenance of slight negative pressure inside the chamber will prevent leakage of the test substance into the surrounding areas.

(7) *Observation of animals.* (i) Each animal should be handled and its physical condition appraised at least once each day.

(ii) Additional observations should be made daily with appropriate actions taken to minimize loss of animals to the study (e.g., necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals).

(iii) Clinical signs and mortality should be recorded for all animals. Special attention should be paid to tumor development. The time of onset, location, dimensions, appearance and progression of each grossly visible or palpable tumor should be recorded.

(iv) Body weights should be recorded individually for all animals once a week during the first 13 weeks of the test period and at least once every 4 weeks thereafter unless signs of clinical toxicity suggest more frequent weighings to facilitate monitoring of health status.

(v) When the test substance is administered in the food or drinking water, measurements of food or water consumption, respectively, should be determined weekly during the first 13 weeks of the study and then at approximately monthly intervals unless health status or body weight changes dictate otherwise.

(vi) At the end of the study period all survivors are sacrificed. Moribund animals should be removed and sacrificed when noticed.

(8) *Physical measurements.* For inhalation studies, measurements or monitoring should be made of the following:

(i) The rate of air flow should be monitored continuously, but should be recorded at intervals of at least once every 30 minutes.

(ii) During each exposure period the actual concentrations of the test substance should be held as constant as practicable, monitored continuously and recorded at least three times during the test period: at the beginning, at an intermediate time and at the end of the period.

(iii) During the development of the generating system, particle size analysis should be performed to establish the stability of aerosol concentrations. During exposure, analyses should be conducted as often as necessary to determine the consistency of particle size distribution and homogeneity of the exposure stream.

(iv) Temperature and humidity should be monitored continuously, but shoud be recorded at intervals of at least once every 30 minutes.

(9) *Clinical examinations.* At 12 months, 18 months, and at sacrifice, a blood smear should be obtained from all animals. A differential blood count should be performed on blood smears from those animals in the highest dosage group and the controls. If these data, or data from the pathological examination indicate a need, then the 12- and 18-

month blood smears from other dose levels should also be examined. Differential blood counts should be performed for the next lower group(s) only if there is a major discrepancy between the highest group and the controls. If clinical observations suggest a deterioration in health of the animals during the study, a differential blood count of the affected animals should be performed.

(10) *Gross necropsy.* (i) A complete gross examination should be performed on all animals, including those which died during the experiment or were killed in moribund conditions.

(ii) The following organs and tissues or representative samples thereof, should be preserved in a suitable medium for possible future histopathological examination: all gross lesions and tumors of all animals should be preserved; brain—including sections of medulla/pons, cerebellar cortex and cerebral cortex; pituitary; thyroid/parathyroid; thymus; lungs; trachea; heart; spinal cord at three levels—cervical, midthoracic and lumbar; sternum and/or femur with bone marrow; salivary glands; liver; spleen; kidneys; adrenals; esophagus; stomach; duodenum; jejunum; ileum; cecum; colon; rectum; urinary bladder; representative lymph nodes; pancreas; gonads; uterus; accessory genital organs (epididymis, prostate, and, if present, seminal vesicles); female mammary gland; skin; musculature; peripheral nerve; and eyes. In special studies such as inhalation studies, the entire respiratory tract should be preserved, including nasal cavity, pharynx, larynx and paranasal sinuses. In dermal studies, skin from sites of skin painting should be examined and preserved.

(iii) Inflation of lungs and urinary bladder with a fixative is the optimal method for preservation of these tissues. The proper inflation and fixation of the lungs in inhalation studies is a necessary requirement for appropriate and valid histopathological examination.

(iv) If other clinical examinations are carried out, the information obtained from these procedures should be available before microscopic examination, since they may provide significant guidance to the pathologist.

(11) *Histopathology.* (i) The following histopathology should be performed:

(A) Full histopathology on organs and tissues listed above of all animals in the control and high dose groups and all animals that died or were killed during the study.

(B) All gross lesions in all animals.

(C) Target organs in all animals.

(ii) If a significant difference is observed in hyperplastic, pre-neoplastic or neoplastic lesions between the highest dose and control groups, microscopic examination should be made on that particular organ or tissue of all animals in the study.

(iii) If excessive early deaths or other problems occur in the high dose group, compromising the significance of the data, the next lower dose level should be examined for complete histopathology.

(iv) In case the results of an experiment give evidence of substantial alteration of the animals' normal longevity or the induction of effects that might affect a neoplastic response, the next lower dose level should be examined fully as described in this section.

(v) An attempt should be made to correlate gross observations with microscopic findings.

(c) *Data and reporting*—(1) *Treatment of results.* (i) Data should be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals showing lesions, the types of lesions and the percentage of animals displaying each type of lesion.

(ii) All observed results, quantitative and incidental, should be evaluated by an appropriate statistical method. Any generally accepted statistical method may be used; the statistical methods should be selected during the design of the study.

(2) *Evaluation of study results.* (i) The findings of an oncogenic toxicity study should be evaluated in conjunction with the findings of preceding studies and considered in terms of the toxic effects, the necropsy and histopathological findings. The evaluation should include the relationship between the dose of the test substance and the presence, incidence and severity of abnormalities (including behavioral and clinical abnormalities), gross lesions, identified target organs, body weight changes, effects on mortality and any other general or specific toxic effects.

(ii) In any study which demonstrates an absence of toxic effects, further investigation to establish absorption and bioavailability of the test substance should be considered.

(iii) In order for a negative test to be acceptable, it should meet the following criteria: no more than 10 percent of any group is lost due to autolysis, cannibalism, or management problems; and survival in each group is no less than 50 percent at 18 months for mice and hamsters and at 24 months for rats.

(3) *Test report.* (i) In addition to the reporting requirements as specified under 40 CFR Part 792, Subpart J the following specific information should be reported:

(A) *Group animal data.* Tabulation of toxic response data by species, strain, sex and exposure level for:

(*1*) Number of animals dying.

(*2*) Number of animals showing signs of toxicity.

(*3*) Number of animals exposed.

(B) *Individual animal data.*

(*1*) Time of death during the study or whether animals survived to termination.

(*2*) Time of observation of each abnormal sign and its subsequent course.

(*3*) Body weight data.

(*4*) Food and water consumption data, when collected.

(*5*) Results of ophthalmological examination, when performed.

(*6*) Hematological tests employed and all results.

(*7*) Clinical biochemistry tests employed and all results.

(*8*) Necropsy findings.

(*9*) Detailed description of all histopathological findings.

(*10*) Statistical treatment of results, where appropriate.

(*11*) Historical control data, if taken into account.

(ii) In addition, for inhalation studies the following should be reported:

(A) *Test conditions.* (*1*) Description of exposure apparatus including design, type, dimensions, source of air, system for generating particulates and aerosols, method of conditioning air, treatment of exhaust air and the method of housing the animals in a test chamber.

(*2*) The equipment for measuring temperature, humidity, and particulate aerosol concentrations and size should be described.

(B) *Exposure data.* These should be tabulated and presented with mean values and a measure of variability (e.g., standard deviation) and should include:

(*1*) Airflow rates through the inhalation equipment.

(*2*) Temperature and humidity of air.

(*3*) Nominal concentration (total amount of test substance fed into the inhalation equipment divided by volume of air).

(*4*) Actual concentration in test breathing zone.

(*5*) Particle size distribution (e.g., median aerodynamic diameter of particles with standard deviation from the mean).

(d) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Department of Health and Welfare. *The Testing of Chemicals for Carcinogenicity, Mutagenicity, Teratogenicity.* Minister of Health and Welfare. (Canada: Department of Health and Welfare, 1975).

(2) Food and Drug Administration Advisory Committee on Protocols for Safety Evaluation: Panel on Carcinogenesis. "Report on Cancer Testing in the Safety of Food Additives and Pesticides," *Toxicology and Applied Pharmacology.* 20:419–438 (1971).

(3) International Union Against Cancer. "Carcinogenicity Testing," *IUCC Technical Report Series.* Vol. 2. Ed. I. Berenblum. (Geneva: International Union Against Cancer, 1969).

(4) Leong, B.K.J., Laskin, S. "Number and Species of Experimental Animals for Inhalation Carcinogenicity Studies" Paper presented at Conference on Target Organ Toxicity, September 1975, Cincinnati, Ohio.

(5) National Academy of Sciences. "Principles and Procedures for Evaluating the Toxicity of Household Substances." A report prepared by the Committee for the Revision of NAS Publication 1138, under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC (1977).

(6) National Cancer Institute. *Report of the Subtask Group on Carcinogen Testing to the Interagency Collaborative Group on Environmental Carcinogenesis.* (Bethesda: United States National Cancer Institute, 1976).

(7) National Center for Toxicological Research. "Appendix B," *Report of Chronic Studies Task Force Committee.* April 13–21 (Rockville: National Center for Toxicological Research, 1972).

(8) Page, N.P. "Chronic Toxicity and Carcinogenicity Guidelines," *Journal Environmental Pathology and Toxicology.* 1:161–182 (1977).

(9) Page, N.P. "Concepts of a Bioassay Program in Environmental Carcinogenesis," *Advances in Modern Toxicology Vol. 3).* Ed. Kraybill and Mehlman. (Washington, D.C.: Hemisphere Publishing Corporation, 1977) pp. 87–171.

(10) Sontag, J.M., Page N.P., Saffiotti, U. *Guidelines for Carcinogen Bioassay in Small Rodents.* NCI–CS–TR–1. (Bethesda: United States Cancer Institute, Division of Cancer Control and Prevention, Carcinogenesis Bioassay Program, 1976).

(11) United States Pharmaceutical Manufacturers Association. *Guidelines for the Assessment of Drug and Medical Device Safety in Animals.* (1977).

(12) World Health Organization. "Principles for the Testing and Evaluation of Drugs for Carcinogenicity," *WHO Technical Report Series No. 426.* (Geneva: World Health Organization, 1969).

(13) World Health Organization. "Part I. Environmental Health Criteria 6," *Principles and Methods for Evaluating the Toxicity of Chemicals.* (Geneva: World Health Organization, 1978).

§ 798.3320 Combined Chronic Toxicity/Oncogenicity.

(a) *Purpose.* The objective of a combined chronic toxicity/oncogenicity study is to determine the effects of a substance in a mammalian species following prolonged and repeated exposure. The application of this guideline should generate data which identify the majority of chronic and oncogenic effects and determine dose-response relationships. The design and conduct should allow for the detection of neoplastic effects and a determination of oncogenic potential as well as general toxicity, including neurological, physiological, biochemical, and hematological effects and exposure-related morphological (pathology) effects.

(b) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* Preliminary studies providing data on acute, subchronic, and metabolic responses should have been carried out to permit an appropriate choice of animals (species and strain). As discussed in other guidelines, the mouse and rat have been most widely used for assessment of oncogenic potential, while the rat and dog have been most often studied for chronic toxicity. The rat is the species of choice for combined chronic toxicity and oncogenicity studies. The provisions of this guideline are designed primarily for use with the rat as the test species. If other species are used, the tester should provide justification/reasoning for their selection. The strain selected should be susceptible to the oncogenic or toxic effect of the class of substances being tested, if known, and provided it does not have a spontaneous background too high for meaningful assessment. Commonly used laboratory strains should be employed.

(ii) *Age.* (A) Dosing of rats should begin as soon as possible after weaning, ideally before the rats are 6 weeks old, but in no case more than 8 weeks old.

(B) At commencement of the study, the weight variation of animals used should not exceed ±20 percent of the mean weight for each sex.

(C) Studies using prenatal or neonatal animals may be recommended under special conditions.

(iii) *Sex.* (A) Equal numbers of animals of each sex should be used at each dose level.

(B) The females should be nulliparous and nonpregnant.

(iv) *Numbers.* (A) At least 100 rodents (50 females and 50 males) should be used at each dose level and concurrent control for those groups not intended for early sacrifice. At least 40 rodents (20 females and 20 males) should be used for satellite dose group(s) and the satellite control group. The purpose of the satellite group is to allow for the evaluation of pathology other than neoplasia.

(B) If interim sacrifices are planned, the number of animals should be increased by the number of animals scheduled to be sacrificed during the course of the study.

(C) The number of animals at the termination of each phase of the study should be adequate for a meaningful and valid statistical evaluation of long term exposure. For a valid interpretation of negative results, it is essential that survival in all groups not fall below 50 percent at the time of termination.

(2) *Control groups.* (i) A concurrent control group (50 females and 50 males) and a satellite control group (20 females and 20 males) are recommended. These groups should be untreated or sham treated control groups or, if a vehicle is used in administering the test substance, vehicle control groups. If the toxic properties of the vehicle are not known or cannot be made available, both untreated and vehicle control groups are recommended. Animals in the satellite control group should be sacrificed at the same time the satellite test group is terminated.

(ii) In special circumstances such as inhalation studies involving aerosols or the use of an emulsifier of uncharacterized biological activity in oral studies, a concurrent negative control group should be utilized. The negative control group should be treated in the same manner as all other test animals, except that this control group should not be exposed to the test substance or any vehicle.

(iii) The use of historical control data (i.e., the incidence of tumors and other suspect lesions normally occuring under the same laboratory conditions and in the same strain of animals employed in the test) is desirable for assessing the significance of changes observed in exposed animals.

(3) *Dose levels and dose selection.* (i) For risk assessment purposes, at least three dose levels should be used, in addition to the concurrent control group.

Dose levels should be spaced to produce a gradation of effects.

(ii) The highest dose level in rodents should elicit signs of toxicity without substantially altering the normal life span due to effects other than tumors.

(iii) The lowest dose level should produce no evidence of toxicity. Where there is a usable estimation of human exposure, the lowest dose level should exceed this even though this dose level may result in some signs of toxicity.

(iv) Ideally, the intermediate dose level(s) should produce minimal observable toxic effects. If more than one intermediate dose is used the dose levels should be spaced to produce a gradation of toxic effects.

(v) For rodents, the incidence of fatalities in low and intermediate dose groups and in the controls should be low to permit a meaningful evaluation of the results.

(vi) For chronic toxicological assessment, a high dose treated satellite and a concurrent control satellite group should be included in the study design. The highest dose for satellite animals should be chosen so as to produce frank toxicity, but not excessive lethality, in order to elucidate a chronic toxicological profile of the test substance. If more than one dose level is selected for satellite dose groups, the doses should be spaced to produce a gradation of toxic effects.

(4) *Exposure conditions.* The animals are dosed with the test substance ideally on a 7-day per week basis over a period of at least 24 months for rats, and 18 months for mice and hamsters, except for the animals in the satellite groups which should be dosed for 12 months.

(5) *Observation period.* It is necessary that the duration of the oncogenicity test comprise the majority of the normal life span of the animals to be used. It has been suggested that the duration of the study should be for the entire lifetime of all animals. However, a few animals may greatly exceed the average lifetime and the duration of the study may be unnecessarily extended and complicate the conduct and evaluation of the study. Rather, a finite period covering the majority of the expected life span of the strain is preferred since the probability is high that, for the great majority of chemicals, induced tumors will occur within such an observation period. The following guidelines are recommended:

(i) Generally, the termination of the study should be at 18 months for mice and hamsters and 24 months for rats; however, for certain strains of animals with greater longevity and/or low spontaneous tumor rate, termination should be at 24 months for mice and hamsters and at 30 months for rats. For longer time periods, and where any other species are used, consultation with the Agency in regard to duration of the test is advised.

(ii) However, termination of the study is acceptable when the number of survivors of the lower doses or of the control group reaches 25 percent. In the case where only the high dose group dies prematurely for obvious reasons of toxicity, this should not trigger termination of the study.

(iii) The satellite groups and the concurrent satellite control group should be retained in the study for at least 12 months. These groups should be scheduled for sacrifice for an estimation of test-substance-related pathology uncomplicated by geriatric changes.

(6) *Administration of the test substance.* The three main routes of administration are oral, dermal, and inhalation. The choice of the route of administration depends upon the physical and chemical characteristics of the test substance and the form typifying exposure in humans.

(i) *Oral studies.* (A) The animals should receive the test substance in their diet, dissolved in drinking water, or given by gavage or capsule for a period of at least 24 months for rats and 18 months for mice and hamsters.

(B) If the test substance is administered in the drinking water, or mixed in the diet, exposure is continuous.

(C) For a diet mixture, the highest concentration should not exceed 5 percent.

(ii) *Dermal studies.* (A) The animals are treated by topical application with the test substance, ideally for at least 6 hours per day.

(B) Fur should be clipped from the dorsal area of the trunk of the test animals. Care should be taken to avoid abrading the skin which could alter its permeability.

(C) The test substance should be applied uniformly over a shaved area which is approximately 10 percent of the total body surface area. With highly toxic substances, the surface area covered may be less, but as much of the area as possible should be covered with as thin and uniform a film as possible.

(D) During the exposure period, the test substance may be held, if necessary, in contact with the skin with a porous gauze dressing and nonirritating tape. The test site should be further covered in a suitable manner to retain the gauze dressing and test substance and ensure that the animals cannot ingest the test substance.

(iii) *Inhalation studies.* (A) The animals should be tested with inhalation equipment designed to sustain a dynamic air flow of 12 to 15 air changes per hour, ensure an adequate oxygen content of 19 percent and an evenly distributed exposure atmosphere. Where a chamber is used, its design should minimize crowding of the test animals and maximize their exposure to the test substance. This is best accomplished by individual caging. As a general rule, to ensure stability of a chamber atmosphere, the total "volume" of the test animals should not exceed 5 percent of the volume of the test chamber. Alternatively, oro-nasal, head only, or whole body individual chamber exposure may be used.

(B) The temperature at which the test is performed should be maintained at 22 °C (±2°). Ideally, the relative humidity should be maintained between 40 to 60 percent, but in certain instances (e.g., tests of aerosols, use of water vehicle) this may not be practicable.

(C) Food and water should be withheld during each daily 6-hour exposure period.

(D) A dynamic inhalation system with a suitable analytical concentration control system should be used. The rate of air flow should be adjusted to ensure that conditions throughout the equipment are essentially the same. Maintenance of slight negative pressure inside the chamber will prevent leakage of the test substance into the surrounding areas.

(7) *Observation of animals.* (i) Each animal should be handled and its physical condition appraised at least once each day.

(ii) Additional observations should be made daily with appropriate actions taken to minimize loss of animals to the study (e.g., necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals).

(iii) Clinical signs and mortality should be recorded for all animals. Special attention should be paid to tumor development. The time of onset, location, dimensions, appearance and progression of each grossly visible or palpable tumor should be recorded.

(iv) Body weights should be recorded individually for all animals once a week during the first 13 weeks of the test period and at least once every 4 weeks thereafter, unless signs of clinical toxicity suggest more frequent weighings to facilitate monitoring of health status.

(v) When the test substance is administered in the food or drinking water, measurements of food or water consumption, respectively, should be determined weekly during the first 13 weeks of the study and then at approximately monthly intervals unless health status or body weight changes dictate otherwise.

(vi) At the end of the study period, all survivors are sacrificed. Moribund animals should be removed and sacrificed when noticed.

(8) *Physical measurements.* For inhalation studies, measurements or monitoring should be made of the following:

(i) The rate of airflow should be monitored continuously, but should be recorded at intervals of at least once every 30 minutes.

(ii) During each exposure period the actual concentrations of the test substance should be held as constant as practicable, monitored continuously and recorded at least three times during the test period: at the beginning, at an intermediate time and at the end of the period.

(iii) During the development of the generating system, particle size analysis should be performed to establish the stability of aerosol concentrations. During exposure, analyses should be conducted as often as necessary to determine the consistency of particle size distribution and homogeneity of the exposure stream.

(iv) Temperature and humidity should be monitored continuously, but should be recorded at intervals of at least once every 30 minutes.

(9) *Clinical examinations.* (i) The following examinations should be made on at least 20 rodents of each sex per dose level:

(A) Certain hematology determinations (e.g., hemoglobin content, packed cell volume, total red blood cells, total white blood cells, platelets, or other measures of clotting potential) should be performed at termination and should be performed at 3 months, 6 months and at approximately 6-month intervals thereafter (for those groups on test for longer than 12 months) on blood samples collected from 20 rodents per sex of all groups. These collections should be from the same animals at each interval. If clinical observations suggest a deterioration in health of the animals during the study, a differential blood count of the affected animals should be performed. A differential blood count should be performed on samples from animals in the highest dosage group and the controls. Differential blood counts should be performed for the next lower group(s) if there is a major discrepancy between the highest group and the controls. If hematological effects were noted in the subchronic test, hematological testing should be performed at 3, 6, 12, 18 and 24 months for a year study.

(B) Certain clinical biochemistry determinations on blood should be carried out at least three times during the test period: just prior to initiation of dosing (baseline data), near the middle and at the end of the test period. Blood samples should be drawn for clinical measurements from at least ten rodents per sex of all groups; if possible, from the same rodents at each time interval. Test areas which are considered appropriate to all studies: electrolyte balance, carbohydrate metabolism and liver and kidney function. The selection of specific tests will be influenced by observations on the mode of action of the substance and signs of clinical toxicity. Suggested chemical determinations: calcium, phosphorus, chloride, sodium, potassium, fasting glucose (with period of fasting appropriate to the species), serum glutamic-pyruvic transaminase (now known as serum alanine aminotransferase), serum glutamic oxaloacetic transaminase (now known as serum aspartate aminotransferase), ornithine decarboxylase, gamma glutamyl transpeptidase, blood urea nitrogen, albumen, creatinine phosphokinase, total cholesterol, total bilirubin and total serum protein measurements. Other determinations which may be necessary for an adequate toxicological evaluation include analyses of lipids, hormones, acid/base balance, methemoglobin and cholinesterase activity. Additional clinical biochemistry may be employed where necessary to extend the investigation of observed effects.

(ii) The following should be performed on at least 10 rodents of each sex per dose level:

(A) Urine samples from the same rodents at the same intervals as hematological examination above, should be collected for analysis. The following determinations should be made from either individual animals or on a pooled sample/sex/group for rodents: appearance (volume and specific gravity), protein, glucose, ketones, bilirubin, occult blood (semi-quantitatively) and microscopy of sediment (semi-quantitatively).

(B) Ophthalmological examination, using an ophthalmoscope or equivalent suitable equipment, should be made prior to the administration of the test substance and at the termination of the study. If changes in the eyes are detected, all animals should be examined.

(10) *Gross necropsy.* (i) A complete gross examination should be performed on all animals, including those which died during the experiment or were killed in moribund conditions.

(ii) The liver, kidneys, adrenals, brain and gonads should be weighed wet, as soon as possible after dissection to avoid drying. For these organs, at least 10 rodents per sex per group should be weighed.

(iii) The following organs and tissues, or representative samples thereof, should be preserved in a suitable medium for possible future histopathological examination: all gross lesions and tumors; brain-including sections of medulla/pons, cerebellar cortex, and cerebral cortex; pituitary; thyroid/parathyroid; thymus; lungs; trachea; heart; sternum and/or femur with bone marrow; salivary glands; liver; spleen; kidneys; adrenals; esophagus; stomach; duodenum; jejunum; ileum; cecum; colon; rectum; urinary bladder; representative lymph nodes; pancreas; gonads; uterus; accessory genital organs (epididymis, prostate, and, if present, seminal vesicles); female mammary gland; aorta; gall bladder (if present); skin; musculature; peripheral nerve; spinal cord at three levels—cervical, midthoracic, and lumbar; and eyes. In inhalation studies, the entire respiratory tract, including nose, pharynx, larynx and paranasal sinuses should be examined and preserved. In dermal studies, skin from sites of skin painting should be examined and preserved.

(iv) Inflation of lungs and urinary bladder with a fixative is the optimal method for preservation of these tissues. The proper inflation and fixation of the lungs in inhalation studies is considered essential for appropriate and valid histopathological examination.

(v) If other clinical examinations are carried out, the information obtained from these procedures should be available before microscopic examination, since they may provide significant guidance to the pathologist.

(11) *Histopathology.* (i) The following histopathology should be performed:

(A) Full histopathology on the organs and tissues, listed above, of all non-rodents, of all rodents in the control and high dose groups and of all rodents that died or were killed during the study.

(B) All gross lesions in all animals.

(C) Target organs in all animals.

(D) Lungs, liver and kidneys of all animals. Special attention to examination of the lungs of rodents should be made for evidence of infection since this provides an assessment of the state of health of the animals.

(ii) If excessive early deaths or other problems occur in the high dose group compromising the significance of the data, the next dose level should be examined for complete histopathology.

(iii) In case the results of the experiment give evidence of substantial alteration of the animals' normal longevity or the induction of effects that might affect a toxic response, the next lower dose level should be examined as described above.

(iv) An attempt should be made to correlate gross observations with microscopic findings.

(c) *Data and reporting*—(1) *Treatment of results.* (i) Data should be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals showing lesions, the types of lesions and the percentage of animals displaying each type of lesion.

(ii) All observed results, quantitative and incidental, should be evaluated by an appropriate statistical method. Any generally accepted statistical methods may be used; the statistical methods should be selected during the design of the study.

(2) *Evaluation of study results.* (i) The findings of a combined chronic toxicity/oncogenicity study should be evaluated in conjunction with the findings of preceding studies and considered in terms of the toxic effects, the necropsy and histopathological findings. The evaluation will include the relationship between the dose of the test substance and the presence, incidence and severity of abnormalities (including behavioral and clinical abnormalities), gross lesions, identified target organs, body weight changes, effects on mortality and any other general or specific toxic effects.

(ii) In any study which demonstrates an absence of toxic effects, further investigation to establish absorption and bioavailablity of the test substance should be considered.

(iii) In order for a negative test to be acceptable, it should meet the following criteria: no more than 10 percent of any group is lost due to autolysis, cannibalism, or management problems; and survival in each group is no less than 50 percent at 18 months for mice and hamsters and at 24 months for rats.

(3) *Test report.* (i) In addition to the reporting requirements as specified under 40 CFR Part 792, Subpart J the following specific information should be reported:

(A) *Group animal data.* Tabulation of toxic response data by species, strain, sex and exposure level for:

(*1*) Number of animals dying.

(*2*) Number of animals showing signs of toxicity.

(*3*) Number of animals exposed.

(B) *Individual animal data.* (*1*) Time of death during the study or whether animals survived to termination.

(*2*) Time of observation of each abnormal sign and its subsequent course.

(*3*) Body weight data.

(*4*) Food and water consumption data, when collected.

(*5*) Results of ophthalmological examination, when performed.

(*6*) Hematological tests employed and all results.

(*7*) Clinical biochemistry tests employed and all results.

(*8*) Necropsy findings.

(*9*) Detailed description of all histopathological findings.

(*10*) Statistical treatment of results where appropriate.

(*11*) Historical control data, if taken into account.

(ii) In addition, for inhalation studies the following should be reported:

(A) *Test Conditions.* (*1*) Description of exposure apparatus including design, type, dimensions, source of air, system for generating particulates and aerosols, method of conditioning air, treatment of exhaust air and the method of housing the animals in a test chamber.

(*2*) The equipment for measuring temperature, humidity, and particulate aerosol concentrations and size should be described.

(B) *Exposure data.* These should be tabulated and presented with mean values and a measure of variability (e.g. standard deviation) and should include:

(*1*) Airflow rates through the inhalation equipment.

(*2*) Temperature and humidity of air.

(*3*) Nominal concentration (total amount of test substance fed into the inhalation equipment divided by volume of air).

(*4*) Actual concentration in test breathing zone.

(*5*) Particle size distribution (e.g. median aerodynamic diameter of particles with standard deviation from the mean).

(d) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Benitz, K.F. "Measurement of Chronic Toxicity," *Methods of Toxicology.* Ed. G.E. Paget. (Oxford: Blackwell Scientific Publications, 1970) pp. 82–131.

(2) D'Aguanno, W. "Drug Safety Evaluation—Pre-Clinical Considerations," "*Industrial Pharmacology: Neuroleptics.* Vol. I Ed. S. Fielding and H. Lal. (Mt. Kisco, New York: Futura Publishing Co., 1974) pp. 317–332.

(3) Department of Health and Welfare. *The Testing of Chemicals for Carcinogenicity, Mutagenicity, Teratogenicity. Minister of Health and Welfare. (Canada: Department of Health and Welfare, 1975).*

(4) Fitzhugh, O.G. "Chronic Oral Toxicity," *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics.* The Association of Food and Drug Officials of the United States (1959, 3rd Printing 1975). pp. 36–45.

(5) Food and Drug Administration Advisory Committee on Protocols for Safety Evaluation: Panel on Carcinogenesis. "*Report on Cancer Testing in the Safety of Food Additives and Pesticides,*" *Toxicology and Applied Pharmacology.* 20:419–438 (1971).

(6) Goldenthal, E.I., and D'Aguanno, W. "Evaluation of Drugs," *Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics.* The Association of Food and Drug Officials of the United States (1959, 3rd printing 1975) pp.60–67.

(7) International Union Against Cancer. "Carcinogenicity Testing," *IUCC Technical Report Series* Vol. 2, Ed. I. Berenblum. (Geneva: International Union Against Cancer, 1969).

(8) Leong, B.K.J., and Laskin, S. "Number and Species of Experimental Animals for Inhalation Carcinogenicity Studies," Paper presented at Conference on Target Organ Toxicity. September, 1975, Cincinnati, Ohio.

(9) National Academy of Sciences. "Principles and Procedures for Evaluating the Toxicity of Household Substances," A report prepared by the Committee for the Revision of NAS Publication 1138, under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC (1977).

(10) National Cancer Institute. *Report of the Subtask Group on Carcinogen Testing to the Interagency Collaborative Group on Environmental Carcinogenesis.* (Bethesda: United States National Cancer Institute, 1976).

(11) National Center for Toxicological. *Report of Chronic Studies Task Force Research Committee. "Appendix B, (Rockville: National Center for Toxicological Research, 1972)).*

(12) Page, N.P. "Chronic Toxicity and Carcinogenicity Guidelines," *Journal Environmental Pathology and Toxicology.* 1:161–182 (1977).

(13) Page, N.P. "Concepts of a Bioassay Program in Environmental Carcinogenesis," *Advances in Modern Toxicology* Volume 3, Ed. Kraybill and Mehlman. (Washington, D.C.: Hemisphere Publishing Corp., 1977) pp. 87–171.

(14) Schwartz, E. 1974. "Toxicology of Neuroleptic Agents," *Industrial Pharmacology: Neuroleptics.* Ed. S. Fielding and H. Lal. (Mt. Kisco, New

York: Futura Publishing Co, 1974) pp. 203–221.

(15) Sontag, J.M., Page, N.P., and Saffiotti, U. *Guidelines for Carcinogen Bioassay in Small Rodents.* NCI–CS–TR–1 (Bethesda: United States Cancer Institute, Division of Cancer Control and Prevention, Carcinogenesis Bioassay Program, 1976).

(16) United States Pharmaceutical Manufacturers Association. *Guidelines for the Assessment of Drug and Medical Device Safety in Animals.* (1977).

(17) World Health Organization. "Principles for the Testing and Evaluation of Drugs for Carcinogenicity," *WHO Technical Report Series No. 426.* (Geneva: World Health Organization, 1969).

(18) World Health Organization. "Guidelines for Evaluation of Drugs for Use in Man," *WHO Technical Report Series No. 563.* (Geneva: World Health Organization, 1975).

(19) World Health Organization. "Part I. Environmental Health Criteria 6," *Principles and Methods for Evaluating the Toxicity of Chemicals.* (Geneva: World Health Organization, 1978).

(20) World Health Organization. "Principles for Pre-Clinical Testing of Drug Safety," *WHO Technical Report Series No. 341.* (Geneva: World Health Organization, 1966).

Subpart E—Specific Organ/Tissue Toxicity

§ 798.4100 Dermal sensitization.

(a) *Purpose.* In the assessment and evaluation of the toxic characteristics of a substance, determination of its potential to provoke skin sensitization reactions is important. Information derived from tests for skin sensitization serves to identify the possible hazard to a population repeatedly exposed to a test substance. While the desirability of skin sensitization testing is recognized, there are some real differences of opinion about the best method to use. The test selected should be a reliable screening procedure which should not fail to identify substances with significant allergenic potential, while at the same time avoiding false negative results.

(b) *Definitions.* (1) Skin sensitization (allergic contact dermatitis) is an immunologically mediated cutaneous reaction to a substance. In the human, the responses may be characterized by pruritis, erythema, edema, papules, vesicles, bullae or a combination of these. In other species the reactions may differ and only erythema and edema may be seen.

(2) Induction period is a period of at least 1 week following a sensitization exposure during which a hypersensitive state is developed.

(3) Induction exposure is an experimental exposure of a subject to a test substance with the intention of inducing a hypersensitive state.

(4) Challenge exposure is an experimental exposure of a previously treated subject to a test substance following an induction period, to determine whether the subject will react in a hypersensitive manner.

(c) *Principle of the test method.* Following initial exposure(s) to a test substance, the animals are subsequently subjected, after a period of not less than 1 week, to a challenge exposure with the test substance to establish whether a hypersensitive state has been induced. Sensitization is determined by examining the reaction to the challenge exposure and comparing this reaction to that of the initial induction exposure.

(d) *Test procedures.* (1) Any of the following seven test methods is considered to be acceptable. It is realized, however, that the methods differ in their probability and degree of reaction to sensitizing substances.

(i) Freund's complete adjuvant test.

(ii) Guinea-pig maximization test.

(iii) Split adjuvant technique.

(iv) Buehler test.

(v) Open epicutaneous test.

(vi) Mauer optimization test.

(vii) Footpad technique in guinea pig.

(2) Removal of hair is by clipping, shaving, or possibly by depilation, depending on the test method used.

(3) *Animal selection*—(i) *Species and strain.* The young adult guinea pig is the preferred species. Commonly used laboratory strains should be employed. If other species are used, the tester should provide justification/reasoning for their selection.

(ii) *Number and sex.* (A) The number and sex of animals used will depend on the method employed.

(B) The females should be nulliparous and nonpregnant.

(4) *Control animals.* (i) Periodic use of a positive control substance with an acceptable level of reliability for the test system selected is recommended;

(ii) Animals may act as their own controls or groups of induced animals can be compared to groups which have received only a challenge exposure.

(5) *Dose levels.* The dose level will depend upon the method selected.

(6) *Observation of animals.* (i) Skin reactions should be graded and recorded after the challenge exposures at the time specified by the methodology selected. This is usually at 24, 48, and 72, hours. Additional notations should be made as necessary to fully describe unusual responses;

(ii) Regardless of method selected, initial and terminal body weights should be recorded.

(7) *Procedures.* The procedures to be used are those described by the methodology chosen.

(e) *Data and reporting.* (1) Data should be summarized in tabular form, showing for each individual animal the skin reaction, results of the induction exposure(s) and the challenge exposure(s) at times indicated by the method chosen. As a minimum, the erythema and edema should be graded and any unusual finding should be recorded.

(2) *Evaluation of the results.* The evaluation of results will provide information on the proportion of each group that became sensitized and the extent (slight, moderate, severe) of the sensitization reaction in each individual animal.

(3) *Test report.* In addition to the reporting requirements as specified under 40 CFR Part 792, Subpart J, the following specific information should be reported:

(i) A description of the method used and the commonly accepted name.

(ii) Information on the positive control study, including positive control used, method used, and time conducted.

(iii) The number and sex of the test animals.

(iv) Species and strain.

(v) Individual weights of the animals at the start of the test and at the conclusion of the test.

(vi) A brief description of the grading system.

(vii) Each reading made on each individual animal.

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Buehler, E.V. "Delayed Contact Hypersensitivity in the Guinea Pig," *Archives Dermatology.* 91:171 (1965).

(2) Draize, J.H. "Dermal Toxicity," *Food Drug Cosmetic Law Journal.* 10:722–732 (1955).

(3) Klecak, G. "Identification of Contact Allergens: Predictive Tests in Animals," *Advances in Modern Toxicology: Dermatology and Pharmacology.* Ed. F.N. Marzulli and H.I. Maibach. (Washington, D.C.: Hemisphere Publishing Corp., 1977) 4:305–339).

(4) Klecak, G., Geleick, H., Grey, J.R. "Screening of Fragrance Materials for Allergenicity in the Guinea Pig.–1. Comparison of Four Testing Methods," *Journal of the Society of Cosmetic Chemists.* 28:53–64 (1977).

(5) Magnusson, B., Kligman, A.M. "The Identification of Contact Allergens by Animal Assay," The Guinea Pig Maximization Test. *The Journal of Investigative Dermatology.* 52:268–276 (1973).

(6) Maguire, H.C. "The Bioassay of Contact Allergens in the Guinea Pig" *Journal of the Society of Cosmetic Chemists.* 24:151–162 (1973).

(7) Maurer, T., Thomann, P., Weirich, E.G., Hess, R. "The Optimization Test in the Guinea Pig. A Method for the Predictive Evaluation of the Contact Allergenicity of Chemicals," *Agents and Actions.* (Basel: Birkhauser Verlag, 1975) Vol. 5/2.

(8) Maurer, T., Thomann, P., Weirich, E.G., Hess, R. "The Optimization Test in the Guinea Pig: A Method for the Predictive Evaluation of the Contact Allergenicity of Chemicals," *International Congress Series Excerpta Medica No. 376,* (1975) Vol. 203.

§ 798.4350 Inhalation developmental toxicity study.

(a) *Purpose.* In the assessment and evaluation of the toxic characteristics of an inhalable material such as a gas, volatile substance, or aerosol/particulate, determination of the potential developmental toxicity is important. The inhalation developmental toxicity study is designed to provide information on the potential hazard to the unborn which may arise from exposure of the mother during pregnancy.

(b) *Definitions.* (1) Developmental toxicity is the property of a chemical that causes in utero death, structural or functional abnormalities or growth retardation during the period of development.

(2) "Aerodynamic diameter" applies to the behavioral size of particles of aerosols. It is the diameter of a sphere of unit density which behaves aerodynamically like the particles of the test substance. It is used to compare particles of different sizes, shapes, and densities and to predict where in the respiratory tract such particles may be deposited. This term is used in contrast to "optical," "measured" or "geometric" diameters which are representation of actual diameters which in themselves cannot be related to deposition within the respiratory tract.

(3) "Geometric mean diameter" or "median diameter" is the calculated aerodynamic diameter which divides the particles of an aerosol in half based on the weight of the particles. Fifty percent of the particles by weight will be larger than the median diameter and 50 percent of the particles will be smaller than the median diameter. The median diameter and its geometeric standard deviation are used to statistically describe the particle size distribution of any aerosol based on the weight and size of the particles.

(4) "Inhalable diameter" refers to that aerodynamic diameter of a particle which is considered to be inhalable for the organism. It is used to refer to particles which are capable of being inhaled and may be deposited anywhere within the respiratory tract from the trachea to the deep lung (the alveoli). For man, the inhalable diameter is considered here as 15 micrometers or less.

(5) Concentration refers to an exposure level. Exposure is expressed as weight or volume of test substance per volume of air (mg/1), or as parts per million (ppm).

(6) No-observed-effect level is the maximum concentration in a test which produces no observed adverse effects. A no-observed-effect level is expressed in terms of weight or volume of test substance given daily per unit volume of air.

(c) *Principle of the test method.* The test substance is administered in graduated concentrations, for at least that part of the pregnancy covering the major period of organogenesis, to several groups of pregnant experimental animals, one exposure level being used per group. Shortly before the expected date of delivery, the pregnant females are sacrificed, the uteri removed, and the contents examined for embryonic or fetal deaths, and live fetuses.

(d) *Limit test.* If a test at an exposure of 5 mg/1 (actual concentration of respirable substances) or, where this is not possible due to physical or chemical properties of the test substance, the maximum attainable concentration, produces no observable developmental toxicity, then a full study using three exposure levels might not be necessary.

(e) *Test procedures*— (1) *Animal selection*—(i) *Species and strain.* Testing should be performed in at least two mamalian species. Commonly used species include the rat, mouse, rabbit, and hamster. If other mamalian species are used, the tester should provide justification/reasoning for their selection. Commonly used laboratory strains should be employed. The strain should not have low fecundity and should preferably be characterized for its sensitivity to developmental toxins.

(ii) *Age.* Young adult animals (nulliparous females) should be used.

(iii) *Sex.* Pregnant female animals should be used at each exposure level.

(iv) *Number of animals.* At least 20 pregnant rats, mice, or hamsters or 12 pregnant rabbits are recommended at each exposure level. The objective is to ensure that sufficient pups are produced to permit meaningful evaluation of the potential developmental toxicity of the test substance.

(2) *Control group.* A concurrent control group should be used. This group should be exposed to clean, filtered air under conditions identical to those used for the group exposed to the substance of interest. In addition, a vehicle-exposed group may be necessary when the substance under study requires a vehicle for delivery. It is recommended that during preliminary range finding studies, air vs. vehicle exposure be compared. If there is no substantial difference, air exposure itself would be an appropriate control. If vehicle and air exposure yield different results, both vehicle and air exposed control groups are recommended.

(3) *Concentration levels and concentration selection.* (i) At least three concentration levels with a control and, where appropriate, a vehicle control, should be used.

(ii) The vehicle should neither be developmentally toxic nor have effects on reproduction.

(iii) To select the appropriate concentration levels, a pilot or trial study may be advisable. Since pregnant animals have an increased minute ventilation as compared to non-pregnant animals, it is recommended that the trial study be conducted in pregnant animals. Similarly, since presumably the minute ventilation will vary with progression of pregnancy, the animals should be exposed during the same period of gestation as in the main study. In the trial study, the concentration producing embryonic or fetal lethalities or maternal toxicity should be determined.

(iv) Unless limited by the physical/chemical nature or biological properties of the substance, the highest concentration level should induce some overt maternal toxicity such as slight weight loss, but not more than 10 percent maternal deaths.

(v) The lowest concentration level should not produce any grossly observable evidence of either maternal or developmental toxicity.

(vi) Ideally, the intermediate concentration level(s) should produce minimal observable toxic effects. If more than one intermediate concentration is used, the concentration levels should be spaced to produce a gradation of toxic effects.

(4) *Exposure duration.* The duration of exposure should be at least six hours daily allowing appropriate additional time for chamber equilibrium.

(5) *Observation period.* Day 0 in the test is the day on which a vaginal plug and/or sperm are observed. The exposure period should cover the period of major organogenesis. This may be taken as days 6 to 15 for rat and mouse, 6 to 14 for hamster, or 6 to 18 for rabbit.

(6) *Inhalation exposure.* (i)(A) The animals should be tested in inhalation equipment designed to sustain a dynamic air flow of 12 to 15 air changes per hour and ensure an adequate oxygen content of 19 percent and an evenly distributed exposure atmosphere. Where a chamber is used, its design should minimize crowding of the test animals and maximize their exposure to the test substance. This is best accomplished by individual caging. As a general rule, to ensure stability of a chamber atmosphere, the total "volume" of the test animals should not exceed 5 percent of the volume of the test chamber.

(B) Pregnant animals should be subjected to the minimum amount of stress. Since whole-body exposure appears to be the least stressful mode of exposure it is the method preferred. In general, oro-nasal or head-only exposure, which is sometimes used to avoid concurrent exposure by the dermal or oral routes, is not recommended because of the associated stress accompanying the restraining of the animals. However, there may be specific instances where it may be more appropriate than whole-body exposure. The tester should provide justification/reasoning for its selection.

(ii) A dynamic inhalation system with a suitable analytical concentration control system should be used. The rate of air flow should be adjusted to ensure that conditions throughout the equipment exposure chamber are essentially the same. Maintenance of slight negative pressure inside the chamber will prevent leakage of the test substance into the surrounding areas.

(iii) The temperature at which the test is performed should be maintained at 22 °C (±2°) for rodents or 20 °C (±3°) for rabbits. Ideally, the relative humidity should be maintained between 40 to 60 percent, but in certain instances (e.g., tests of aerosols, use of water vehicle) this may not be practicable.

(7) *Physical measurements.* Measurements or monitoring should be made of the following:

(i) The rate of airflow should be monitored continuously but should be recorded at least every 30 minutes.

(ii) The actual concentrations of the test substance should be measured in the breathing zone. During the exposure period the actual concentrations of the test substance should be held as constant as practicable, monitored continuously and measured at least at the beginning, at an intermediate time and at the end of the exposure period.

(iii) During the development of the generating system, particle size analysis should be performed to establish the stability of aerosol concentrations. During exposure, analysis should be conducted as often as necessary to determine the consistency of particle size distribution.

(iv) Temperature and humidity should be monitored continuously but should be recorded at least every 30 minutes.

(8) *Food and water during exposure period.* Food should be withheld during exposure. Water may or may not be withheld. If it is not withheld it should not come in direct contact with the test atmospheres.

(9) *Observation of animals.* (i) A gross examination should be made at least once each day.

(ii) Additional observations should be made daily with appropriate actions taken to minimize loss of animals to the study (e.g., necropsy or refrigeration of animals found dead and isolation or sacrifice of weak or moribund animals).

(iii) Signs of toxicity should be recorded as they are observed, including the time of onset, the degree and duration.

(iv) During the treatment and observation periods, cage-side observations should include, but not be limited to: changes in skin and fur, eye and mucous membranes, as well as respiratory, autonomic and central nervous systems, somatomotor activity and behavioral pattern. Particular attention should be directed to observation of tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma.

(v) Measurements should be made weekly of food consumption for all animals in the study.

(vi) Animals should be weighed at least weekly.

(vii) Females showing signs of abortion or premature delivery should be sacrificed and subjected to a thorough macroscopic examination.

(10) *Gross necropsy.* (i) At the time of sacrifice or death during the study, the dam should be examined macroscopically for any structural abnormalities or pathological changes which may have influenced the pregnancy.

(ii) Immediately after sacrifice or death, the uterus should be removed, weighed, and the contents examined for embryonic or fetal deaths and the number of viable fetuses. The degree of resorption should be described in order to help estimate the relative time of death.

(iii) The number of corpora lutea should be determined for all species except mice.

(iv) The sex of the fetuses should be determined and they should be weighed individually, the weights recorded, and the mean fetal weight derived.

(v) Following removal, each fetus should be examined externally.

(vi) For rats, mice and hamsters, one-third to one-half of each litter should be prepared and examined for skeletal anomalies, and the remaining part of each litter should be prepared and examined for soft tissue anomalies using appropriate methods.

(vii) For rabbits, each fetus should be examined by careful dissection for visceral anomalies and then examined for skeletal anomalies.

(f) *Data and reporting*—(1) *Treatment of results.* Data should be summarized in tabular form, showing for each test group: the number of animals at the start of the test, the number of pregnant animals, the number and percentages of live fetuses and the number of fetuses with any soft tissue or skeletal abnormalities.

(2) *Evaluation of results.* The findings of a developmental toxicity study should be evaluated in terms of the observed effects and the exposure levels producing effects. It is necessary to consider the historical developmental toxicity data on the species/strain tested. A properly conducted developmental toxicity study should provide a satisfactory estimation of a no-effect level.

(3) *Test report.* In addition to the reporting requirements as specified under 40 CFR Part 792, Subpart J, the following specific information should be reported:

(i) *Test conditions.* (A) Description of exposure apparatus including design, type, dimensions, source of air, system for generating particulates and aerosols, methods of conditioning air, and the method of housing the animals in a test chamber when this apparatus is used.

(B) The equipment for measuring temperature, humidity, and particulate aerosol concentrations and size shall be described.

(ii) *Exposure data.* These shall be tabulated and presented with mean values and a measure of variability (e.g., standard deviation) and should include:

(A) Airflow rates through the inhalation equipment.

(B) Temperature of air.

(C) Nominal concentration—total amount of test substance fed into the inhalation equipment divided by volume of air (no standard deviation).

(D) Measured total concentrations (particulate and/or gaseous phases) in test breathing zone.

(E) Particle size distribution (e.g., median aerodynamic diameter of particles with geometric standard deviation) including estimates of the percents of inhalable and non-inhalable portions for the test animals.

(iii) *Animal data.* (A) Toxic response data by concentration.

(B) Species and strain.

(C) Time of death during the study or whether animals survived to termination.

(D) Time of onset and duration of each abnormal sign and its subsequent course.

(E) Food, body weight and uterine weight data.

(F) Pregnancy and litter data.

(G) Fetal data (live/dead, sex, soft tissue and sketetal defects, resorptions).

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Department of Health and Welfare. *The Testing of Chemicals for Carcinogenicity, Mutagenicity and Teratogenicity.* Minister of Health and Welfare (Canada: Department of Health and Welfare, 1975).

(2) National Academy of Sciences. "Principles and Procedures for Evaluating the Toxicity of Household Substances." A report prepared by the Committee for the Revision of NAS Publication 1138, under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC (1977).

(3) World Health Organization. *Principles for the Testing of Drugs for Teratogenicity.* WHO Technical Report Series No. 364. (Geneva: World Health Organization, 1967).

§ 798.4420 Preliminary developmental toxicity screen.

(a) *Purpose.* The *in vivo* developmental toxicity assay is designed to assess the potential of agents to induce toxic effects in the conceptus after administration to the mother during pregnancy. This test should be used only to prioritize environmental agents for testing by more rigorous standard protocols.

(b) *Definitions.* Development toxicity is the capability of an agent to induce *in utero* death, structural or functional abnormalities, or growth retardation after contact with the pregnant animal.

(c) *Principle of the test method.* The test substance is administered to pregnant animals during a significant portion of the major period of organogenesis. A single dose level is administered. This dose level should be high enough to elicit significant maternal toxicity. The dams are allowed to give birth and the neonates are counted and weighed on days 1 and 3 postpartum (day 1 is the day after birth). The underlying hypothesis for this assay is that most prenatal insults will manifest themselves as reduced viability and/or growth during the postnatal period.

(d) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* Testing must be performed in a mammalian species and strain which will allow human handling of newborn pups without cannibalization or abandonment. The preferred species would be either rat or mouse, and of necessity, a strain that does not exhibit the behavior referred to above. The strain should be commonly used and should not have low fecundity.

(ii) *Age.* Young adult animals (nulliparous females) should be used.

(iii) *Sex.* Pregnant female animals should be used.

(iv) *Number of animals.* At least 30 pregnant animals should be used for each compound. The objective is to ensure that sufficient litters are produced to permit meaningful evaluation of the potential developmental toxicity of the test substance.

(2) *Control group.* A concurrent control group should be used. This group should be an untreated or sham treated control group, or, if a vehicle is used in administering the test substance, a vehicle control group. Except for treatment with the test substance, animals in the control group(s) should be handled in an identical manner to test group animals.

(3) *Dose levels and dose selection.* (i) A single dose level with a concurrent control and, when appropriate, a vehicle control, should be used.

(ii) The vehicle should be neither developmentally toxic nor have effects on reproduction.

(iii) To select the appropriate dose levels, a pilot or trial study may be advisable. It is not always necessary to carry out a trial study in pregnant animals. Comparison of the results from a trial study in non-pregnant, and the main study in pregnant animals will demonstrate whether or not the test substance is more toxic in pregnant animals.

(iv) Unless limited by the physical/chemical nature or biological properties of the substance, the dose level used should be:

(A) high enough to cause overt maternal toxicity as evidenced by significant death, weight loss or neurotoxic manifestations, or

(B) 10 gm/kg, if lower dose levels fail to induce maternal toxicity.

(4) *Observation period.* Day 0 in the test is the day in which a vaginal plug and/or sperm are observed. The dose period should encompass a significant portion of the period of major organogenesis. This may be taken as days 7–11 for rat and mouse. When there is evidence of rapid clearance it may be advisable to extend the dosing period for 2 days to cover the critical period of palatal closure.

(5) *Administration of test substance.* The test substance or vehicle is usually administered orally, by intubation unless the chemical or physical characteristics of the test substance or pattern of human exposure suggest a more appropriate route of administration. The test substance should be administered at the same time each day.

(6) *Exposure conditions.* The female test animals are treated with the test substance daily throughout the appropriate treatment period. When given by gavage, the dose may be based on the weight of the females at the start of substance administration, or, alternatively, in view of the rapid weight gain which takes place during pregnancy, the animals may be weighed periodically and the dosage based on the most recent weight determination.

(7) *Observation of pregnant animals.* (i) A gross examination of the dams should be made at least once prior to parturition.

(ii) Pregnant animals should be weighed the day prior to the beginning of treatment, and that day on which treatment ends.

(iii) During the treatment and observation periods, cage-side observations should include, but not be limited to: change in skin and fur, eye and mucous membranes, as well as respiratory, autonomic and central nervous systems, somatomotor activity and behavioral pattern.

(iv) Signs of toxicity should be recorded as they are observed, including the time of onset, the degree and duration.

(v) During the dosing period females that die or are sacrificed because they are moribund should be examined for signs of pregnancy and details of the conditions of the uterus and/or its contents recorded. Animals that have not delivered two days after expected date of parturition should be sacrificed and similar examinations made.

(8) *Observation of dams after birth.* Dams should be observed for signs of overt toxicity during the postpartum

period at the same time neonatal examinations are being made.

(9) *Neonatal examinations.* (i) Dams are allowed to give birth and the litters are examined for gross anomalies and presence of milk, counted, and weighed on postpartum days 1 and 3.

(ii) Dead pups should be necropsied and abnormalities noted.

(iii) For those compounds that induce only neonatal growth reduction it may be advisable to normalize litter size on postpartum day 3 (to approximately four females and four males) and leave them with the dam through weaning. This procedure will determine if the growth reduction is transient or if it represents a permanent functional alteration.

(e) *Data and reporting*—(1) *Treatment of results.* Data should be summarized in tabular form, showing for each test group: the number of animals at the start of the test, the number of pregnant animals, the maternal weight during the treatment period, the average number of live neonates on days 1 and 3, the average neonatal weight on days 1 and 3, and the average weight gained during that period.

(2) *Evaluation of results.* The findings of this bioassay should be evaluated in terms of the types of effects noted. All data analyses should compare treatment groups and their concurrent controls. Statistical treatment of the results should involve analysis of variance, and the number of live pups on days 1 and 3 should be used as a covariate in the analyses of postnatal body weight so as to correct for differences in pup weights due to litter size. The number of animals going to term must be sufficiently large to allow for a reasonable detection of compound-induced deficiencies. Conditions which significantly reduce the number of dams going to term (e.g. lack of pregnancy or compound-induced maternal death) should lead to a repeat of the study.

(3) *Test report.* In addition to the reporting requirements as specified under 40 CFR Part 792, Subpart J the following specific information should be reported:

(i) Toxic response data.

(ii) Species and strain.

(iii) Time of maternal death during the study or whether animals survived to termination.

(iv) Time of onset and duration of each abnormal sign and its subsequent course.

(v) Pregnancy data.

(vi) Litter data including number live and dead; and average litter weight on days 1 and 3 postpartum.

(vii) Necropsy data on dead pups.

(f) *References.* For additional background information on this test guideline the following references should be consulted.

(1) Chernoff, N. and Kavlock, R. "An *in vivo* teratology screen utilizing pregnant mice," *Journal of Toxicology and Environmental Health* 10: 541–550 (1982).

(2) Doe, J. E., Samuels, D. M., Tinston, D. J. and De Silva Wickramaratne, G. A. "Comparative aspects of the reproductive toxicology by inhalation in rats of ethylene glycol monomethyl ether and propylene glycol monomethyl ether," *Toxicology and Applied Pharmacology.* 69(1): 43–47 (1983).

§ 798.4470 Primary dermal irritation.

(a) *Purpose.* In the assessment and evaluation of the toxic characteristics of a substance, determination of the irritant and/or corrosive effects on skin of mammals is an important initial step. Information derived from this test serves to indicate the existence of possible hazards likely to arise from exposure of the skin to the test substance.

(b) *Definitions.* (1) Dermal irritation is the production of reversible inflammatory changes in the skin following the application of a test substance.

(2) Dermal corrosion is the production of irreversible tissue damage in the skin following the application of the test substance.

(c) *Principle of the test methods.* (1) The substance to be tested is applied in a single dose to the skin of several experimental animals, each animal serving as its own control. The degree of irritation is read and scored at specified intervals and is further described to provide a complete evaluation of the effects. The duration of the study should be sufficient to permit a full evaluation of the reversibility or irreversibility of the effects observed but need not exceed 14 days.

(2) When testing solids (which may be pulverized if considered necessary), the test substance should be moistened sufficiently with water or, where necessary, a suitable vehicle, to ensure good contact with the skin. When vehicles are used, the influence of the vehicle on irritation of skin by the test substance should be taken into account. Liquid test substances are generally used undiluted.

(3) Strongly acidic or alkaline substances, for example with a demonstrated pH of 2 or less, or 11.5 or greater, need not be tested for primary dermal irritation, owing to their predictable corrosive properties.

(4) The testing of materials which have been shown to be highly toxic by the dermal route is unnecessary.

(d) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* The albino rabbit is recommended as the preferred species. If another mammalian species is used, the tester should provide justification/reasoning for its selection.

(ii) *Number of animals.* At least 6 healthy adult animals should be used unless, justification/reasoning for using fewer animals is provided.

(2) *Control animals.* Separate animals are not recommended for an untreated control group. Adjacent areas of untreated skin of each animal may serve as a control for the test.

(3) *Dose level.* A dose of 0.5 ml of liquid or 500 mg of solid or semi-solid is applied to the test site.

(4) *Preparation of animals' skins.* Approximately 24 hours before the test, fur should be removed from the test area by clipping or shaving from the dorsal area of the trunk of the animals. Care should be taken to avoid abrading the skin. Only animals with healthy intact skin should be used.

(5) *Application of the test substance.* (i) The recommended exposure duration is 4-hours. Longer exposure may be indicated under certain conditions (e.g. expected pattern of human use and exposure). At the end of the exposure period, residual test substance should generally be removed, where practicable, using water or an appropriate solvent, without altering the existing response or the integrity of the epidermis.

(ii) The test substance should be applied to a small area (approximately 6 cm^2) of skin and covered with a gauze patch, which is held in place with non-irritating tape. In the case of liquids or some pastes, it may be necessary to apply the test substance to the gauze patch and then apply that to the skin. The patch should be loosely held in contact with the skin by means of a suitable semiocclusive dressing for the duration of the exposure period. However, the use of an occlusive dressing may be consided appropriate in some cases. Access by the animal to the patch and resultant ingestion/inhalation of the test substance should be prevented.

(6) *Observation period.* The duration of the observation period should be at least 72 hours, but should not be rigidly fixed. It should be sufficient to fully evaluate the reversibility or irreversiblity of the effects observed. It need not exceed 14 days after application.

(7) *Clinical examination and scoring.* (i) After removal of the patch, animals should be examined for signs of

erythema and edema and the responses scored within 30–60 minutes, and then at 24, 48 and 72 hours after patch removal.

(ii) Dermal irritation should be scored and recorded according to the grades in the following Table 1. Further observations may be needed, as necessary, to establish reversibility. In addition to the observation of irritation, any lesions and other toxic effects should be fully described.

TABLE 1—EVALUATION OF SKIN REACTION

Formation	Value
Erythema and Eschar Formation:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Maximum possible	4
Edema Formation:	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 millimeter)	3
Severe edema (raised more than 1 millimeter and extending beyond area of exposure	4
Maximum possible	4

(e) *Data and reporting*—(1) *Data summary.* Data should be summarized in tabular form, showing for each individual animal the irritation scores for erythema and edema at 30 to 60 minutes, 24, 48 and 72 hours after patch removal, any lesions, a description of the degree and nature of irritation, corrosion or reversibility, and any other toxic effects observed.

(2) *Evaluation of results.* The dermal irritation scores should be evaluated in conjunction with the nature and reversibility or otherwise of the responses observed. The individual scores do not represent an absolute standard for the irritant properties of a material. They should be viewed as reference values which are only meaningful when supported by a full description and evaluation of the observations. The use of an occlusive dressing is a severe test and the results are relevant to very few likely human exposure conditions.

(3) *Test report.* In addition to the reporting recommendations as specified under 40 CFR Part 792, Subpart J, the following specific information should be reported:

(i) Physical nature and where appropriate, concentration, and pH value for the test substance.

(ii) Species and strain.

(iii) Tabulation of irritation response data for each individual animal for each observation time period (e.g. 30 to 60 minutes, 24, 48, and 72 hours after patch removal).

(iv) Description of any lesions observed.

(v) Narrative description of the degree and nature of irritation observed.

(vi) Description of any toxic effects other than dermal irritation.

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Draize, J.H. "Dermal Toxicity," *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics.* Association of Food and Drug Officials of the United States (1959, 3rd printing 1975) pp. 46–59.

(2) Draize, J.H. Woodward, G., Calvery, H.O. "Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes" *Journal of Pharmacology and Experimental Therapeutics.* 83:377–390 (1944).

(3) Marzulli, F.N., Maibach, H.I. "Dermatotoxicology and Pharmacology," *Advances in Modern Toxicology.* Vol. 4. (New York: Hemisphere Publishing Corp., 1977).

(4) National Academy of Sciences. "Principles and Procedures for Evaluating the Toxicity of Household Substances," A report prepared by the Committee for the Revision of NAS Publication 1138, Under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC, (1978).

(5) World Health Organization. "Part I. Environmental Health Criteria 6," *Principles and Methods for Evaluating the Toxicity of Chemicals.* (Geneva: World Health Organization, 1978).

§ 798.4500 Primary eye irritation.

(a) *Purpose.* In the assessment and evaluation of the toxic characteristics of a substance, determination of the irritant and/or corrosive effects on eyes of mammals is an important initial step. Information derived from this test serves to indicate the existence of possible hazards likely to arise from exposure of the eyes and associated mucous membranes to the test substance.

(b) *Definitions*—(1) *Eye irritation.* The production of reversible changes in the eye following the application of a test substance to the anterior surface of the eye.

(2) *Eye corrosion.* The production of irreversible tissue damage in the eye following application of a test substance to the anterior surface of the eye.

(c) *Principle of the test method.* (1) The substance to be tested is applied in a single dose to one of the eyes in each of several experimental animals; the untreated eye is used to provide control information. The degree of irritation/corrosion is evaluated and scored at specified intervals and is fully described to provide a complete evaluation of the effects. The duration of the study should be sufficient to permit a full evaluation of the reversibility or irreversibility of the effects observed but need not exceed 21 days.

(2) Strongly acidic or alkaline substances, for example, with a demonstrated pH of 2 or less, or 11.5 or greater, need not be tested owing to their predictable corrosive properties.

(3) Materials which have demonstrated definite corrosion or severe irritation in a dermal study need not be further tested for eye irritation. It may be presumed that such substances will produce similarly severe effects in the eyes.

(d) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* A variety of experimental animals have been used, but it is recommended that testing should be performed using healthy adult albino rabbits. Commonly used laboratory strains should be used. If another mammalian species is used, the tester should provide justification/reasoning for its selection.

(ii) *Number of animals.* At least six animals should be used, unless justification/reasoning for using fewer animals is provided.

(2) *Dose level.* For testing liquids, a dose of 0.1 ml is recommended. In testing solids, pastes, and particulate substances, the amount used should have a volume of 0.1 ml, or a weight of not more than 100 mg (the weight must always be recorded). If the test material is solid or granular, it should be ground to a fine dust. The volume of particulates should be measured after gently compacting them (e.g. by tapping the measuring container). To test a substance contained in a pressurized aerosol container, the eye should be held open and the test substance administered in a single burst of about one second from a distance of 10 cm directly in front of the eye. The dose may be estimated by weighing the container before and after use. Care should be taken not to damage the eye. Pump sprays should not be used but instead the liquid should be expelled and 0.1 ml collected and instilled into the eye as described for liquids.

(3) *Examination of eyes prior to test.* Both eyes of each experimental animal provisionally selected for testing should

be examined within 24 hours before testing starts by the same procedure to be used during the test examination. Animals showing eye irritation, ocular defects or preexisting corneal injury should not be used.

(4) *Application of the test substance.* (i) The test substance should be placed in the conjunctival sac of one eye of each animal after gently pulling the lower lid away from the eyeball. The lids are then gently held together for about one second in order to limit loss of the material. The other eye, which remains untreated, serves as a control. If it is thought that the substance may cause extreme pain, local anesthetic may be used prior to instillation of the test substance. The type and concentration of the local anesthetic should be carefully selected to ensure that no significant differences in reaction to the test substance will result from its use. The control eye should be similarly anesthetized.

(ii) The eyes of the test animals should not be washed out for 24 hours following instillation of the test substance. At 24 hours, a washout may be used if considered appropriate.

(5) *Observation period.* The duration of the observation period is at least 72 hours, but should not be fixed rigidly. It should be sufficient to evaluate fully the reversibility or irreversibility of the effects observed. It normally need not exceed 21 days after instillation.

(6) *Clinical examination and scoring.* (i) The eyes should be examined at 1, 24, 48, and 72 hours. If there is no evidence of irritation at 72 hours, the study may be ended. Extended observation may be necessary if there is persistent corneal involvement or other ocular irritation in order to determine the progress of the lesions and their reversibility or irreversibility. In addition to the observations of the cornea, iris and conjunctivae, any other lesions which are noted should be recorded and reported. The grades of ocular reaction using the following Table I should be recorded at each examination.

Table I—Grades for Ocular Lesions

Cornea	
Opacity: degree of density (area most dense taken for reading). No ulceration or opacity	0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible	[1]1
Easily discernible translucent area, details of iris slightly obscured	[1]2
Nacrous area, no details or iris visible, size of pupil barely discernible	[1]3
Opaque cornea, iris not discernible through the opacity	[1]4
Iris	
Normal	0
Markedly deepened rugae, congestion, swelling moderate circumcorneal hyperemia, or injection, any of these or combination of any thereof, iris still reacting to light (sluggish reaction is positive)	[1]1
No reaction to light, hemorrhage, gross destruction (any or all of these)	[1]2
Conjunctivae	
Redness (refers to palpebral and bulbar conjunctivae, cornea and iris).	
Blood vessels normal	0
Some blood vessels definitely hyperemic (injected)	1
Diffuse, crimson color, individual vessels not easily discernible	[1]2
Diffuse beefy red chemosis: lids and/or nictitating membranes	[1]3
No swelling	0
Any swelling above normal (includes nictitating membranes)	1
Obvious swelling with partial eversion of lids	[1]2
Swelling with lids about half closed	[1]3
Swelling with lids more than half closed	[1]4

[1] Indicates positive effect.

(ii) Examination of reactions can be facilitated by use of a binocular loupe, hand slit-lamp, biomicroscope, or other suitable device. After recording the observations at 24 hours, the eyes of any or all rabbits may be further examined with the aid of fluorescein.

(iii) The grading of ocular responses is subject to various interpretations. To promote harmonization and to assist testing laboratories and those involved in making and interpreting the observations, an illustrated guide in grading eye irritation should be used. (Such an illustrated guide is in use in the United States and can be obtained from the Consumer Product Safety Commission, Washington, D.C. 20207.)

(e) *Data and reporting.*—(1) *Data summary.* Data should be summarized in tabular form, showing for each individual animal the irritation scores at the designated observation time; a description of the degree and nature of irritation; the presence of serious lesions and any effects other than ocular which were observed.

(2) *Evaluation of the results.* The ocular irritation scores should be evaluated in conjunction with the nature and reversibility or otherwise of the responses observed. The individual scores do not represent an absolute standard for the irritant properties of a material. They should be viewed as reference values and are only meaningful when supported by a full description and evaluation of the observations.

(3) *Test report.* In addition to the reporting requirements as specified under 40 CFR Part 792, Subpart J the following specific information should be reported:

(i) Physical nature and, where appropriate, concentration and pH value for the test substance.

(ii) Species and strain.

(iii) Tabulation of irritant/corrosive response data for each individual animal at each observation time point (e.g., 1, 24, 48, and 72 hours).

(iv) Description of any lesions observed.

(v) Narrative description of the degree and nature of irritation or corrosion observed.

(vi) Description of the method used to score the irritation at 1, 24, 48, and 72 hours (e.g., hand slit-lamp, biomicroscope, fluorescein).

(vii) Description of any non-ocular effects noted.

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Buehler, EV., Newmann, E.A. "A Comparison of Eye Irritation in Monkeys and Rabbits," *Toxicology and Applied Pharmacology.* 6:701–710 (1964).

(2) Draize, J.H. "Dermal Toxicity," *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics.* The Association of Food and Drug Officials of the United States (1959, 3rd printing 1975), pp. 49–52.

(3) Draize, J.H., Woodward, G., Calvery, H.O. "Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes," *Journal of Pharmacology and Experimental Therapeutics.* 83:377–390 (1944).

(4) Loomis, T.A. *Essentials of Toxicology.* Second, (Philadelphia: Lea and Febicer, 1974), pp. 207–213.

(5) National Academy of Sciences. "Principles and Procedures for Evaluating the Toxicity of Household Substances." A report propared by the Committee for the revision of NAS Publication 1138, under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC (1977).

(6) World Health Organization. "Part I. Environmental Health Criteria 6," *Principles and Methods for Evaluating the Toxicity of Chemicals.* (Geneva: World Health Organization, 1978).

§ 798.4700 Reproduction and fertility effects.

(a) *Purpose.* This guideline for two-generation reproduction testing is designed to provide general information concerning the effects of a test substance on gonadal function, conception, parturition, and the growth and development of the offspring. The study may also provide information about the effects of the test substance on neonatal morbidity, mortality, and preliminary data on teratogenesis and serve as a guide for subsequent tests.

(b) *Principle of the test method.* The test substance is administered to parental (P) animals prior to their mating, during the resultant pregnancies, and through the weaning of their F_1 offspring. The substance is then administered to selected F_1 offspring during their growth into adulthood, mating, and production of an F_2 generation, up until the F_2 generation is weaned.

(c) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* The rat is the preferred species. If another mammalian species is used, the tester should provide justification/reasoning for its selection. Strains with low fecundity should not be used.

(ii) *Age.* Parental (P) animals should be about 5 to 8 weeks old at the start of dosing.

(iii) *Sex.* (A) For an adequate assessment of fertility, both males and females should be studied.

(B) The females should be nulliparous and non-pregnant.

(iv) *Number of animals.* Each test and control group should contain at least 20 males and a sufficient number of females to yield at least 20 pregnant females at or near term.

(2) *Control groups.* (i) A concurrent control group should be used. This group should be an untreated or sham treated control group or if a vehicle is used in administering the test substance, a vehicle control group.

(ii) If a vehicle is used in administering the test substance, the control group should receive the vehicle in the highest volume used.

(iii) If a vehicle or other additive is used to facilitate dosing, it should not interfere with absorption of the test substance or produce toxic effects.

(3) *Dose levels and dose selection.* (i) At least three dose levels and a concurrent control should be used.

(ii) The highest dose level should induce toxicity but not high levels of mortality in the parental (P) animals.

(iii) The lowest dose level should not produce any grossly observable evidence of toxicity.

(iv) Ideally the intermediate dose level(s) should produce minimal observable toxic effects. If more than one intermediate dose is used, dose levels should be spaced to produce a gradation of toxic effects.

(4) *Exposure conditions.* The animals should be dosed with the test substance, ideally, on a 7 days per week basis.

(i) Dosing, mating, delivery, and sacrifice schedule.

(A) Daily dosing of the parental (P) males and females should begin when they are 5 to 8 weeks old. For both sexes, dosing should be continued for at least 10 weeks before the mating period.

(B) Dosing of P males should continue through the 3 week mating period. At the end of the mating period, P males may be sacrificed and examined, or may be retained for possible production of a second litter. If these animals are retained for a second litter, dosing should be continued. Dosing of the F_1 males saved for mating should continue from the time they are weaned through the period they are mated with the F_1 females (11 weeks). F_1 males may be sacrificed after the F_1 mating period.

(C) Daily dosing of the P females should continue through the three week mating period, pregnancy, and to the weaning of the F_1 offspring. Dosing of the F_1 females saved for mating should continue from the time they are weaned, through the period they are mated with the F_1 males (11 weeks from the time of weaning) pregnancy, and to the weaning of the F_2 offspring.

(ii) All animals are sacrificed as scheduled.

(A) All P males should be sacrificed at the end of the 3 week mating period, or may be retained for possible production of a second litter. If these animals are retained for a second litter, dosing should be continued.

(B) F_1 males selected for mating should be sacrificed at the end of the three week period of the F_1 mating.

(C) F_1 males and females not selected for mating should be sacrified when weaned.

(D) The P females should be sacrificed upon weaning of their F_1 offspring.

(E) F_1 dams and their F_2 offspring are sacrificed when the offspring are weaned.

(5) *Administration of the test substance*—(i) *Oral studies.* (A) It is recommended that the test substance be administered in the diet or drinking water.

(B) If administered by gavage or capsule, the dosage administered to each animal prior to mating should be based on the individual animal's body weight and adjusted weekly. During pregnancy the dosage should be based on the body weight at day 0 and 6 of pregnancy.

(ii) If another route of administration is used, the tester should provide justification and reasoning for its selection.

(6) *Mating procedure*—(i) *Parental.* (A) For each mating, each female should be placed with a single male from the same dose level until pregnancy occurs or 1 week has elapsed. If mating has not ocurred after 1 week, the female should be placed with a different male. Paired matings should be clearly identified.

(B) Those pairs that fail to mate should be evaluated to determine the cause of the apparent infertility. This may involve such procedures as additional opportunities to mate with proven fertile males or females, histological examination of the reproductive organs, and examination of the estrus or spermatogenic cycles.

(C) Each day, the females should be examined for presence of sperm or vaginal plugs. Day 0 of pregnancy is defined as the day vaginal plugs or sperm are found.

(ii) *F_1 cross.* (A) For mating the F_1 offspring, one male and one female are randomly selected at weaning from each litter for cross mating with another pup of the same dose level but different litter, to produce the F_2 generation.

(B) F_1 males and females not selected for mating are sacrificed upon weaning.

(iii) *Special housing.* After evidence of copulation, pregnant animals should be caged separately in delivery or maternity cages and provided with nesting materials.

(iv) *Standardization of litter sizes.* (A) On day 4 after birth, the size of each litter should be adjusted by eliminating extra pups by random selection to yield, as nearly as possible, 4 males and 4 females per litter.

(B) Whenever the number of male or female pups prevents having 4 of each sex per litter, partial adjustment (for example, 5 males and 3 females) is permitted. Adjustments are not appropriate for litters of less than 8 pups.

(C) Elimination of runts only is not appropriate.

(D) Adjustments of the F_2 litters is conducted in the same manner.

(7) *Observation of animals.* (i) A gross examination should be made at least once each day. Pertinent behavioral changes, signs of difficult or prolonged parturition, and all signs of toxicity, including mortality, should be recorded. These observations should be reported for each individual animal. Food consumption for all animals should be monitored weekly.

(ii) The duration of gestation should be calculated from day 0 of pregnancy.

(iii) Each litter should be examined as soon as possible after delivery for the number of pups, stillbirths, live births, sex, and the presence of gross anomalies. Dead pups and pups sacrificed at day 4 should be preserved and studied for possible defects. Live pups should be counted and litters weighed, by weighing each individual pup at birth, or soon thereafter, and on days 4, 7, 14, and 21 after parturition.

(iv) Physical or behavioral abnormalities observed in the dams of offspring should be recorded.

(v) P males and females should be weighed on the first day of dosing and weekly thereafter. F_1 litters should be weighed at birth, or soon thereafter, and on days 4, 7, 14, and 21. In all cases, litter weights should be calculated from the weights of the individual pups.

(8) *Gross necropsy.* (i) A complete gross examination should be performed on all animals, including those which died during the experiment or were killed in moribund conditions.

(ii) Special attention should be directed to the organs of the reproductive system.

(iii) The following organs and tissues, or representative samples thereof, should be preserved in a suitable medium for possible future histopathological examination: vagina; uterus; ovaries; testes; epididymides; seminal vesicles; prostate, pituitary gland; and, target organ(s) of all P and F_1 animals selected for mating.

(9) *Histopathology.* The following histopathology should be performed:

(i) Full histopathology on the organs listed above for all high dose, and control P_1 and F_1 animals selected for mating.

(ii) Organs demonstrating pathology in these animals should then be examined in animals from the other dose groups.

(iii) Microscopic examination should be made of all tissues showing gross pathological changes.

(d) *Data and reporting*—(1) *Treatment of results.* Data should be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals pregnant, the types of change and the percentage of animals displaying each type of change.

(2) *Evaluation of study results.* (i) An evaluation of test results, including the statistical analysis, based on the clinical findings, the gross necropsy findings, and the microscopic results, should be made and supplied. This should include an evaluation of the relationship, or lack thereof, between the animals' exposure to the test substance and the incidence and severity of all abnormalities.

(ii) In any study which demonstrates an absence of toxic effects, further investigation to establish absorption and bioavailability of the test substance should be considered.

(3) *Test report.* In addition to the reporting requirements as specified under 40 CFR Part 792, Subpart J the following specific information should be reported:

(i) Toxic response data by sex and dose, including fertility, gestation, viability and lactation indices, and length of gestation.

(ii) Species and strain.

(iii) Time of death during the study or whether animals survived to termination.

(iv) Toxic or other effects on reproduction, offspring, or postnatal growth.

(v) Time of observation of each abnormal sign and its subsequent course.

(vi) Body weight data for P, F_1, and F_2 animals.

(vii) Necropsy findings.

(viii) Detailed description of all histopathological findings.

(ix) Statistical treatment of results where appropriate.

(e) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Clermont, Y., Perry, B. "Quantitative Study of the Cell Population of the Seminiferous Tubules in Immature Rats," *American Journal of Anatomy.* 100:241–267 (1957).

(2) Goldenthal, E.I. *Guidelines for Reproduction Studies for Safety Evaluation of Drugs for Human Use.* Drug Review Branch, Division of Toxicological Evaluation, Bureau of Science, Food and Drug Administration, Washington, DC (1966).

(3) Hasegawa, T., Hayashi, M., Ebling, F.J.G., Henderson, I.W. *Fertility and Sterility.* (New York: American Elsevier Publishing Co., Inc., 1973).

(4) Oakberg, E.F. "Duration of Spermatogenesis in the Mouse and Timing of Stages of the Cycle of the Seminiferous Epithelium," *American Journal of Anatomy.* 9:507–516 (1956).

(5) Roosen-Runge, E.C. "The Process of Spermatogenesis in Mammals," *Biological Review.* 37:343–377 (1962).

§ 798.4900 Developmental toxicity study.

(a) *Purpose.* In the assessment and evaluation of the toxic characteristics of a chemical, determination of the potential developmental toxicity is important. The developmental toxicity study is designed to provide information on the potential hazard to the unborn which may arise from exposure of the mother during pregnancy.

(b) *Definitions.* (1) Developmental toxicity is the property of a chemical that causes in utero death, structural or functional abnormalities or growth retardation during the period of development.

(2) Dose is the amount of test substance administered. Dose is expressed as weight of test substance (g, mg) per unit weight of a test animal (e.g., mg/kg).

(3) No-observed-effect level is the maximum concentration in a test which produces no observed adverse effects. A no-observed-effect level is expressed in terms of weight of test substance given daily per unit weight of test animal (mg/kg)

(c) *Principle of the test method.* The test substance is administered in graduated doses for at least part of the pregnancy covering the major period of organogenesis, to several groups of pregnant experimental animals, one dose level being used per group. Shortly before the expected date of delivery, the pregnant females are sacrificed, the uteri removed, and the contents examined for embryonic or fetal deaths, and live fetuses.

(d) *Limit test.* If a test at an exposure of at least 1000 mg/kg body weight, using the procedures described for this study, produces no observable developmental toxicity, then a full study using three dose levels might not be necessary.

(e) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* Testing should be performed in at least 2 mammalian species. Commonly used species include the rat, mouse, rabbit, and hamster. If other mammalian species are used, the tester should provide justification/reasoning for their selection. Commonly used laboratory strains should be employed. The strain should not have low fecundity and should preferably be characterized for its sensitivity to developmental toxins.

(ii) *Age.* Young adult animals (nulliparous females) should be used.

(iii) *Sex.* Pregnant female animals should be used at each dose level.

(iv) *Number of animals.* At least 20 pregnant rats, mice or hamsters or 12 pregnant rabbits are recommended at each dose level. The objective is to ensure that sufficient pups are produced to permit meaningful evaluation of the potential developmental toxicity of the test substance.

(2) *Control group.* A concurrent control group should be used. This group should be an untreated or sham treated

control group, or, if a vehicle is used in administering the test substance, a vehicle control group. Except for treatment with the test substance, animals in the control group(s) should be handled in an identical manner to test group animals.

(3) *Dose levels and dose selection.* (i) At least 3 dose levels with a control and, where appropriate, a vehicle control, should be used.

(ii) The vehicle should neither be developmentally toxic nor have effects on reproduction.

(iii) To select the appropriate dose levels, a pilot or trial study may be advisable. It is not always necessary to carry out a trial study in pregnant animals. Comparison of the results from a trial study in non-pregnant, and the main study in pregnant animals will demonstrate if the test substance is more toxic in pregnant animals. If a trial study is carried out in pregnant animals, the dose producing embryonic or fetal lethalities or maternal toxicity should be determined.

(iv) Unless limited by the physical/chemical nature or biological properties of the substance, the highest dose level should induce some overt maternal toxicity such as slight weight loss, but not more than 10 percent maternal deaths.

(v) The lowest dose level should not produce any grossly observable evidence of either maternal or developmental toxicity.

(vi) Ideally, the intermediate dose level(s) should produce minimal observable toxic effects. If more than one intermediate concentration is used, the concentration levels should be spaced to produce a gradation of toxic effects.

(4) *Observation period.* Day 0 in the test is the day on which a vaginal plug and/or sperm are observed. The dose period should cover the period of major organogenesis. This may be taken as days 6 to 15 for rat and mouse, 6 to 14 for hamster, or 6 to 18 for rabbit.

(5) *Administration of test substance.* The test substance or vehicle is usually administered orally, by oral intubation unless the chemical or physical characteristics of the test substance or pattern of human exposure suggest a more appropriate route of administration. The test substance should be administered at the same time each day.

(6) *Exposure conditions.* The female test animals are treated with the test substance daily throughout the appropriate treatment period. When given by gavage, the dose may be based on the weight of the females at the start of substance administration, or, alternatively, in view of the rapid weight gain which takes place during pregnancy, the animals may be weighed periodically and the dosage based on the most recent weight determination.

(7) *Observation of animals.* (i) A gross examination should be made at least once each day.

(ii) Additional observations should be made daily with appropriate actions taken to minimize loss of animals to the study (e.g., necropsy or refrigeration of those animals found dead and isolation or sacrifice of weak or moribund animals).

(iii) Signs of toxicity should be recorded as they are observed, including the time of onset, the degree and duration.

(iv) During the treatment and observation periods, cage-side observations should include, but not be limited to: changes in skin and fur, eye and mucous membranes, as well as respiratory, autonomic and central nervous systems, somatomotor activity and behavioral pattern.

(v) Measurements should be made weekly of food consumption for all animals in the study.

(vi) Animals should be weighed at least weekly.

(vii) Females showing signs of abortion or premature delivery should be sacrificed and subjected to a thorough macroscopic examination.

(8) *Gross necropsy.* (i) At the time of sacrifice or death during the study, the dam should be examined macroscopically for any structural abnormalities or pathological changes which may have influenced the pregnancy.

(ii) Immediately after sacrifice or death, the uterus should be removed, weighed and the contents examined for embryonic or fetal deaths and the number of viable fetuses. The degree of resorption should be described in order to help estimate the relative time of death.

(iii) The number of corpora lutea should be determined for all species except mice.

(iv) The sex of the fetuses should be determined and they should be weighed individually, the weights recorded, and the mean fetal weight derived.

(v) Following removal, each fetus should be examined externally.

(vi) For rats, mice and hamsters, one-third to one-half of each litter should be prepared and examined for skeletal anomalies, and the remaining part of each litter should be prepared and examined for soft tissue anomalies using appropriate methods.

(vii) For rabbits, each fetus should be examined by careful dissection for visceral anomalies and then examined for skeletal anomalies.

(f) *Data and reporting*—(1) *Treatment of results.* Data should be summarized in tablular form, showing for each test group: the number of animals at the start of the test, the number of pregnant animals, the number and percentages of live fetuses and the number of fetuses with any soft tissue or skeletal abnormalities.

(2) *Evaluation of results.* The findings of a developmental toxicity study should be evaluated in terms of the observed effects and the exposure levels producing effects. It is necessary to consider the historical developmental toxicity data on the species/strain tested. A properly conducted developmental toxicity study should provide a satisfactory estimation of a no-effect level.

(3) *Test report.* In addition to the reporting requirements as specified under 40 CFR Part 792, Subpart J the following specific information should be reported:

(i) Toxic response data by concentration.

(ii) Species and strain.

(iii) Time of death during the study or whether animals survived to termination.

(iv) Time of onset and duration of each abnormal sign and its subsequent course.

(v) Food, body weight and uterine weight data.

(vi) Pregnancy and litter data.

(vii) Fetal data (live/dead, sex, soft tissue and skeletal defects, resorptions).

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Department of Health and Welfare. *The Testing of Chemicals for Carcinogenicity, mutagenicity and Teratogenicity.* Minister of Health and Welfare (Canada: Department of Health and Welfare, 1975).

(2) National Academy of Sciences. "Principles and Procedures for Evaluating the Toxicity of Household Substances." A report prepared by the Committee for the Revision of NAS Publication 1138, under the auspices of the Committee on Toxicology, National Research Council, National Academy of Sciences, Washington, DC (1977).

(3) World Health Organization. *Principles for the Testing of Drugs for Teratogenicity.* WHO Technical Report Series No. 364. (Geneva: World Health Organization, (1967).

Subpart F—Genetic Toxicity

§ 798.5100 Escherichia coli WP2 and WP2 uvrA reverse mutation assays

(a) *Purpose.* The *E. coli* tryptophan (*trp*) reversion system is a microbial assay which measures trp−trp$^+$ reversion induced by chemicals which cause mutations in the genome of this organism.

(b) *Definition.* A reverse mutation assay in *E. coli* detects mutation in a gene of tryptophan requiring strain to produce a tryptophan independent strain of this organism.

(c) *Reference substances.* These may include, but need not be limited to, 4-nitroquinoline oxide, methyl methanesulfonate, or 2-aminoanthracene.

(d) *Test method*—(1) *Principle.* Bacteria are exposed to test chemical with and without metabolic activation and plated onto minimal medium. After a suitable period of incubation, revertant colonies are counted and compared to the number of spontaneous revertants in an untreated and/or vehicle control culture.

(2) *Description.* Several methods for performing the test have been described. Among those used are:

(i) The direct plate incorporation method.

(ii) The preincubation method.

(iii) The treat and plate method.

(iv) The modified fluctuation test.

(v) The desiccator method.

The procedure described here is for the direct plate incorporation method.

(3) *Strain selection*—(i) *Designation.* At the present time, three strains, WP2, WP2 *uvrA* and WP2 *uvrA*/pKM101 should be used. Other strains may be utilized when appropriate.

(ii) *Preparation and storage.* Recognized methods of stock culture preparation and storage should be used. The requirement of tryptophan for growth should be demonstrated for each strain. Other phenotypic characteristics should be checked using such methods as sensitivity to mitomycin C and resistance to ampicillin. Spontaneous reversion frequency should be in the range expected either as reported in the literature or as established in the laboratory by historical control values.

(iii) *Bacterial growth.* Fresh cultures of bacteria should be grown up to the late exponential or early stationary phase of growth (approximately 10^8–10^9 cells per ml).

(4) *Metabolic activation.* Bacteria should be exposed to the test substance both in the presence and absence of an appropriate metabolic activation system. The most commonly used system is a cofactor supplemented postmitochondrial fraction prepared from the livers of rodents treated with enzyme inducing agents. The use of other species, tissues or techniques may also be appropriate.

(5) *Control groups*—(i) *Concurrent controls.* Concurrent positive and negative (untreated and/or vehicle) controls should be included in each experiment.

(ii) *Direct acting positive controls.* Examples of positive controls for assays performed without metabolic activation include methyl methanesulfonate and 4-nitroquinoline oxide.

(iii) *Positive controls to ensure the efficacy of the activation system.* The positive control reference substance for tests including a metabolic activation system should be selected on the basis of the type of activation system used in the test. 2-Aminoanthracene is an example of a positive control compound in tests using postmitochondrial fractions from the livers of rodents treated with enzyme inducing agents such as Aroclor-1254.

(iv) *Other positive controls.* Other positive control reference substances may be used.

(6) *Test chemicals*—(i) *Vehicle.* Test chemicals and positive control reference substances should be dissolved in an appropriate vehicle and then further diluted in vehicle for use in the assay.

(ii) *Exposure concentrations.* (A) The test should initially be performed over a broad range of concentrations. Among the criteria to be taken into consideration for determinating the upper limits of test chemical concentration are cytotoxicity and solubility. Cytotoxicity of the test chemical may be altered in the presence of metabolic activation systems. Toxicity may be evidenced by a reduction in the number of spontaneous revertants, a clearing of the background lawn or by the degree of survival of treated cultures. Relatively insoluble chemicals should be tested up to the limits of solubility. For freely soluble nontoxic chemicals, the upper test chemical concentration should be determined on a case by case basis.

(B) Generally, a maximum of 5 mg/plate for pure substances is considered acceptable. At least 5 different amounts of test substance should be tested with adequate intervals between the test points.

(C) When appropriate, a positive response should be confirmed by testing over a narrow range of concentrations.

(e) *Test performance*—(1) *Direct plate incorporation method.* (i) For this test without metabolic activation, test chemical and 0.1 ml of a fresh bacterial culture should be added to 2.0 ml of overlay agar.

(ii) For tests with metabolic activation, 0.5 ml of activation mixture containing an adequate amount of postmitochondrial fraction should be added to the overlay agar after the addition of test chemical and bacteria. Contents of each tube should be mixed and poured over the surface of a selective agar plate. Overlay agar should be allowed to solidify before incubation. At the end of the incubation period, revertant colonies per plate should be counted.

(2) *Other methods.* Other methods may also be appropriate.

(3) *Media.* An appropriate selective medium with an adequate overlay agar should be used.

(4) *Incubation conditions.* All plates in a given experiment should be incubated for the same time period. This incubation period should be for 48–72 hours at 37 °C.

(5) *Number of cultures.* All plating should be done at least in triplicate. If scientifically justified, the use of duplicates may be acceptable. All results should be confirmed in an independent experiment.

(f) *Data and report*—(1) *Treatment of results.* Data should be presented as number of revertant colonies per plate for each replicate and dose. The numbers of revertant colonies on both negative (untreated and/or vehicle) and positive control plates should also be presented. Individual plate counts, the mean number of revertant colonies per plate and standard deviation should be presented for test chemical and positive and negative (untreated and/or vehicle) controls.

(2) *Statistical evaluation.* Data should be evaluated by appropriate statistical methods.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of revertants. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test substance concentrations.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of revertants or a statistically significant and reproducible positive response at any one of the test points is considered non-mutagenic in the system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* (i) Positive results from the *E. coli* reverse mutation assay indicate that, under the test conditions, the test substance induces mutations in the genome of this organism.

(ii) Negative results indicate that under the test conditions the test substance is not mutagenic in *E. coli.*

(5) *Test report.* In addition to the reporting recommendations as specified under 40 CFR Part 792, Subpart J the following specific information should be reported:

(i) Bacterial strain used.

(ii) Details of both the protocol used to prepare the metabolic activation system and of its use in the assay.

(iii) Dose levels and rationale for selection of dose.

(iv) Positive and negative controls.

(v) Individual plate counts, mean number of revertant colonies per plate, standard deviation.

(vi) Dose-response relationship, if applicable.

(g) *References.* For additional background information on this test guideline, the following references should be consulted:

(1) Ames, B.N. McCann, J., Yamasaki, E. "Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test," *Mutation Research* 31:347–364 (1975).

(2) Brusick, D.J., Simmon, V.F., Rosenkranz, H.S., Ray, V.A., Stafford, R.S. An evaluation of the *Escherichia coli* WP2 and WP2 *uvrA* reverse mutation assay," *Mutation Research* 76:169–190 (1980).

(3) Green, M.H.L., Muriel, W.J. "Mutagen testing using trp^+ in *Escherichia coli.,*" *Mutation Research* 38:3–32 (1976).

(4) Vogel, H.J., Bonner, D.M. "Acetylornithinase of *E. coli:* partial purification and some properties," *Journal of Biological Chemistry* 218:97–106 (1956).

§ 798.5140 Gene mutation in Aspergillus nidulans.

(a) *Purpose. Aspergillus nidulans* is a eukaryotic fungus which has been developed to detect and study a variety of genetic phenomena including chemically induced mutagenesis. *A. nidulans* can be used to detect both forward and reverse gene mutation. These mutations are detected by changes in colonial morphology or nutritional requirements in treated populations. The methionine and 2–thioxanthine forward mutation systems can be used to detect mutations in *A. nidulans.*

(b) *Definition.* A forward mutation is a gene mutation from the wild (parent) type to the mutant condition.

(c) *Reference substances.* These may include, but need not be limited to, ethyl methanesulfonate, cyclophosphamide or aflatoxin B_1.

(d) *Test method*—(1) *Principle.* Conidia are exposed to test chemical both with and without metabolic activation and plated on selective medium to determine changes in colonial morphology or nutritional requirements. At the end of a suitable incubation period, mutant colonies are counted and compared to the number of spontaneous mutants in an untreated control culture. Simultaneous determination of survival permits calculation of mutation frequency.

(2) *Description.* Tests for mutation in *A. nidulans* are performed in liquid suspension. Treated conidia are plated on selective medium to determine changes in nutritional requirements or colonial morphology.

(3) *Strain selection*—(i) *Designation.* For the methionine and 2-thioxanthine systems the hapliod Glascow *biAl; meth G1* strain is the most commonly used strain although other strains may be appropriate. Any translocation-free strain which produces green colonies on thioxanthine free medium and yellow colonies on medium containing thioxanthine may be used in the thioxanthine system.

(ii) *Preparation and storage.* Stock culture preparation and storage, growth requirements, method of strain identification and demonstration of appropriate phenotypic requirements should be performed using good microbiological techniques and should be documented.

(iii) *Media.* Any medium which supports growth and a characteristic colonial morphology may be used in the assay.

(4) *Preparation of conidia.* Prior to chemical treatment, conidia from 4–5 single colonies of the appropriate strain are grown at 37 °C on complete medium. At the end of the incubation period, conidia are collected, conidial chains broken up, mycelial debris removed and conidia concentrated prior to removal of the germination inhibitory substance. Germination inhibitory substance should be removed by Tween 80 or diethyl ether.

(5) *Metabolic activation.* Conidia should be exposed to test substance both in the presence and absence of an appropriate metabolic activation system.

(6) *Control groups.* Concurrent positive and negative untreated and/or vehicle) controls both with and without metabolic activation should be included in each experiment.

(7) *Test chemicals*—(i) *Vehicle.* Test chemicals and positive control reference substances should be dissolved in an appropriate vehicle and then further diluted in vehicle for use in the assay.

(ii) *Exposure concentrations.* (A) The test should initially be performed over a broad range of concentrations selected on the basis of a preliminary assay. Effective treatment times should also be selected in the preliminary assay.

(B) Each test should include five treatment points, two at fixed concentrations for different time periods, and three at varying concentrations for fixed periods of time.

(C) Among the criteria to be taken into consideration for determining the upper limits of test chemical concentration are cytotoxicity and solubility. Cytotoxicity of the test chemical may be altered in the presence of a metabolic activation system. Relatively insoluble chemicals should be tested up to the limits of solubility. For freely soluble nontoxic chemicals, the upper test chemical concentration should be determined on a case by case basis.

(D) When appropriate, a positive response should be confirmed by using a narrow range of test concentrations.

(e) *Test Performance*—(1) *Treatment.* Germinating or quiescent conidia in liquid suspension should be exposed to the test chemical at 37 °C under conditions of yellow light and controlled pH and oxygen tension. At the end of the exposure period, treatment should be terminated by repeated centrifugation and washing of the conidia or by dilution. Chemical neutralization of the test agent may also be used but is not recommended.

(2) *Media*—(i) *Methionine system.* For the methionine system, conidia should be plated on methionine deficient medium for mutant selection and on medium supplemented with methionine to determine survival.

(ii) *Thioxanthine system.* (A) For the 2-thioxanthine system, treated conidia should be plated on nitrogen-free glucose and salts minimal medium containing 2-thioxanthine.

(B) After incubation, green colonies should be counted and isolated by restreaking. The isolated colonies should be classified on the basis of genetic criteria. Yellow, wild-type colonies will grow on the same plate. This permits concurrent determination of survival and an estimation of mutation frequency.

(3) *Determination of mutation frequency and viability.* In both systems, mutation frequency and

viability should be determined immediately before and immediately after chemical treatment.

(4) *Incubation conditions.* All incubations should be at 37 °C. Incubation time will vary depending upon system and endpoint (mutation or viability) being determined.

(5) *Number of cultures.* (i) At least 10 independent plates per concentration with no more than 20 colonies per plate should be used in the methionine system.

(ii) Fifteen to 20 plates per concentration are preferred for the 2-thioxanthine system.

(f) *Data and report*—(1) *Treatment of results.* Individual plate counts for test substance and controls should be presented for both mutation induction and survival. The mean number of colonies per plate and standard deviation should also be presented. Data should be presented in tabular form indicating, as applicable, numbers of colonies counted, and numbers and classification of mutants identified. Sufficient detail should be provided for verification of survival and mutation frequencies.

(2) *Statistical evaluation.* Data should be evaluated by appropriate statistical methods.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of mutant colonies. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test points.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of mutant colonies or a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* (i) Positive results from the methionine and 2-thioxanthine systems in *A. nidulans* indicate that, under the test conditions, the test substance causes gene (point) mutations in the DNA of this organism caused by base pair changes and small deletions in the genome.

(ii) Negative results indicate that under the test conditions the test chemical is not mutagenic in *A. nidulans.*

(5) *Test report.* In addition to the reporting recommendations as specified under 40 CFR Part 792, Subpart J the following specific information should be reported:

(i) Strain of organism used in the assay.

(ii) Test chemical vehicle, doses used and rationale for dose selection, toxicity data.

(iii) Method used for preparation of conidia.

(iv) Treatment conditions, including length of exposure and method used to stop treatment.

(v) Details of both the protocol used to prepare the metabolic activation system and of its use in the assay.

(vi) Incubation times and temperature.

(vii) Positive and negative controls.

(viii) Dose-response relationship, if applicable.

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Ames, B.N., McCann, J., Yamasaki, E. "Methods for detecting carcinogens and mutagens with the *Salmonella/* mammalian-microsome mutagenicity test," *Mutation Research* 31:347–364 (1975).

(2) Kafer, E., Scott, B.R., Dorn, G.L., Stafford, R.S. "*Aspergillus nidulans:* systems and results of tests for chemical induction of mitotic segregation and mutation. I. Diploid and duplication assay systems: a report of the U.S. EPA's Gene-Tox Program," *Mutation Research* 98:1–48 (1982).

(3) Munson, R.J., Goodhead, D.T. "Relation between induced mutation frequency and cell survival: a theoretical approach and an examination of experimental data for eukaryotes," *Mutation Research* 42:145–159 (1977).

(4) Scott, B.R., Dorn, G.L., Kafer, E., Stafford, R.S. "*Aspergillus nidulans:* systems and results of tests for mitotic segregation and mutation. II. Haploid assay systems and overall response of all systems: a report of the U.S. EPA's Gene-Tox Program," *Mutation Research* 98:49–94 (1982).

§ 798.5200 Mouse visible specific locus test.

(a) *Purpose.* The mouse visible specific locus test (MSLT) may be used to detect and quantitate mutations in the germ line of a mammalian species.

(b) *Definitions.* (1) A visible specific locus mutation is a genetic change that alters factors responsible for coat color and other visible characteristics of certain mouse strains.

(2) The germ line is the cells in the gonads of higher eukaryotes which are the carriers of the genetic information for the species.

(c) *Reference substances.* Not applicable.

(d) *Test method*—(1) *Principle.* (i) The principle of the MSLT is to cross individuals who differ with respect to the genes present at certain specific loci, so that a genetic alteration involving the standard gene at any one of these loci will produce an offspring detectably different from the standard heterozygote. The genetic change may be detectable by various means, depending on the loci chosen to be marked.

(ii) Three variations of the method currently exist for detecting newly arising point mutations in mouse germ cells:

(A) The visible specific locus test using either 5 or 7 loci.

(B) The biochemical specific locus test using up to 20 enzymes.

(C) The test for mutations at histocompatibility loci.

(iii) Of the three tests, the visible specific locus test has been most widely used in assessing genetic hazard due to environmental agents. It is the method described in this guideline.

(2) *Description.* For technical reasons, males rather than females are generally treated with the test agent. Treated males are then mated to females which are genetically homozygous for certain specific visible marker loci. Offspring are examined in the next generation for evidence that a new mutation has arisen.

(3) *Animal selection*—(i) *Species and strain.* Mice are recommended as the test species. Male mice should be either $(C_3H \times 101)F_1$ or $(101 \times C_3H)F_1$ hybrids. Females should be T stock virgins.

(ii) *Age.* Healthy sexually mature animals should be used.

(iii) *Number.* A decision on the minimum number of treated animals should take into account the spontaneous variation of the biological characterization being evaluated. Other considerations should include:

(A) The use of either historical or concurrent controls.

(B) The power of the test.

(C) The minimal rate of induction required.

(D) The use of positive controls.

(E) The level of significance desired.

(iv) *Assignment to groups.* Animals should be randomized and assigned to treatment and control groups.

(4) *Control groups*—(i) *Concurrent controls.* The use of positive or spontaneous controls is left to the discretion of the investigator. However, any laboratory which has had no prior experience with the test should, at its first attempt, produce a negative control sample of 20,000 and a positive control,

using 100 mg/kg 1-ethyl-nitrosourea, in a sample of 5,000 offspring.

(ii) *Historical controls.* Long term, accumulated spontaneous control data of 43/801,406 are available for comparative purposes.

(5) *Test chemicals*—(i) *Vehicle.* When possible, test chemicals should be dissolved or suspended in distilled water or isotonic saline buffered appropriately, if needed, for stability. Water insoluble chemicals should be dissolved or suspended in appropriate vehicles. The vehicle used should neither interfere with the test compound nor produce major toxic effects. Fresh preparations of the test chemical should be employed.

(ii) *Dose levels.* Usually, only one dose level need be tested. This should be the highest dose tolerated without toxic effects, provided that any temporary sterility induced due to elimination of spermatagonia is of only moderate duration, as determined by a return of males to fertility within 80 days after treatment.

(iii) *Route of administration.* Acceptable routes of administration include gavage, inhalation, admixture with food or water, and IP or IV injections.

(e) *Test performance*—(1) *Treatment and mating.* Hybrid F_1 ($C_3H \times 101$ or $101 \times C_3H$) male mice should be treated with the test substance and immediately mated to virgin T stock females. Each treated male should be mated to a fresh group of 2 to 4 virgin females each week for 7 weeks, after which he should be returned to the first group of females and rotated through the seven sets of females repeatedly. This mating schedule generally permits sampling of all postspermatagonial stages of germ cell development during the first 7 weeks and rapid accumulation of data for exposed spermatagonial stem cells thereafter.

(2) *Examination of offspring.* (i) Offspring may be examined at (or soon after) birth but must be examined at about 3 weeks of age at which time the numbers of mutant and nonmutant offspring in each litter should be recorded.

(ii) Nonmutant progeny should be discarded. Mutant progeny should be subjected to genetic tests for verification.

(f) *Data and report*—(1) *Treatment of results.* Data should be presented in tabular form and should permit independent analysis of cell stage specific effects, and dose dependent phenomena. The data should be recorded and analyzed in such a way that clusters of identical mutations are clearly identified. The individual mutants detected should be thoroughly described. In addition, positive and negative control data, if they are available, should be tabulated so that it is possible to differentiate between concurrent (when available) and long term, accumulated mutation frequencies.

(2) *Statistical evaluation.* Data should be evaluated by appropriate statistical methods.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of specific locus mutations. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test points.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of specific locus mutations or a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* (i) Positive results in the MSLT indicate that under the test conditions the test substance induces heritable gene mutations in the test species.

(ii) Negative results indicate that under the test conditions the test substance does not induce heritable gene mutations in the test species.

(5) *Test report.* In addition to the reporting recommendations as specified under 40 CFR Part 792, Subpart J the following specific information should be reported:

(i) Strain, age and weight of animals used, number of animals of each sex in experimental and control groups.

(ii) Test chemical vehicle, doses used and rationale for dose selection, toxicity data.

(iii) Route and duration of exposure.

(iv) Mating schedule.

(v) Time of examination for mutant progeny.

(vi) Criteria for scoring mutants.

(vii) Use of concurrent or negative controls.

(viii) Dose response relationship, if applicable.

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Russell, L.B., Shelby, P.B., von Halle, E., Sheridan, W., Valcovic, L. The mouse specific locus test with agents other than radiations: interpretation of data and recommendations for future work: a report of the U.S. EPPT Gene-Tox Program," *Mutation Research*, 86:329–354 (1981).

(2) [Reserved]

§ 798.5250 Gene mutation in Neurospora crassa.

(a) *Purpose. Neurospora crassa* is a eukaryotic fungus which has been developed to detect and study a variety of genetic phenomena including chemically induced mutagenesis. *N. crassa* can be used to detect both forward and reverse gene mutation. These mutations are detected by biochemical or morphological changes in the treated population. The most commonly used mutation assay in *N. crassa* measures forward mutation in the *ad-3* region of the genome.

(b) *Definition.* A forward mutation is a gene mutation from the wild (parent) type to the mutant condition.

(c) *Reference substances.* These may include, but need not be limited to, ethyl- or methyl methanesulfonate.

(d) *Test method*—(1) *Principle.* The detection of forward mutations at the *ad-3* locus in either homokaryons or heterokaryons may be used. However, use of two component heterokaryons is recommended because of the greater range of mutations which can be recovered. In either case, the test relies on the identification of purple (mutant) colonies among a large number of white (wild-type) colonies. A representative sample of purple colonies can be recovered and thoroughly analyzed genetically.

(2) *Description.* Forward mutations at the *ad-3* locus can be detected using noncolonial strains of *N. crassa* grown on media containing sorbose as well as glucose. Under these conditions, colonies are formed and reproducible colonial morphology results. Adenine-requiring mutants which accumulate a reddish-purple pigment can be readily identified and counted.

(3) *Strain selection*—(i) *Designation.* At the present time, heterokaryon 12 is recommended for use in this assay. The use of other strains may also be appropriate.

(ii) *Preparation and storage.* Stock culture preparation and storage, growth requirements, method of strain identification and demonstration of appropriate phenotypic requirements should be performed using good microbiological techniques and should be documented.

(iii) *Media.* Frie's No. 3 minimal medium or Westgaard's Synthetic medium with 1.5 percent agar or any medium known to support growth and characteristic colonial morphology may be used in the assay.

(4) *Preparation of conidia.* Stock cultures should be grown on minimal medium to select for single colonies with noncolonial morphology. Single colony isolates then should be inoculated into agar flasks and incubated at 35 C for 48 hrs to select colonies with spreading growth patterns in which mycelia cover the entire flask. Flasks should be incubated at 23–25 C and those with bright orange conidia selected for preparation of conidial suspensions. Suspensions should be diluted for use in distilled water.

(5) *Metabolic activation.* Conidia should be exposed to test substance both in the presence and absence of an appropriate metabolic activation system.

(6) *Control groups.* Concurrent positive and negative (untreated and/or vehicle) controls both with and without metabolic activation should be included in each experiment.

(7) *Test chemicals*—(i) *Vehicle.* Test chemicals and positive control reference substances should be dissolved in an appropriate vehicle and then further diluted in vehicle for use in the assay.

(ii) *Exposure concentrations.* (A) The test should initially be performed over a broad range of concentrations selected on the basis of a preliminary assay. Effective treatment times should also be selected in the preliminary assay.

(B) Among the criteria to be taken into consideration for determining the upper limits of test chemical concentration are cytotoxicity and solubility. Cytotoxicity of the test chemical may be altered in the presence of metabolic activation systems. For toxic chemicals, the highest concentration tested should not reduce survival below 10 percent of that seen in the control cultures. Relatively insoluble chemicals should be tested up to the limits of solubility. For freely soluble nontoxic chemicals, the upper test chemical concentration should be determined on a case by case basis.

(C) Each test should include five treatment points; two at fixed concentrations for different time periods, and three at varying concentrations for fixed periods of time.

(D) When appropriate, a positive response should be confirmed by testing over a narrow range of concentrations.

(e) *Test performance*—(1) *Treatment.* (i) Growing or nongrowing conidia should be exposed to the test chemical with and without metabolic activation. At the end of the exposure period, treatment should be terminated by chemical quenching. The quenching solution may contain 0.1 percent sodium thiosulfate.

(ii) Conidia should then be plated on the appropriate media to determine mutation induction and viability. At the end of the incubation period, colonies should be scored for viability and mutation induction.

(iii) Mutants should be classified according to color and morphology.

(iv) Both mutation frequency and viability should be determined both immediately before and immediately after chemical treatment.

(2) *Incubation conditions.* All plates in a given test should be incubated for the same time period. This incubation period may be from 2 to 7 days at 30 °C.

(3) *Number of cultures.* Generally, 15 to 20 individual plates per concentration should be used.

(f) *Data and report*—(1) *Treatment of results.* Individual plate counts for test substance and controls should be presented for both mutation induction and survival. The mean number of colonies per plate and standard deviation should be presented. Data should be presented in tabular form indicating, as applicable, numbers of colonies counted, numbers of mutants identified and classification of mutants (e.g., color segregants). Sufficient detail should be provided for verification of survival and mutation frequencies.

(2) *Statistical evaluation.* Data should be evaluated by appropriate statistical techniques.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of mutant colonies. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test points.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of mutant colonies or a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* (i) Positive results from the *ad-3* system in *N. crassa* indicate that, under the test conditions, the test substance causes mutations in the DNA of this organism.

(ii) Negative results indicate that under the test conditions the test substance is not mutagenic in *N. crassa.*

(5) *Test report.* In addition to the reporting recommendations as specified under 40 CFR Part 792, Subpart J the following specific information should be reported:

(i) Strain of organism used in the assay.

(ii) Test chemical vehicle, doses used and rationale for dose selection.

(iii) Method used for preparation of conida.

(iv) Treatment conditions, including length of exposure and method used to stop treatment.

(v) Incubation times and temperature.

(vi) Details of both the protocol used to prepare the metabolic activation system and of its use in the assay.

(vii) Dose-response relationship, if applicable.

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Brockman, H.E., de Serres, F.J. "Induction of *ad-3* mutants of *Neurospora crassa* by 2-aminopurine," *Genetics* 48: 597–604 (1963).

(2) de Serres, F.J., Malling, H.V. "Measurement of recessive lethal damage over the entire genome and at two specific loci in the *ad-3* region of a two-component heterokaryon of *Neurospora crassa,*" *Chemical mutagens: principles and methods for their detection.* Vol. 2, Ed. Hollaender, A. (New York and London: Plenum Press, 1971) pp. 311–342.

(3) Matzinger, P.K. Ong, T–M. "In vitro activation of aflatoxin B_1 to metabolites mutagenic in *Neurospora crassa.*" *Mutation Research,* 37:27–32 (1976).

§ 798.5265 The Salmonella typhimurium reverse mutation assay.

(a) *Purpose.* The *Salmonella typhimurium* histidine (his) reversion system is a microbial assay which measures $his^- \rightarrow his^+$ reversion induced by chemicals which cause base changes or frameshift mutations in the genome of this organism.

(b) *Definitions.* (1) A reverse mutation assay in *Salmonella typhimurium* detects mutation in a gene of a histidine requiring strain to produce a histidine independent strain of this organism.

(2) Base pair mutagens are agents which cause a base change in the DNA. In a reversion assay, this change may occur at the site of the original mutation or at a second site in the chromosome.

(3) Frameshift mutagens are agents which cause the addition or deletion of single or multiple base pairs in the DNA molecule.

(c) *Reference substances.* These may include, but need not be limited to, sodium azide, 2-nitrofluorene, 9-aminoacridine, 2-aminoanthracene, congo red, benzopurpurin 4B, trypan blue or direct blue 1.

(d) *Test method*—(1) *Principle.* Bacteria are exposed to test chemical with and without a metabolic activation system and plated onto minimal medium. After a suitable period of incubation, revertant colonies are counted and compared to the number of spontaneous revertants in an untreated and/or vehicle control culture.

(2) *Description.* Several methods for performing the test have been described. Among those used are:

(i) The direct plate incorporation method.

(ii) The preincubation method.

(iii) The azo-reduction method.

The procedures described here are for the direct plate incorporation method and the azo-reduction method.

(3) *Strain selection*—(i) *Designation.* At the present time four strains, TA 1535, TA 1537, TA 98 and TA 100 should be used. The use of other strains in addition to these four is left to the discretion of the investigator.

(ii) *Preparation and storage.* Recognized methods of stock culture preparation and storage should be used. The requirement of histidine for growth should be demonstrated for each strain. Other phenotypic characteristics should be checked using such methods as crystal violet sensitivity and resistance to ampicillin. Spontaneous reversion frequency should be in the range expected either as reported in the literature or as established in the laboratory by historical control values.

(iii) *Bacterial growth.* Fresh cultures of bacteria should be grown up to the late exponential or early stationary phase of growth (approximately 10^8-10^9 cells per ml).

(4) *Metabolic activation.* Bacteria should be exposed to the test substance both in the presence and absence of an appropriate metabolic activation system. For the direct plate incorporation method, the most commonly used system is a cofactor supplemented postmitochondrial fraction prepared from the livers of rodents treated with enzyme inducing agents such as Aroclor 1254. For the azo-reduction method, a cofactor supplemented postmitochondrial fraction prepared from the livers of untreated hamsters is preferred. For this method, the cofactor supplement should contain flavin mononucleotide, exogenous glucose 6-phosphate dehydrogenase, NADH and excess of glucose-6-phosphate.

(5) *Control groups*—(i) *Concurrent controls.* Concurrent positive and negative (untreated and/or vehicle) controls should be included in each experiment. Positive controls should ensure both strain responsiveness and efficacy of the metabolic activation system.

(ii) *Strain specific positive controls.* Strain specific positive controls should be included in the assay. Examples of strain specific positive controls are as follows:

(A) Strain TA 1535, TA 100, sodium azide.

(B) TA 98, 2-nitrofluorene.

(C) TA 1537, 9-aminoacridine.

(iii) *Positive controls to ensure the efficacy of the activation system.* The positive control reference substance for tests including a metabolic activation system should be selected on the basis of the type of activation system used in the test. 2-Aminoanthracene is an example of a positive control compound in plate-incorporation tests using postmitochondrial fractions from the livers of rodents treated with enzyme inducing agents such as Aroclor-1254. Congo red is an example of a positive control compound in the azo-reduction method. Other positive control reference substances may be used.

(iv) *Class-specific positive controls.* The azo-reduction method should include positive controls from the same class of compounds as the test agent wherever possible.

(6) *Test chemicals*— (i) *Vehicle.* Test chemicals and positive control reference substances should be dissolved in an appropriate vehicle and then further diluted in vehicle for use in the assay.

(ii) *Exposure concentrations.* (A) The test should initially be performed over a broad range of concentrations. Among the criteria to be taken into consideration for determining the upper limits of test chemical concentration are cytotoxicity and solubility. Cytotoxicity of the test chemical may be altered in the presence of metabolic activation systems. Toxicity may be evidenced by a reduction in the number of spontaneous revertants, a clearing of the background lawn or by the degree of survival of treated cultures. Relatively insoluble compounds should be tested up to the limits of solubility. For freely soluble nontoxic chemicals, the upper test chemical concentration should be determined on a case by case basis.

(B) Generally, a maximum of 5 mg/plate for pure substances is considered acceptable. At least 5 different amounts of test substance should be tested with adequate intervals between test points.

(C) When appropriate, a positive response should be confirmed by testing over a narrow range of concentrations.

(e) *Test performance*—(1) *Direct plate incorporation method.* For this test without metabolic activation, test chemical and 0.1 ml of a fresh bacterial culture should be added to 2.0 ml of overlay agar. For tests with metabolic activation, 0.5 ml of activation mixture containing an adequate amount of postmitochondrial fraction should be added to the agar overlay after the addition of test chemical and bacteria. Contents of each tube should be mixed and poured over the surface of a selective agar plate. Overlay agar should be allowed to solidify before incubation. At the end of the incubation period, revertant colonies per plate should be counted.

(2) *Azo-reduction method.* (i) For this test with metabolic activation, 0.5 ml of S–9 mix containing 150 μl of S–9 and 0.1 ml of bacterial culture should be added to a test tube kept on ice. One-tenth ml of chemical should be added and the tubes should be incubated with shaking at 30 °C for 30 min. At the end of the incubation period, 2.0 ml of agar should be added to each tube, the contents mixed and poured over the surface of a selective agar plate. Overlay agar should be allowed to solidify before incubation. At the end of the incubation period, revertant colonies per plate should be counted.

(ii) For tests without metabolic activation, 0.5 ml of buffer should be used in place of the 0.5 ml of S–9 mix. All other procedures should be the same as those used for the test with metabolic activation.

(3) *Other methods.* Other methods may also be appropriate.

(4) *Media.* An appropriate selective medium with an adequate overlay agar should be used.

(5) *Incubation conditions.* All plates within a given experiment should be incubated for the same time period. This incubation period should be for 48–72 hours at 37 °C.

(6) *Number of cultures.* In general, all plating should be done at least in triplicate. If scientifically justified, the use of duplicates may be acceptable. All results should be confirmed in an independent experiment.

(f) *Data and report*— (1) *Treatment of results.* Data should be presented as number of revertant colonies per plate for each replicate and dose. The numbers of revertant colonies on both negative (untreated and/or vehicle) and positive control plates should also be presented. Individual plate counts, the mean number of revertant colonies per plate and standard deviation should be presented for test chemical and positive and negative (untreated and/or vehicle) controls.

(2) *Statistical evaluation.* Data should be evaluated by appropriate statistical methods.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive result, one of which is a

statistically significant dose-related increase in the number of revertants. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test substance concentrations.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of revertants of a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* (i) Positive results from the *S. typhimurium* reverse mutation assay indicate that, under the test conditions, the test substance induces point mutations by base changes or frameshifts in the genome of this organism.

(ii) Negative results indicate that under the test conditions the test substance is not mutagenic in *S. typhimurium.*

(5) *Test report.* In addition to the reporting recommendations as specified under 40 CFR Part 792, Subpart J, the following specific information should be reported:

(i) Bacterial strain used.

(ii) Metabolic activation system used and its preparation for use in the assay; for S-9 preparations this should include cofactor cocktail (contents, storage conditions and amount used); source and amount of S-9 and details of preparation and storage of S-9 mix.

(iii) Dose levels and rationale for selection of dose.

(iv) Positive and negative controls.

(v) Individual plate counts, mean number of revertant colonies per plate, standard deviation.

(vi) Dose-response relationship, if applicable.

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Ames, B.N., McCann, J., Yamasaki, E. "Methods for detecting carcinogens and mutagens with the *Salmonella/* mammalian-microsome mutagenicity test," *Mutation Research* 31:347–364 (1975).

(2) de Serres, F.J., Shelby, M.D. "The *Salmonella* mutagenicity assay: recommendations," *Science* 203:563–565 (1979).

(3) Prival, M.J., Mitchell, V.D. "Analysis of a method for testing azo dyes for mutagenic activity in *Salmonella typhimurium* in the presence of flavin mononucleotide and hamster liver S-9," *Mutation Research* 97:103–116 (1982).

(4) Vogel, H.J., Bonner, D.M. "Acetylornithinase of *E. coli:* partial purification and some properties," *Journal of Biological Chemistry*. 218:97–106 (1956).

§ 789.5275 Sex-linked recessive lethal test in Drosophila melanogaster.

(a) *Purpose.* The sex-linked recessive lethal (SLRL) test using *Drosophila melanogaster* detects the occurrence of mutations, both point mutations and small deletions, in the germ line of the insect. This test is a forward mutation assay capable of screening for mutations at about 800 loci on the X-chromosome. This represents about 80 percent of all X-chromosome loci. The X-chromosome represents approximately one-fifth of the entire haploid genome.

(b) *Definitions.* (1) Lethal mutation is a change in the genome which, when expressed, causes death to the carrier.

(2) Recessive mutation is a change in the genome which is expressed in the homozygous or hemizygous condition.

(3) Sex-Linked genes are present on the sex (X or Y) chromosomes. Sex-linked genes in the context of this guideline refer only to those located on the X-chromosome.

(c) *Reference substances.* These may include, but need not be limited to, ethyl methanesulfonate or N-nitrosodimethylamine.

(d) *Test method*—(1) *Principle.* Mutations in the X-chromosome of *D. melanogaster* are phenotypically expressed in males carrying the mutant gene. When the mutation is lethal in the hemizygous condition, its presence is inferred from the absence of one class of male offspring out of the two that are normally produced by a heterozygous female. The SLRL test takes advantage of these facts by means of specially marked and arranged chromosomes.

(2) *Description.* Wild-type males are treated and mated to appropriate females. Female offspring are mated individually to their brothers, and in the next generation the progeny from each separate dose are scored for phenotypically wild-type males. Absence of these males indicates that a sex-linked recessive lethal mutation has occurred in a germ cell of the P_1 male.

(3) *Drosophila stocks.* Males of a well-defined wild type stock and females of the Muller-5 stock may be used. Other appropriately marked female stocks with multiple inverted X-chromosomes may also be used.

(4) *Control groups*—(i) *Concurrent controls.* Concurrent positive and negative (vehicle) controls should be included in each experiment.

(ii) *Positive controls.* Examples of positive controls include ethyl methanesulfonate and N-nitrosodimethylamine.

(iii) *Other positive controls.* Other positive control reference substances may be used.

(iv) *Negative controls.* Negative (vehicle) controls should be included. The size of the negative (vehicle) control group should be determined by the availability of appropriate laboratory historical control data. If the historical control data are of sufficient numbers, concurrent controls may not be necessary.

(5) *Test chemicals*—(i) *Vehicle.* Test chemicals should be dissolved in water. Compounds which are insoluble in water may be dissolved or suspended in appropriate vehicles (e.g., a mixture of ethanol and Tween-60 or 80) and then diluted in water or saline prior to administration. Dimethylsulfoxide should br avoided as a vehicle.

(ii) *Dose levels.* For the initial assessment of mutagenicity, it may be sufficient to test a single dose of the test substance. This dose should be the maximum tolerated dose or that which produces some indication of toxicity. If the test is being used to verify mutagenic activity in this system at least two additional exposure levels should be used.

(iii) *Route of administration.* Exposure may be oral, by injection or by exposure to gases or vapors. Feeding of the test compound may be done in sugar solution. When necessary, substances may be dissolved in 0.7 percent NaCl solution and injected into the thorax or abdomen.

(e) *Test performance*—(1) *Treatment and mating.* Wild-type males (3 to 5 days old) should be treated with the test substance and mated individually to an excess of virgin females from the Muller-5 stock or females from another appropriately marked (with multiply-inverted X-chromosomes) stock. The females should be replaced with fresh virgins every 2 to 3 days to cover the entire germ cell cycle. The offspring of these females are scored for lethal effects corresponding to the effects on mature sperm, mid or late stage spermatids, early spermatids, spermatocytes and spermatogonia at the time of treatment.

(2) *F1 matings.* Heterozygous F_1 females from the above crosses should be allowed to mate individually (i.e., one female per vial) with their brothers. In the F_2 generation, each culture should be scored for the absence of wild-type

males. If a culture appears to have arisen from an F_1 female carrying a lethal in the parental X-chromosome (i.e., no males with the treated chromosome are observed), daughters of that female with the same genotype should be tested to ascertain if the lethality is repeated in the next generation.

(3) *Number of matings.* (i) The test should be designed with a predetermined sensitivity and power. The number of flies in each group should reflect these defined parameters. The spontaneous mutant frequency observed in the appropriate control group will strongly influence the number of treated chromosomes that must be analysed to detect substances which show mutation rates close to those of the controls.

(ii) Test results should be confirmed in a separate experiment.

(f) *Data and report*—(1) *Treatment of results.* Data should be tabulated to show the number of chromosomes tested, the number of nonfertile males and the number of lethal chromosomes at each exposure concentration and for each mating period for each male treated. Numbers of clusters of different size per male should be reported.

(2) *Statistical evaluation.* Data should be evaluated by appropriate statistical techniques.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of sex-lined recessive lethals. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test points.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of sex-linked recessive lethals or a statistically significant and reproducible positive response at any one of the test points is considered non-mutagenic in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* (i) Positive results in the SLRL test in *D. melanogaster* indicate that under the test conditions the test agent causes mutations in germ cells of this insect.

(ii) Negative results indicate that under the test conditions the test substance is not mutagenic in *D. melanogaster.*

(5) *Test report.* In addition to the reporting recommendations as specified under 40 CFR Part 792, Subpart J the following specific information should be reported.

(i) *Drosophila* stock used in the assay, age of insects, number of males treated, number of sterile males, number of F_2 cultures established, number of F_2 cultures without progeny.

(ii) Test chemical vehicle, treatment and sampling schedule, exposure levels, toxicity data, negative (vehicle) and positive controls, if appropriate.

(iii) Criteria for scoring lethals.

(iv) Number of chromosomes tested, number of chromosomes scored, number of chromosomes carrying a lethal mutation.

(v) Historical control data, if available.

(vi) Dose-response relationship, if applicable.

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Sobels, F.H., Vogel, E. "The capacity of *Drosophila* for detecting relevant genetic damage," *Mutation Research* 41:95–106 (1976).

(2) Wurgler F.E., Sobels F.H., Vogel E. "*Drosophila* as assay system for detecting genetic changes," *Handbook of mutagenicity test procedures.* Eds. Kilbey, B.J., Legator, M., Nichols, W., Ramel, C., (Amsterdam: Elsevier/North Holland Biomedical Press, 1977) pp. 335–373.

§ 798.5300 Detection of gene mutations in somatic cells in culture.

(a) *Purpose.* Mammalian cell culture systems may be used to detect mutations induced by chemical substances. Widely used cell lines include L5178Y mouse lymphoma cells and the CHO and V–79 lines of Chinese hamster cells. In these cell lines the most commonly used systems measure mutation at the thymidine kinase (TK), hypoxanthine-guanine-phosphoribosyl transferase (HPRT) and Na^+/K^+ ATPase loci. The TK and HPRT mutational systems detect base pair mutations, frameshift mutations, and small deletions; the Na^+/K^+ ATPase system detects base pair mutations only.

(b) *Definitions.* (1) A forward mutation assay detects a gene mutation from the parental type to the mutant form which gives rise to a change in an enzymatic or functional protein.

(2) Base pair mutagens are agents which cause a base change in the DNA.

(3) Frameshift mutagens are agents which cause the addition or deletion of single or multiple base pairs in the DNA molecule.

(4) Phenotypic expression time is a period during which unaltered gene products are depleted from newly mutated cells.

(c) *Reference substances.* These may include, but need not be limited to, ethyl methanesulfonate, N-nitrosodimethylamine, 2-acetylaminofluorene, 7,12-dimethylbenzanthracene or hycanthone.

(d) *Test method*—(1) *Principle.* Cells are exposed to test substance, both with and without metabolic activation, for a suitable period of time and subcultured to determine cytotoxicity and to allow phenotypic expression prior to mutant selection. Cells deficient in thymidine kinase (TK) due to the forward mutation $TK^+ \rightarrow TK^-$ are resistant to the cytotoxic effects of pyrimidine analogues such as bromodeoxyuridine (BrdU), fluorodeoxyuridine (FdU) or trifluorothymidine (TFT). The deficiency of the "salvage" enzyme thymidine kinase means that these antimetabolites are not incorporated into cellular nucleotides and the nucleotides needed for cellular metabolism are obtained solely from *de novo* synthesis. However, in the presence of thymidine kinase, BrdU, FdU or TFT are incorporated into the nucleotides, resulting in inhibition of cellular metabolism and cytotoxicity. Thus mutant cells are able to proliferate in the presence of BrdU, FdU or TFT whereas normal cells, which contain thymidine kinase, are not. Similarly cells deficient in HPRT are selected by resistance to 8-azaguanine (AG) or 6-thioguanine (TG) and cells with altered Na^+/K^+ ATPase are selected by resistance to ouabain.

(2) *Description.* Cells in suspension or monolayer culture are exposed to the test substance, both with and without metabolic activation, for a defined period of time. Cytotoxicity is determined by measuring the colony forming ability or growth rate of the cultures after the treatment period. The treated cultures are maintained in growth medium for a sufficient period of time—characteristic of each selected locus—to allow near-optimal phenotypic expression of induced mutations. Mutant frequency is determined by seeding known numbers of cells in medium containing the selective agent to detect mutant cells, and in medium without selective agent to determine the cloning efficiency. After a suitable incubation time, cell colonies are counted. The number of mutant colonies in selective medium is adjusted by the number of colonies in nonselective medium to derive the mutant frequency.

(3) *Cells*—(i) *Type of cells used in the assay.* A variety of cell lines are available for use in this assay including subclones of L5178Y, CHO cells or V–79 cells. Cell types used in this assay should have a demonstrated sensitivity

to chemical mutagens, a high cloning efficiency and a low spontaneous mutation frequency. Cells should be checked for *Mycoplasma* contamination and may be periodically checked for karyotype stability.

(ii) *Cell growth and maintenance.* Appropriate culture media and incubation conditions (culture vessels, CO_2 concentrations, temperature and humidity) should be used.

(4) *Metabolic activation.* Cells should be exposed to test substance both in the presence and absence of an appropriate metabolic activation system.

(5) *Control groups.* Positive and negative (untreated and/or vehicle) controls should be included in each experiment. When metabolic activation is used, the positive control substance should be known to require such activation.

(6) *Test chemicals*—(i) *Vehicle.* Test substances may be prepared in culture media or dissolved or suspended in appropriate vehicles prior to treatment of the cells. The final concentration of the vehicle should not interfere with cell viability or growth rate.

(ii) *Exposure concentrations.* (A) The test should be designed to have a predetermined sensitivity and power. The number of cells, cultures, and concentrations of test substance used should reflect these defined parameters. The number of cells per culture is based on the expected background mutant frequency; a general guide is to use a number which is 10 times the inverse of this frequency.

(B) Several concentrations (usually at least four) of the test substance should be used. These should yield a concentration-related toxic effect. The highest concentration should produce a low level of survival and the survival in the lowest concentration should approximate the negative control. Cytotoxicity should be determined after treatment with the test substance both in the presence and in the absence of an exogenous metabolic activation system. Relatively insoluble substances should be tested up to their limit of solubility under culture conditions. For freely-soluble nontoxic substances the highest concentration used should be determined on a case-by-case basis.

(e) Test performance. (1) Cells should be exposed to the test substance both with and without metabolic activation. Exposure should be for a suitable period of time, in most cases 1 to 5 hours is effective; exposure time may be extended over one or more cell cycles.

(2) At the end of the exposure period, cells should be washed and cultured to determine viability and to allow for expression of the mutant phenotype.

(3) At the end of the expression period, which should be sufficient to allow near optimal phenotypic expression of induced mutants, cells should be grown in medium with and without selective agent(s) for determination of numbers of mutants and closing efficiency, respectively.

(4) Results should be confirmed in an independent experiment.

(f) *Data and report*—(1) *Treatment of results.* Data should be presented in tabular form. Indivtdual colony counts for the treated and control groups should be presented for both mutation induction and survival. Survival and cloning efficiencies should be given as a percentage of the controls. Mutant frequency should be expressed as number of mutants per number of surviving cells.

(2) *Statistical evaluation.* Data should be evaluated by appropriate statistical methods.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive result, one of which is a statistically significant concentration-related increase in the mutant frequency. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test substance concentrations.

(ii) A test substance which does not produce either a statistically significant concentration-related increase in the mutant frequency or a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* (i) Positive results for an in vitro mammalian cell gene mutation test indicate that, under the test conditions, a substance induces gene mutations in the cultured mammalian cells used.

(ii) Negative results indicate that, under the test conditions, the test substance does not induce gene mutations in the cultured mammalian cells used.

(5) *Test report.* In addition to the reporting recommendations as specified under 40 CFR Part 792, Subpart J the following specific information should be reported:

(i) Cell type used, number of cell cultures, methods used for maintenance of cell cultures.

(ii) Rationale for selection of concentrations and number of cultures.

(iii) Test conditions: composition of media, CO_2 concentration, concentration of test substance, vehicle, incubation temperature, incubation time, duration of treatment, cell density during treatment, type of metabolic activation system, positive and negative controls, length of expression period (including number of cells seeded and subculture and feeding schedules, if appropriate), selective agent(s).

(iv) Methods used to enumerate numbers of viable and mutant cells.

(v) Dose-response relationship, where possible.

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Amacher, D.E., Paillet, S.C., Ray, V. "Point mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells. I. Application to genetic toxicology testing," *Mutation Research*, 64:391–406 (1979).

(2) Amacher, D.E., Paillet, S.C., Turner, G.N., Ray, V.A. Salsburg, V.A. "Point mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells. II. Test validation and interpretation," *Mutation Research*, 72:447–474 (1980).

(3) Bradley, M.O., Bhuyan B., Francis, M.C., Langenback, R., Peterson, A., Huberman, E. "Mutagenesis by chemical agents in V–79 Chinese hamster cells: a review and analysis of the literature: a report of the Gene-Tox Program," *Mutation Research*, 87:81–142 (1981).

(4) Clive, D., Johnson, K.O., Spector, J.F.S., Batson, A.G., Brown, M.M. "Validation and characterization of the L5178Y $TK^+/^-$ mouse lymphoma mutagen assay system," *Mutation Research*, 59:61–108 (1979).

(5) Clive, D., Spector, J.F.S. "Laboratory procedures for assessing specific locus mutations at the TK locus in cultured L5178Y mouse lymphoma cells," *Mutation Research*, 31:17–29 (1975).

(6) Hsie, A.W., Casciano, D.A., Couch, D.B., Krahn, D.F., O'Neill, J.P., Whitfield, B.L. "The use of Chinese hamster ovary cells to quantify specific locus mutation and to determine mutagenicity of chemicals: a report of the U.S. EPA's Gene-Tox Program," *Mutation Research*, 86:193–214 (1981).

§ 798.5375 In vitro mammalian cytogenetics.

(a) *Purpose.* The in vitro cytogenetics test is a mutagenicity test system for the detection of chromosomal aberrations in cultured mammalian cells. Chromosomal aberrations may be either structural or numerical. However, because cytogenetic assays are usually designed to analyse cells at their first post-treatment mitosis and numerical aberrations require at least one cell division to be visualized, this type of aberration is generally not observed in a routine cytogenetics assay. Structural aberrations may be of two types, chromosome or chromatid.

(b) *Definitions.* (1) Chromosome-type aberrations are changes which result from damage expressed in both sister chromatids at the same time.

(2) Chromatid-type aberrations are damage expressed as breakage of single chromatids or breakage and/or reunion between chromatids.

(c) *Reference substances.* Not applicable.

(d) *Test method*—(1) *Principle.* In vitro cytogenetics assays may employ cultures of established cell lines, cell strains or primary cell cultures. Cell cultures are exposed to the test substance both with and without metabolic activation. Following exposure of cell cultures to test substances, they are treated with a spindle inhibitor (e.g., colchicine or Colcemid®) to arrest cells in a metaphase-like stage of mitosis (c-metaphase). Cells are then harvested and chromosome preparations made. Preparations are stained and metaphase cells are analyzed for chromosomal aberrations.

(2) *Description.* Cell cultures are exposed to test compounds and harvested at various intervals after treatment. Prior to harvesting, cells are treated with a spindle inhibitor (e.g., colchicine or Colcemid®) to accumulate cells in c-metaphase. Chromosome preparations from cells are made, stained and scored for chromosomal aberrations.

(3) *Cells*—(i) *Type of cells used in the assay.* There are a variety of cell lines or primary cell cultures, including human cells, which may be used in the assay. Established cell lines and strains should be checked for *Mycoplasma* contamination and may be periodically checked for karyotype stability.

(ii) *Cell growth and maintenance.* Appropriate culture media, and incubation conditions (culture vessels CO_2 concentrations, temperature and humidity) should be used.

(4) *Metabolic activation.* Cells should be exposed to test substance both in the presence and absence of an appropriate metabolic activation system.

(5) *Control groups.* Positive and negative (untreated and/or vehicle) controls both with and without metabolic activation should be included in each experiment. When metabolic activation is used, the positive control substance should be known to require such activation.

(6) *Test chemicals*—(i) *Vehicle.* Test substances may be prepared in culture media or dissolved or suspended in appropriate vehicles prior to treatment of the cells. Final concentration of the vehicle should not interfere with cell viability or growth rate.

(ii) *Exposure concentrations.* Multiple concentrations of the test substance over a range adequate to define the response should be tested. The highest test substance concentration tested with and without metabolic activation should show evidence or cytotoxicity or reduced mitotic activity. Relatively insoluble substances should be tested up to the limit of solubility. For freely soluble nontoxic chemicals, the upper test chemical concentration should be determined on a case by case basis.

(e) *Test performance*—(1) *Established cell lines and strains.* Prior to use in the assay, cells should be generated from stock cultures, seeded in culture vessels at the appropriate density and incubated at 37 °C.

(2) *Human lymphocyte cultures.* Heparinized or acid-citrate-dextrose whole blood should be added to culture medium containing a mitogen, e.g., phytohemagglutinin (PHA) and incubated at 37 °C. White cells sedimented by gravity (buffy coat) or lymphocytes which have been purified on a density gradient may also be utilized.

(3) *Treatment with test substance.* For established cell lines and strains, cells in the exponential phase of growth should be treated with test substances in the presence and absence of a metabolic activation system. Mitogen-stimulated human lymphocyte cultures may be treated with the test substance in a similar manner.

(4) *Number of cultures.* At least two independent cultures should be used for each experimental point.

(5) *Culture harvest time.* (i) For established cell lines and strains multiple harvest times are recommended. However, for screening purposes, a single harvest time, e.g. 24 hrs, may be appropriate. If the test chemical changes the cell cycle length, the fixation intervals should be changed accordingly.

(ii) For human lymphocyte cultures, the substance to be tested may be added to the cultures at various times after mitogen stimulation so that there is a single harvest time after the initiation of the cell culture. Alternatively, a single treatment may be followed by multiple harvest times. Harvest time should be extended for those chemicals which induce an apparent cell cycle delay. Because the population of human lymphocytes is only partially synchronized, a single treatment, at, or close to, the time when metaphase stages first appear in the culture will include cells in all phases of the division cycle. Therefore, a single harvest at the time of second mitosis may be carried out for screening purposes.

(iii) Cell cultures are treated with a spindle inhibitor, (e.g. colchicine or Colcemid®). 1 or 2 hours prior to harvesting. Each culture is harvested and processed separately for the preparation of chromosomes.

(6) *Chromosome preparation.* Chromosome preparation involves hypotonic treatment of the cells, fixation and staining.

(7) *Analysis.* Slides should be coded before analysis. The number of cells to be analysed should be based upon the spontaneous control frequency, defined sensitivity and the power chosen for the test before analysis. In human lymphocytes, only cells containing 46 centromeres should be analysed. In established cell lines and strains, only metaphases containing ±2 centromeres of the modal number should be analysed. Uniform criteria for scoring aberrations should be used.

(f) *Data and report*—(1) *Treatment of results.* Data should be presented in a tabular form. Different types of structural chromosomal aberrations should be listed with their numbers and frequencies for experimental and control groups. Data should be evaluated by appropriate statistical methods. Gaps or achromatic lesions are recorded separately and not included in the total aberration frequency.

(2) *Statistical evaluation.* Data should be evaluated by appropriate statistical methods.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of structural chromosomal aberrations. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test substance concentrations.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of structural chromosomal aberrations or a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* (i) Positive results in the in vitro cytogenetics assay indicate that under the test conditions the test substance induces chromosomal aberrations in cultured mammalian somatic cells.

(ii) Negative results indicate that under the test conditions the test substance does not induce chromosomal aberrations in cultured mammalian somatic cells.

(5) *Test report.* In addition to the reporting recommendations as specified under 40 CFR Part 792, Subpart J the following specific information should be reported:

(i) Cells used, density and passage number at time of treatment, number of cell cultures.

(ii) Methods used for maintenance of cell cultures including medium, temperature and CO_2 concentration.

(iii) Test chemical vehicle, concentration and rationale for the selection of the concentrations used in the assay, duration of treatment.

(iv) Details of both the protocol used to prepare the metabolic activation system and of its use in the assay.

(v) Identity of spindle inhibitor, its concentration and duration of treatment.

(vi) Time of cell harvest.

(vii) Positive and negative controls.

(viii) Methods used for preparation of slides for microscopic examination.

(ix) Number of metaphases analysed.

(x) Mitotic index where applicable.

(xi) Criteria for scoring aberrations.

(xii) Type and number of aberrations, given separately for each treated and control culture, total number of aberrations per group; frequency distribution of number of chromosomes in established cell lines and strains.

(xiii) Dose-response relationship, if applicable.

(g) *References.* For additional background information on this test guideline the following references should be consulted.

(1) Ames, B.N., McCann, J., Yamasaki, E. "Methods for detecting carcinogens and mutagens with the *Salmonella/* mammalian-microsome mutagenicity test," *Mutation Research,* 31:347–364 (1975).

(2) Evans, H.J. "Cytological methods for detecting chemical mutagens," *Chemical mutagens, principles and methods for their detection,* Vol. 4, Ed. A. Hollaender (New York, London: Plenum Press, 1976) pp. 1–29.

(3) Howard, P.N., Bloom, A.D., Krooth, R.S. "Chromosomal aberrations induced by N-methyl-N'-nitro-N-nitrosoguanidine in mammalian cells," *In Vitro* 7:359–365 (1972).

(4) Ishidate, M. Jr., Odashima, S. "Chromosome tests with 134 compounds on Chinese hamster cells in vitro: a screening for chemical carcinogens," *Mutation Research,* 48:337–354 (1975).

(5) Preston, R.J., Au, W., Bender, M.A., Brewen, J.G., Carrano, A.V., Heddle, J.A., McFee, A.F., Wolff, S., Wassom, J.S., "Mammalian in vivo and in vitro cytogenetic assays: a report of the Gene-tox Program," *Mutation Research,* 87:143–188 (1981).

§ 798.5385 In vivo mammalian bone marrow cytogenetics tests: Chromosomal analysis.

(a) *Purpose.* The *in vivo* bone marrow cytogenetic test is a mutagenicity test for the detection of structural chromosomal aberrations. Chromosomal aberrations are generally evaluated in first post-treatment mitoses. With the majority of chemical mutagens, induced aberrations are of the chromatid type but chromosome type aberrations also occur.

(b) *Definitions.* (1) Chromosome-type aberrations are changes which result from damage expressed in both sister chromatids at the same time.

(2) Chromatid-type aberrations are damage expressed as breakage of single chromatids or breakage and/or reunion between chromatids.

(c) *Reference substances.* Not applicable.

(d) *Test method*—(1) *Principle.* Animals are exposed to test chemicals by appropriate routes and are sacrificed at sequential intervals. Chromosome preparations are made from bone marrow cells. The stained preparations are examined and metaphase cells are scored for chromosomal aberrations.

(2) *Description.* The method employs bone marrow of laboratory rodents which have been exposed to test chemicals. Prior to sacrifice, animals are further treated with a spindle inhibitor, (e.g., colchicine or Colcemid ®) to arrest the cells in c-metaphase. Chromosome preparations from the cells are stained and scored for chromosomal aberrations.

(3) *Animal selection*—(i) *Species and strain.* Any appropriate mammalian species may be used. Examples of commonly used rodent species are rats, mice, and hamsters.

(ii) *Age.* Healthy young adult animals should be used.

(iii) *Number and sex.* At least five female and five male animals per experimental and control group should be used. Thus, 10 animals would be sacrificed per time per group treated with the test compound if several test times after treatment are included in the experimental schedule. The use of a single sex or different number of animals should be justified.

(iv) *Assignment to groups.* Animals should be randomized and assigned to treatment and control groups.

(4) *Control groups*—(i) *Concurrent controls.* Concurrent positive and negative (vehicle) controls should be included in the assay.

(ii) *Positive controls.* A single dose positive control showing a significant response at any one time point is adequate. A compound known to produce chromosomal aberrations in vivo should be employed as the positive control.

(5) *Test chemicals*—(i) *Vehicle.* When possible, test chemicals should be dissolved in isotonic saline or distilled water. Water insoluble chemicals may be dissolved or suspended in appropriate vehicles. The vehicles used should neither interfere with the test chemical nor produce toxic effects. Fresh preparations of the test compound should be employed.

(ii) *Dose levels.* For an initial assessment, one dose of the test substance may be used, the dose being the maximum tolerated dose or that producing some indication of cytotoxicity (e.g., partial inhibition of mitosis). Additioinal dose levels may be used. For determintation of dose-response, at least three dose levels should be used.

(iii) *Route of administration.* The usual routes are oral or by intraperitoneal injection. Other routes may be appropriate.

(iv) *Treatment schedule.* In general, test substances should be administered once only. However, based on toxicological information a repeated treatment schedule may be employed.

(e) *Test performance*—(1) *Generally the test may be performed in two assays.* (i) Animals are treated with the test substance once at the selected dose(s). Samples are taken at three times after treatment. For rodents, the central sampling interval is 24 hours. Since cell cycle kinetics can be influenced by the test substance, one earlier and one later sampling interval adequately spaced within the range of 6 to 48 hours should be applied. Where the additional dose levels are tested in a subsequent experiment, samples should be taken at the predetermined most sensitive interval or, if this is not established, at the central sampling time.

(ii) If a repeated treatment schedule is used at the selected dose(s), samples should be taken 6 and 24 hours after the last treatment; other sampling times may be used if justified. Where the additional dose levels are tested in a subsequent experiment, samples should be taken at the predetermined most sensitive interval or, if this is not established, at 6 hours after the last treatment.

(2) *Administration of spindle inhibitor.* Prior to sacrifice, animals should be injected IP with an appropriate dose of a spindle inhibitor (e.g., colchicine or Colcemid®) to arrest cells in c-metaphase.

(3) *Preparation of slides.* Immediately after sacrifice, the bone marrow should be obtained, exposed to hypotonic solution, and fixed. The cells should then be spread on slides and stained. Chromosome preparations should be made following standard procedures.

(4) *Analysis.* The number of cells to be analysed per animal should be based upon the number of animals used, the negative control frequency, the predetermined sensitivity and the power chosen for the test. Slides should be coded before microscopic analysis.

(f) *Data and report*—(1) *Treatment of results.* Data should be presented in tabular form for both cells and animals. Different types of structural chromosomal aberrations should be listed with their numbers and a mean frequency per cell for each animal in all treated and control groups. Gaps (achromatic lesions) should be recorded separately and not included in the total abberration frequency. Differences among animals within each group should be considered before making comparisons between treated and control groups.

(2) *Statistical evaluation.* Data should be evaluated by appropriate statistical methods.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of structual chromosomal aberrations or abnormal metaphase figures. Another criterion may be based upon detection of a reproducible and statistically significant positive response for a least one of the test points.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of chromosomal aberrations or abnormal metaphase figures or a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* (i) Positive results in the *in vivo* bone marrow cytogenetics assay indicate that under the test conditions the test substance induces chromosomal aberrations in the bone marrow of the test species.

(ii) Negative results indicate that under the test conditions, the test substance does not induce chromosomal aberrations in the bone marrow of the test species.

(5) *Test report.* In addition to the reporting recommendations as specified under 40 CFR Part 792, Subpart J the following specific information should be reported:

(i) Species, strain, age, weight, number and sex of animals in each treatment and control group.

(ii) Test chemical vehicle, dose levels used, rationale for dose selection.

(iii) Route of administration, treatment and sampling schedules, toxicity data, negative and positive controls.

(iv) Identity of spindle-inhibitor, its concentration and duration of treatment.

(v) Details of the protocol used for chromosome preparation, number of cells scored per animal, type and number of aberrations given separately for each treated and control animal.

(vi) Mitotic index, where applicable.

(vii) Criteria for scoring aberrations.

(viii) Number and frequency of aberrant cells per animal in each treatment and control groups.

(ix) Total number of aberrations per group.

(x) Number of cells with aberrations per group.

(xi) Dose-response relationship, if applicable.

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Adler, I.D., Ramarao, G., Epstein, S.S. "In vivo cytogenetic effects of trimethyl-phosphate and of TEPA on bone marrow cells of male rats," *Mutation Research,* 13:263–273 (1971).

(2) Evans, H.J. "Cytological methods for detecting chemical mutagens," *Chemical Mutagens: Principles and Methods for Their Detection,* Vol. 4. Ed. A. Hollaender (New York and London: Plenum Press, 1976) pp. 1–29.

(3) Kilian, J.D., Moreland, F.E. Benge, M.C., Legator, M.S., Whorton, E.B. Jr. "A collaborative study to measure intralaboratory variation with the in vivo bone morrow metaphase procedure," *Handbook of mutagenicity test procedures.* Eds. Kilby, B.J., Legator, M. Nichols, C., Ramel, D., (Amsterdam: Elsevier/North Holland Biomedical Press, 1977) 243–260.

(4) Preston, J.R., Au, W., Bender, M.A., Brewen, J.G., Carrano, A.V. Heddle, J.A., McFee, A.F., Wolff, S., Wassom, J. "Mammalian in vivo and vitro cytogenetics assays: report of the Gene-Tox Program," *Mutation Research, 87:143–188 (1981).*

§ 798.5395 In vivo mammalian bone marrow cytogenetics tests: Micronucleus assay.

(a) *Purpose.* The micronucleus test is a mammalian *in vivo* test which detects damage of the chromosomes or mitotic apparatus by chemicals. Polychromatic erythrocytes in the bone marrow of rodents are used in this assay. When the erythroblast develops into an erythrocyte the main nucleus is extruded and may leave a micronucleus in the cytoplasm. The visualization of micronuclei is facilitated in these cells because they lack a nucleus. Micronuclei form under normal conditions. The assay is based on an increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow of treated animals.

(b) *Definition.* Micronuclei are small particles consisting of acentric fragments of chromosomes or entire chromosomes, which lag behind at anaphase of cell division. After telophase, these fragments may not be included in the nuclei of daughter cells and form single or multiple micronuclei in the cytoplasm.

(c) *Reference substances.* Not applicable.

(d) *Test method*—(1) *Principle.* Animals are exposed to test substance by an appropriate route. They are sacrificed, the bone marrow extracted and smear preparations made and stained. Polychromatic erythrocytes are scored for micronuclei under the microscope.

(2) *Description.* The method employs bone marrow of laboratory mammals which are exposed to test substances.

(3) *Animal selection*—(i) *Species and strain.* Mice are recommended. However, any appropriate mammalian species may be used.

(ii) *Age.* Healthy young adult animals should be used.

(iii) *Number and sex.* At least five female and five male animals per experimental and control group should be used. Thus, 10 animals would be sacrificed per time per group if several test times after treatment were included in the experimental schedule. The use of a single sex or a different number of animals should be justified.

(iv) *Assignment to groups.* Animals should be randomized and assigned to treatment and control groups.

(4) *Control groups*—(i) *Concurrent controls.* Concurrent positive and negative (vehicle) controls should be included in each assay.

(ii) *Positive controls.* A compound known to produce micronuclei *in vivo* should be employed as the positive control.

(5) *Test chemicals*—(i) *Vehicle.* Solid and liquid test substances should be dissolved or suspended in distilled water or isotonic saline. Water insoluble chemicals may be dissolved or suspended in appropriate vehicles. The vehicle used should neither interfere with the test compound nor produce toxic effects. Fresh preparations of the test compound should be employed.

(ii) *Dose levels.* For an initial assessment, one dose of the test substance may be used, the dose being the maximum tolerated dose or that producing some indication of cytotoxicity, e.g. a change in the ratio of polychromatic to normochromatic erythrocytes. Additional dose levels may be used. For determination of dose response, at least three does levels should be used.

(iii) *Route of administration.* The usual routes of administration are IP or oral. Other routes may be appropriate.

(iv) *Treatment schedule.* Test substances should generally be administered only once. However, based upon toxicological information a repeated treatment schedule may be employed.

(e) *Test performance*—(1) *Treatment and sampling times.* (i) Animals should be treated with the test substance once at the highest tolerated dose. Sampling times should coincide with the maximum response of the assay which varies with the test substance. Therefore, using the highest dose, bone marrow samples should be taken at least three times, starting not earlier than 12 hours after treatment, with appropriate intervals following the first sample but not extending beyond 72 hours. When other doses are used sampling should be at the maximum sensitive period, or, if that is not known, approximately 24 hours after treatment. Other appropriate sampling times may be used in addition.

(ii) If a repeated treatment schedule is used, samples should be taken at least three times, starting not earlier than 12 hours after the last treatment and at appropriate intervals following the first sample, but not extending beyond 72 hours.

(iii) Bone marrow should be obtained immediately after sacrifice. Cells should be prepared, put on slides, spread as a smear and stained.

(2) *Analysis.* Slides should be coded before microscopic analysis. At least 1000 polychromatic erythrocytes per animal should be scored for the incidence of micronuclei. The ratio of polychromatic to normochromatic erythrocytes should be determined for each animal by counting a total of 1000 erythrocytes. Additional information may be obtained by scoring normochromatic erythrocytes for micronuclei.

(f) *Data and report*—(1) *Treatment of results.* Criteria for scoring micronuclei should be given. Individual data should be presented in a tabular form including positive and negative (vehicle) controls and experimental groups. The number of polychromatic erythrocytes scored, the number of micronucleated polychromatic erythrocytes, the percentage of micronucleated cells, the number of micronucleated normochromatic erythrocytes, and, if applicable, the percentage of micronucleated erythrocytes and the ratio of normochromatic to polychromatic erythrocytes should be listed separately for each experimental and control animal. Absolute numbers should be included if percentages are reported.

(2) *Statistical evaluation.* Data should be evaluated by appropriate statistical methods.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive response, one of which is a statistically significant dose-related increase in the number of micronucleated polychromatic erythrocytes. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test substance concentrations.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of micronucleated polychromatic erythrocytes or a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* (i) The results of the micronucleus test provide information on the ability of a chemical to induce micronuclei in polychromatic erythrocytes of the test species under the conditions of the test. This damage may have been the result of chromosomal damage or damage to the mitotic apparatus.

(ii) Negative results indicate that under the test conditions the test substance does not produce micronuclei in the bone marrow of the test species.

(5) *Test report.* In addition to the reporting recommendations as specified under 40 CFR Part 792, Subpart J, the following specific information should be reported:

(i) Species, strain, age, weight, number and sex of animals in each treatment and control group.

(ii) Test chemical vehicle, dose levels used, rationale for dose selection.

(iii) Rationale for and description of treatment and sampling schedules, toxicity data, negative and positive controls.

(iv) Details of the protocol used for slide preparation.

(v) Criteria for identifying micronucleated erythrocytes.

(vi) Dose-response relationship, if applicable.

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Cihak, R. "Evaluation of benzidine by the micronucleus test," *Mutation Research*, 67: 383–384 (1979).

(2) Cole , R.J., Taylor, N., Cole, J., Arlett, C.F. "Short-term tests for transplacentally active carcinogens. 1. Micronucleus formation in fetal and maternal mouse erythroblasts," *Mutation Research*, 80: 141–157 (1981).

(3) Kliesch, U., Danford, N., Adler, I.D. "Micronucleus test and bone-marrow chromosome analysis. A comparison of 2 methods in vivo for evaluating chemically induced chromosomal alterations," *Mutation Research*, 80: 321–332 (1981).

(4) Matter, B., Schmid, W. "Trenimon-induced chromosomal damage in bone-marrow cells of six mammalian species, evaluated by the micronucleus test," *Mutation Research*, 12: 417–425 (1971).

(5) Schmid, W. "The micronucleus test," *Mutation Research, 31:9–15 (1975).*

(6) Schmid, W. "The micronucleus test for cytogenetic analysis," *Chemical Mutagens, Principles and Methods for their Detection.* Vol. 4 Hollaender A. (Ed. A ed. (New York and London: Plenum Press, (1976) pp. 31–53.

§ 798.5450 Rodent dominant lethal assay.

(a) *Purpose.* Dominant lethal (DL) effects cause embryonic or fetal death. Induction of a dominant lethal event after exposure to a chemical substance indicates that the substance has affected germinal tissue of the test species. Dominant lethals are generally accepted to be the result of chromosomal damage (structural and numerical anomalies) but gene mutations and toxic effects cannot be excluded.

(b) *Definition.* A dominant lethal mutation is one occurring in a germ cell which does not cause dysfunction of the gamete, but which is lethal to the fertilized egg or developing embryo.

(c) *Reference substances.* These may include, but need not be limited to, triethylenemelamine, cyclophosphamide or ethyl methanesulfonate.

(d) *Test method*—(1) *Principle.* Generally, male animals are exposed to the test substance and mated to untreated virgin females. The various germ cell stages can be tested separately by the use of sequential mating intervals. The females are sacrificed after an appropriate period of time and the contents of the uteri are examined to determine the numbers of implants and live and dead embryos. The calculation of the dominant lethal effect is based on comparison of the live implants per female in the treated group to the live implants per female in the control group. The increase of dead implants per female in the treated group over the dead implants per female in the control group reflects the post-implantation loss. The post-implantation loss is calculated by determining the ratio of dead to total implants from the treated group compared to the ratio of dead to total implants from the control group. Pre-implantation loss can be estimated on the basis of corpora lutea counts or by comparing the total implants per female in treated and control groups.

(2) *Description.* (i) Several treatment schedules are available. The most widely used requires single administration of the test substance. Other treatment schedules, such as treatment on five consecutive days, may be used if justified by the investigator.

(ii) Individual males are mated sequentially to virgin females at appropriate intervals. The number of matings following treatment is governed by the treatment schedule and should ensure that germ cell maturation is adequately covered. Females are sacrificed in the second half of pregnancy and the uterine contents examined to determine the total number of implants and the number of live and dead embryos.

(3) *Animal selection*—(i) *Species.* Rats or mice are generally used as the test species. Strains with low background dominant lethality, high pregnancy frequency and high implant numbers are recommended.

(ii) *Age.* Healthy, sexually mature animals should be used.

(iii) *Number.* An adequate number of animals should be used taking into account the spontaneous variation of the biological characteristics being evaluated. The number chosen should be based on the predetermined sensitivity of detection and power of significance. For example, in a typical experiment, the number of males in each group should be sufficient to provide between 30 and 50 pregnant females per mating interval.

(iv) *Assignment to groups.* Animals should be randomized and assigned to treatment and control groups.

(4) *Control groups*—(i) *Concurrent controls.* Generally concurrent positive and negative (vehicle) controls should be included in each experiment. When acceptable positive control results are available from experiments conducted recently (within the last 12 months) in the same laboratory these results can be used instead of a concurrent positive control.

(ii) *Positive controls.* Positive control substances should be used at a dose which demonstrates the test sensitivity.

(5) *Test chemicals*—(i) *Vehicle.* When possible, test substances should be dissolved or suspended in isotonic saline or distilled water. Water insoluble chemicals may be dissolved or suspended in appropriate vehicles. The vehicle used should neither interfere with the test chemical nor produce toxic effects. Fresh preparations of the test chemical should be employed.

(ii) *Dose levels.* Normally, three dose levels should be used. The highest dose should produce signs of toxicity (e.g., slightly reduced fertility). However, in an initial assessment of dominant lethality a single high dose may be sufficient. Nontoxic substances should be tested at 5 g/kg or, if this is not practicable, then at the highest dose attainable.

(iii) *Route of administration.* The usual routes of administration are oral or by IP injection. Other routes may be appropriate.

(e) *Test performance.* (1) Individual males are mated sequentially at appropriate predetermined intervals to one or two virgin females. Females should be left with the males for at least the duration of one estrus cycle or alternatively until mating has occurred as determined by the presence of sperm in the vagina or by the presence of a vaginal plug.

(2) The number of matings following treatment should be governed by the treatment schedule and should ensure that germ cell maturation is adequately covered.

(3) Females should be sacrificed in the second half of pregnancy and uterine contents examined to determine the number of implants and live and dead embryos. The ovaries may be examined to determine the number of corpora lutea.

(f) *Data and report*—(1) *Treatment of results.* Data should be tabulated to show the number of males, the number of pregnant females, and the number of nonpregnant females. Results of each mating, including the identity of each male and female, should be reported individually. For each female, dose level and week of mating, the frequencies of live implants and of dead implants should be enumerated. If the data are recorded as early and late deaths, the tables should make that clear. If pre-implantation loss is estimated, it should be reported. Pre-implantation loss can be calculated as the difference between the number of corpora lutea and the number of implants or as a reduction in the average number of implants per female in comparison with control matings. Where pre-implantation loss is estimated it should be reported.

(2) *Statistical evaluation.* Data should be evaluated by appropriate statistical methods. Differences among animals within the control and treatment groups should be considered before making comparisons between treated and control groups.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of dominant lethals. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test points.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of dominant lethals or a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* (i) A positive DL assay suggests that under the test conditions the test substance may be genotoxic in the germ cells of the treated sex of the test species.

(ii) A negative result suggests that under the conditions of the test the test substance may not be genotoxic in the germ cells of the treated sex of the test species.

(5) *Test Report.* In addition to the reporting recommendations as specified under 40 CFR Part 792, Subpart J the following specific information should be reported:

(i) Species, strain, age and weights of animals used, number of animals of each sex in experimental and control groups.

(ii) Test substance, vehicle used, dose levels and rationale for dosage selection, negative (vehicle) and positive controls, experimental observations, including signs of toxicity.

(iii) Route and duration of exposure.

(iv) Mating schedule.

(v) Methods used to determine that mating has occurred (where applicable).

(vi) Time of sacrifice.

(vii) Criteria for scoring dominant lethals.

(viii) Dose-response relationship, if applicable.

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Brewen, J.G., Payne, H.S., Jones, K.P., Preston, R.J. "Studies on chemically induced dominant lethality. I. The cytogenetic basis of MMS-induced dominant lethality in post-meiotic germ cells" *Mutation Research,* 33:239–250 (1975).

(2) Ehling, U.H., Machemer, L., Buselmaier, E., Dycka, D., Frohberg, H., Kratochvilova, J., Lang, R., Lorke, D., Muller, D., Pheh, J., Rohrborn, G., Roll, R., Schulze-Schencking, M., Wiemann, H. "Standard protocol for the dominant lethal test on male mice. Set up by the Work Group "Dominant lethal mutations of the ad hoc Committee Chemogenetics," *Archives of Toxicology,* 39:173–185 (1978).

§ 798.5460 Rodent heritable translocation assays.

(a) *Purpose.* This test detects transmitted chromosomal damage which manifests as balanced reciprocal translocations in progeny descended from parental males treated with chemical mutagens.

(b) *Definitions.* (1) A heritable translocation is one in which distal segments of nonhomologous chromosomes are involved in a reciprocal exchange.

(2) Diakinesis and metaphase I are stages of meiotic prophase scored cytologically for the presence of multivalent chromosome association characteristic of translocation carriers.

(c) *Reference substances.* Not applicable.

(d) *Test method*—(1) *Principle.* When a balanced reciprocal translocation is induced in a parental male germ cell, the resulting progeny is translocation heterozygote.

(i) *Basis for fertility screening.* Male translocation heterozygotes may be completely sterile. This class consists of two types of translocations:

(A) Translocations between nonhomologous chromosomes in which at least one of the breaks occurs close to one end of a chromosome.

(B) Those that carry multiple translocations. The majority of male translocation heterozygotes are semisterile—they carry one or (rarely) two translocations. The degree of semisterility is dependent upon the proportions of balanced and unbalanced (duplication-deficiency) gametes produced in the ejaculate as a function of meiotic segregation. Balanced and unbalanced sperm are equally capable of fertilizing an egg. Balanced sperm lead to viable progeny. Unbalanced sperm result in early embryonic lethality.

(ii) *Basis for cytological screening.* The great majority of male translocation heterozygotes can be identified cytologically through analysis of diakinesis metaphase I spermatocytes. Translocation heterozygotes are characterized by the presence of multivalent chromosome association such as a ring or chain of four chromosomes held together by chiasmata in paired homologous regions. Some translocation carriers can be identified by the presence of extra long and/or extra short chromosomes in spermatogonial and somatic cell metaphase preparations.

(2) *Description.* Essentially, two methods have been used to screen for translocation heterozygosity; one method uses a mating sequence to identify sterile and semisterile males followed by cytological examination of suspect male individuals; the other method deletes the mating sequence altogether and all F_1 male progeny are examined cytologically for presence of translocation. In the former approach, the mating sequence serves as a screen which eliminates most fully fertile animals for cytological confirmation as translocation heterozygotes.

(3) *Animal selection*—(i) *Species.* The mouse is the species generally used, and is recommended.

(ii) *Age.* Healthy sexually mature animals should be used.

(iii) *Number.* (A) The number of male animals necessary is determined by the following factors:

(*1*) The use of either historical or concurrent controls.

(*2*) The power of the test.

(*3*) The minimal rate of induction required.

(*4*) Whether positive controls are used.

(*5*) The level of significance desired.

(B) [Reserved]

(iv) *Assignment to groups.* Animals should be randomized and assigned to treatment and control groups.

(4) *Control groups*—(i) *Concurrent controls.* No concurrent positive or negative (vehicle) controls are recommended as routine parts of the heritable translocation assay. However, investigators not experienced in performing translocation testing should include a substance known to produce translocations in the assay as a positive control reference chemical.

(ii) *Historical controls.* At the present time, historical control data must be used in tests for significance. When statistically reliable historical controls are not available, negative (vehicle) controls should be used.

(5) *Test chemicals*—(i) *Vehicle.* Solid and liquid test substances should be dissolved or suspended in distilled water or isotonic saline. Water insoluble chemicals may be dissolved or suspended in appropriate vehicles. The vehicle used should neither interfere with the test chemical nor produce toxic effects. Fresh preparations of the test chemical should be employed.

(ii) *Dose levels.* At least two dose levels should be used. The highest dose level should result in toxic effects but should not produce an incidence of fatalities which would prevent a meaningful evaluation.

(iii) *Route of administration.* Acceptable routes of administration include oral, inhalation, admixture with food or water, and IP or IV injection.

(e) *Test performance*—(1) *Treatment and mating.* The animals should be dosed with the test substance 7 days/week over a period of 35 days. After treatment, each male should be caged with 2 untreated females for a period of 1 week. At the end of 1 week, females should be separated from males and caged individually. When females give birth, the day of birth, litter size and sex of progeny are recorded. All male progeny should be weaned and all female progeny should be discarded.

(2) *Testing for translocation heterozygosity.* When males are sexually mature, testing for translocation heterozygosity should begin. One of two methods should be used; the first method involves mating, determining those F_1 progeny which are sterile or semisterile and subsequent cytological analysis of suspect progeny; the other method does not involve mating and determining sterility or semisterility; all progeny are examined cytologically.

(i) *Determination of sterility or semisterility*—(A) *Conventional method.* Females are mated, usually three females for each male, and each female is killed at midpregnancy. Living and dead implantations are counted. Criteria for determining normal and semisterile males are usually established for each new strain because the number of dead implantations varies considerably among strains.

(B) *Sequential method.* Males to be tested are caged individually with females and the majority of the presumably normal males are identified on the basis of a predetermined size of 1 or 2 litters. Breeding pens are examined daily on weekdays beginning 18 days after pairing. Young are discarded immediately after they are scored. Males that sire a litter whose size is the same as or greater than the minimum set for a translocation-free condition are discarded with their litter. If the litter size is smaller than the predetermined number, a second litter is produced with the same rule applying. Males that cannot be classified as normal after production of a second litter are tested further by the conventional method or by cytological confirmation of translocation.

(ii) *Cytological analysis.* For cytological analysis of suspected semisteriles, the air-drying technique is used. Observation of at least 2 diakinesis-metaphase 1 cells with mutivalent association constitutes the required evidence for the presence of a translocation. Sterile males are examined by one of two methods, those with testes of normal size and sperm in the epididymis are examined by the same techniques used for semisteriles. Animals with small testes are examined by squash preparations or, alternatively, by examination of mitotic metaphase preparations. If squash preparations do not yield diakinesis-metaphase 1 cells, analysis of spermatogonia or bone marrow for the presence of unusually long or short chromosomes should be performed.

(f) *Data and report*—(1) *Treatment of results.* (i) Data should be presented in tabular form and should include the number of animals at risk, the germ cell stage treated, the number of partial steriles and semisteriles (if the fertility test is used), the number of cytogenetically confirmed translocation heterozygotes (if the fertility test is used, report the number of confirmed steriles and confirmed partial steriles), the translocation rate, and either the standard error of the rate or the upper 95 percent confidence limit on the rate.

(ii) These data should be presented for both treated and control groups. Historical or concurrent controls should be specified, as well as the randomization procedure used for concurrent controls.

(2) *Statistical evaluation.* Data should be evaluated by appropriate statistical methods.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of heritable translocations. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test points.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of heritable translocations or a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* (i) Positive results in the heritable translocation assay indicate that under the test conditions the test substance causes heritable chromosomal damage in the test species.

(ii) Negative results indicate that under the test conditions the test substance does not cause heritable chromosomal damage in the test species.

(5) *Test report.* In addition to the reporting recommendations as specified under 40 CFR Part 792, Subpart J, the following specific information should be reported:

(i) Species, strain, age, weight and number of animals of each sex in each group.

(ii) Test chemical vehicle, route and schedule of administration, toxicity data.

(iii) Dosing regimen, doses tested and rationale for dosage selection.

(iv) Mating schedule, number of females mated to each male.

(v) The use of historical or concurrent controls.

(vi) Screening procedure including the decision criteria used and the method by which they were determined.

(vii) Dose-response relationship, if applicable.

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Generoso, W.M., Bishop, J.B., Goslee, D.G., Newell, G.W., Sheu, G-J, von Halle, E. "Heritable translocation test in mice," *Mutation Research,* 76:191–215 (1980).

(2) [Reserved]

§ 798.5500 Differential growth inhibition of repair proficient and repair deficient bacteria: "Bacterial DNA damage or repair tests."

(a) *Purpose.* Bacterial DNA damage or repair tests measure DNA damage which is expressed as differential cell killing or growth inhibition of repair deficient bacteria in a set of repair proficient and deficient strains. These tests do not measure mutagenic events *per se.* They are used as an indication of the interaction of a chemical with genetic material implying the potential for genotoxicity.

(b) *Definition.* Test for differential growth inhibition of repair proficient and repair deficient bacteria measure differences in chemically induced cell killing between wild-type strains with full repair capacity and mutant strains deficient in one or more of the enzymes which govern repair of damaged DNA.

(c) *Reference substances.* These may include, but need not be limited to, chloramphenicol or methyl methanesulfonate.

(d) *Test method*—(1) *Principle.* The tests detect agents that interact with cellular DNA to produce growth inhibition or killing. This interaction is recognized by specific cellular repair systems. The assays are based upon the use of paired bacterial strains that differ by the presence of absence of specific DNA repair genes. The response is expressed in the preferential inhibition of growth or the preferential killing of the DNA repair deficient strain since it is incapable of removing certain chemical lesions from its DNA.

(2) *Description.* Several methods for performing the test have been described. Those described here are:

(i) Tests performed on solid medium (diffusion tests).

(ii) Tests performed in liquid culture (suspension tests).

(3) *Strain selection*—(i) *Designation.* At the present time, *Escherichia coli polA* (W3110/p3478) or *Bacillus subtilis rec* (H17/M45) pairs are recommended. Other pairs may be utilized when appropriate.

(ii) *Preparation and storage.* Stock culture preparation and storage, growth requirements, method of strain identification and demonstration of appropriate phenotypic requirements should be performed using good microbiological techniques and should be documented.

(4) *Bacterial growth.* Good microbiological techniques should be used to grow fresh cultures of bacteria. The phase of growth and cell density should be documented and should be adequate for the experimental design.

(5) *Metabolic activation.* Bacteria should be exposed to the test substance both in the presence and absence of an appropriate metabolic activation system. The most commonly used system is a cofactor supplemented postmitochondrial fraction prepared from the livers of rodents treated with enzyme inducing agents. The use of other species, tissues or techniques may also be appropriate.

(6) *Control groups*—(i) *Concurrent controls.* Concurrent positive, negative,

and vehicle controls should be included in each assay.

(ii) *Negative controls.* The negative control should show nonpreferential growth inhibition (i.e., should affect both strains equally). Chloramphenicol is an example of a negative control.

(iii) *Genotype specific controls.* Examples of genotype specific positive controls are methyl methanesulfonate for *polA* strains and mitomycin C for *rec* strains.

(iv) *Positive controls to ensure the efficacy of the activation system.* The positive control reference substance for tests including a metabolic activation system should be selected on the basis of the type of activation system used in the test.

(v) *Other positive controls.* Other positive control reference substances may be used.

(2) *Test chemicals*—(i) *Vehicle.* Test chemicals and positive and negative control reference substances should be dissolved in an appropriate vehicle and then further diluted in vehicle for use in the assay.

(ii) *Exposure concentrations.* The test should initially be performed over a broad range of concentrations. Among the criteria to be taken into consideration for determining the upper limits of test chemical concentration are cytotoxicity and solubility. Cytotoxicity of the test chemical may be altered in the presence of metabolic activation systems. For freely soluble nontoxic chemicals, the upper test chemical concentration should be determined on a case by case basis. Because results are expressed as diameters of zones of growth inhibition in the diffusion test, it is most important that the amounts of chemical on the disc (or in the wells) are exact replicates. When appropriate, a positive response should be confirmed by testing over a narrow range of concentrations.

(e) *Test performance*—(1) *Diffusion assay*—(i) *Disc diffusion assays.* Disc diffusion assays, may be performed in two ways:

(A) A single strain of bacteria may be added to an agar overlay or spread on the surface of the agar and the test chemical placed on a filter disc on the surface of the agar or;

(B) DNA repair proficient and DNA repair deficient bacteria may be streaked in a line on the surface of the agar of the same plate and a disc saturated with test chemical placed on the surface of the agar in contact with the streaks.

(ii) *Well diffusion assays.* In well diffusion assays, bacteria may be either added to the agar overlay or spread onto the surface of the agar. A solution of the test chemical is then placed into a well in the agar.

(2) *Suspension assays.* (i) A bacterial suspension may be exposed to the test chemical and the number of surviving bacteria determined (as colony-forming units) either as a function of time of treatment or as a function of the concentration of test agent.

(ii) Nonturbid suspensions of bacteria may be exposed to serial dilutions of the test agent and a minimal inhibitory concentration for each strain determined, as evidenced by the presence or absence of visible growth after a period of incubation.

(iii) Paired bacterial suspensions (usually with some initial turbidity) may be treated with a single dose of the chemical. Positive results are indicated by a differential inhibition in the rate of increase of turbidity of the paired cultures.

(3) *Number of cultures.* When using a plate diffusion procedure, at least two independent plates should be used at each dilution. In liquid suspension assays, at least two independent specimens for determination of the number of viable cells should be plated.

(4) *Incubation conditions.* All plates in a given test should be incubated for the same time period. This incubation period should be for 18 to 24 hrs at 37 °C.

(f) *Data and report*—(1) *Treatment of results*—(i) *Diffusion assays.* Results should be expressed in diameters of zones of growth inhibition in millimeters or as areas derived therefrom as mm^2. Dose-response data, if available, should be presented using the same units.

(ii) *Liquid suspension assays.* (A) Survival data can be presented as dose responses, preferably as percentage of survivors or fractional survival of each strain or as a relative survival (ratio) of the two strains.

(B) Results can also be expressed as the concentrations required to effect a predetermined survival rate (e.g., D_{37}, the dose permitting 37 percent survival). These data are derived from the survival curve. The concentration should be expressed as weight per volume, as moles, or as molarity.

(C) Similarly, results can be expressed as minimal inhibitory concentration or as minimal lethal dose. The former is determined by the absence of visible growth in liquid medium and the latter is determined by plating dilutions onto semisolid media.

(iii) In all tests, concentrations must be given as the final concentrations during the treatment. Raw data, prior to transformation, should be provided. These should include actual quantities measured, e.g., neat numbers. For measurement of diffusion, the diameters of the discs and/or well should be indicated and the measurements should indicate whether the diameter of the discs and/or well was subtracted. Moreover, mention should be made as to whether the test chemical gave a sharp, diffuse, or double-zone of growth inhibition. If it is the latter, the investigator should indicate whether the inner or the outer zone was measured.

(iv) Viability data should be given as the actual plate counts with an indication of the dilution used and the volume plated or as derived titers (cells per ml). Transformed data alone in the absence of experimental data are not acceptable (i.e, ratios, differences, survival fraction).

(2) *Statistical evaluation.* Data should be evaluated by appropriate statistical methods.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related preferential inhibition or killing of the repair deficient strain. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test points.

(ii) A test substance which does not produce either a statistically significant dose-related preferential inhibition or killing of the repair deficient strain or a statistically significant and reproducible positive response at any one of the test points is considered not to interact with the genetic material of the organisms used in assay.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* DNA damage tests in bacteria do not measure DNA repair *per se* nor do they measure mutations. They measure DNA damage which is expressed as cell killing or growth inhibition. A positive result in a DNA damage test in the absence of a positive result in another system is difficult to evaluate in the absence of a better data base.

(5) *Test report.* In addition to the reporting recommendations as specified under 40 CFR Part 792, Subpart J the following specific information should be reported:

(i) Bacterial strains used.

(ii) Phase of bacterial cell growth at time of use in the assay.

(iii) Media composition.

(iv) Details of both the protocol used to prepare the metabolic activation system and its use in the assay.

(v) Treatment protocol, including doses used and rationale for dose selection, positive and negative controls.

(vi) Method used for determination of degree of cell kill.

(vii) Dose-response relationship, if applicable.

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Ames, B.N., McCann, J., Yamasaki, E. "Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test," *Mutation Research,* 31:347–364 (1975).

(2) Kada, T., Sadie, Y., Tutikawa, K. "In vitro and host-mediated "rec-assay" procedures for screening chemical mutagens; and phloxine, a mutagenic red dye detected," *Mutation Research,* 16:165–174 (1972).

(3) Leifer, Z., Kada, T., Mandel, M., Zeiger, E., Stafford, R., Rosenkranz, H.S. "An evaluation of bacterial DNA repair tests for predicting genotoxicity and carcinogenicity: a report of the U.S. EPA's Gene-Tox Program," *Mutation Research,* 87:211–297 (1981).

(4) Slater, E.E., Anderson, M.D., Rosenkranz, H.S. "Rapid detection of mutagens and carcinogens." *Cancer Research,* 31:970–973 (1971).

§ 798.5550 Unscheduled DNA synthesis in mammalian cells in culture.

(a) *Purpose.* Unscheduled DNA synthesis (UDS) in mammalian cells in culture measures the repair of DNA damage induced by a variety of agents including chemicals, radiation and viruses. UDS may be measured in both in vitro and *in vivo* systems.

(b) *Definition.* In this guideline, unscheduled DNA synthesis in mammalian cells in culture is defined as the incorporation of tritium labelled thymidine (^{3}H-TdR) into the DNA of cells which are

(c) *Reference substances.* These may include, but need not be limited to, 7,12-dimethylbenzanthracene, 2-acetylaminofluorene, 4-nitroquinoline oxide or N-dimethyl-nitrosamine.

(d) *Test method*— (1) *Principle.* Mammalian cells in culture, either primary cultures of rodent hepatocytes or established cell lines, are exposed to the test agent. Established cell lines are treated both with and without metabolic activation. UDS is measured by the uptake of ^{3}H-TdR into the DNA of non-S phase cells. Uptake may be determined by autoradiography or by liquid scintillation counting (LSC) of DNA from treated cells.

(2) *Description*—(i) *Autoradiography.* For autoradiography, coverslip cultures of cells are exposed to test chemical in medium containing ^{3}H-TdR. At the end of the treatment period, cells are fixed, dipped in autoradiographic emulsion, and exposed at 4 °C. At the end of the exposure period, cells are stained and labeled nuclei are counted either manually or with an electronic counter. Established cell lines should be treated both with and without metabolic activation.

(ii) *LSC determinations.* For LSC determinations of UDS, confluent cultures of cells are treated with test chemical both with and without metabolic activation. At the end of the exposure period, DNA is extracted from the treated cells. Total DNA content is determined and extent of ^{3}H-TdR incorporation is determined by scintillation counting.

(3) *Cells*—(i) *Type of cells used in the assay.* (A) A variety of cell lines or primary cell cultures, including human cells, may be used in the assay.

(B) Established cell lines should be checked for *Mycoplasma* contamination and may be periodically checked for karyotype stability.

(ii) *Cell growth and maintenance.* Appropriate culture media and incubation conditions (culture vessels CO_2 concentration), temperature and humidity should be used.

(4) *Metabolic activation.* (i) A metabolic activation system is not used with primary cultures of rodent hepatocytes.

(ii) Established cell lines should be exposed to test substance both in the presence and absence of an appropriate metabolic activation system.

(5) *Control groups.* Concurrent positive and negative (untreated and/or vehicle) controls both with and without metabolic activation as appropriate should be included in each experiment.

(6) *Test chemicals*—(i) *Vehicle.* Test chemicals and positive control reference substances may be prepared in culture media or dissolved or suspended in appropriate vehicles prior to treatment of the cells. Final concentration of the vehicle should not interfere with cell viability or growth rate.

(ii) *Exposure concentrations.* Multiple concentrations of test substance, based upon cytotoxicity and over a range adequate to define the response, should be used. For cytotoxic chemicals, the first dose to elicit a cytotoxic response in a preliminary assay should be the highest dose tested. Relatively insoluble compounds should be tested up to the limits of solubility. For freely soluble nontoxic chemicals, the upper test chemical concentration should be determined on a case by case basis.

(e) *Test performance*—(1) *Primary cultures of rodent hepatocytes.* Freshly isolated rodent hepatocytes should be treated with chemical in medium containing ^{3}H-TdR. At the end of the treatment period, cells should be drained of medium, rinsed, fixed, dried and attached to microscope slides. Slides should be dipped in autoradiographic emulsion, exposed at 4 °C. for an appropriate length of time, developed, stained and counted.

(2) *Established cell lines*—(i) *Autoradiographic techniques.* The techniques for treatment of established cell lines are the same as those for primary cultures of rodent hepatocytes except that cells must not enter S phase prior to treatment. Entry of cells into S phase may be blocked by several methods (e.g. by growth in medium deficient in arginine or low in serum or by treatment with chemical agents such as hydroxyurea). Tests should be done both in the presence and absence of a metabolic activation system.

(ii) *LSC measurement of UDS.* Prior to treatment with test agent, entry of cells into S phase should be blocked as described in paragraph (e)(2)(i) of this section. Cells should be exposed to the test chemical in medium containing ^{3}H-TdR. At the end of the incubation period, DNA should be extracted from the cells by hydrolysis with perchloroacetic acid or by other acceptable methods. One aliquot of DNA is used to determine total DNA content; a second aliquot is used to measure the extent of ^{3}H-TdR incorporation.

(3) *Acceptable background frequencies*—(i) *Autoradiographic determinations.* Net incorporation of ^{3}HTdR into the nucleus of solvent treated control cultures should be less than 1.

(ii) *LSC determinations.* Historical background incorporation rates of ^{3}H-TdR into untreated established cell lines should be established for each laboratory.

(4) *Number of cells counted.* A minimum of 50 cells per culture should be counted for autoradiographic UDS determinations. Slides should be coded before being counted. Several widely separated random fields should be counted on each slide. Cytoplasm adjacent to the nuclear areas should be counted to determine spontaneous background.

(5) *Number of cultures.* Six independent cultures at each concentration and control should be used in LSC UDS determinations.

(F) *Data and report*—(1) *Treatment of results*—(i) *Autoradiographic* *determinations.* For autoradiographic determinations, once untransformed data are recorded, background counts should be subtracted to give the correct nuclear grain count. Values should be reported as net grains per nucleus. Mean, median and mode may be used to describe the distribution of net grains per nucleus.

(ii) *LSC determinations.* For LSC determinations, ^{3}H-TdR incorporation should be reported as dpm/μg DNA. Average dpm/μg DNA with standard deviation or standard error of the mean may be used to describe distribution of incorporation in these studies.

(2) *Statistical evaluation.* Data should be evaluated by appropriate statistical methods.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the incorporation of ^{3}H-TdR into treated cells. Another criterion may be based upon detection of a reproducible and statistically significant positive response for a least one of the test points.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the incorporation of ^{3}H-TdR into treated cells or a statistically significant and reproducible positive response at any one of the test points is considered not to induce UDS in the test system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* (i) Positive results in the UDS assay indicate that under the test conditions the test substance may induce DNA damage in cultured mammalian somatic cells.

(ii) Negative results indicate that under the test conditions the test substance does not induce DNA damage in cultured mammalian somatic cells.

(5) *Test report.* In addition to the reporting recommendations as specified under 40 CFR Part 792, Subpart J the following specific information should be reported:

(i) Cells used, density and passage number at time of treatment, number of cell cultures.

(ii) Methods used for maintenance of cell cultures including medium, temperature and CO_2 concentration.

(iii) Test chemical vehicle, concentration and rationale for selection of concentrations used in the assay.

(iv) Details of both the protocol used preparation of the metabolic activation system and its use in the assay.

(v) Treatment protocol.

(vi) Positive and negative controls.

(vii) Protocol used for autoradiography.

(viii) Details of the method used to block entry of cells into S phase.

(ix) Details of the methods used for DNA extraction and determination of total DNA content in LSC determinations.

(x) Historical background incorporation rates of ^{3}H-TdR in untreated cell lines.

(xi) Dose-response relationship, if applicable.

(g) *References.* For additional background information on this test guideline, the following references should be consulted:

(1) Ames, B.N., McCann, J., Yamasaki, E. "Methods for detecting carcinogens and mutagens with the *Salmonella/* mammalian-microsome mutagenicity test," *Mutation Research,* 31:347–364 (1975).

(2) Rasmussen, R.E., Painter, R.B. "Radiation-stimulated DNA synthesis in cultured mammalian cells," *Journal of Cell Biology,* 29:11–19 (1966).

(3) Stich, H.F., San, P.P.S., Lam, K.J., Koropatnick, D.J., Lo, L.W., Laishes, B.A. "DNA fragmentation and DNA repair as an in vitro and in vivo assay for chemical procarcinogens, carcinogens and carcinogenic nitrosation products," *Screening tests in chemical carcinogenesis.* Eds. Bartsch, H., Tomatis, L., (Lyon: IARC Scientific Publications, No. 12, 1976) pp. 617–636.

(4) Williams, G.M. "Carcinogen-induced DNA repair in primary rat liver cell cultures: a possible screen for chemical carcinogens," *Cancer Letters,* 1:231–236 (1976).

(5) Williams, G.M. "Detection of chemical carcinogens by unscheduled DNA synthesis in rat liver primary cell cultures," *Cancer Research,* 37:1845–1851 (1977).

§ 798.5575 Mitotic gene conversion in Saccharomyces cerevisiae.

(a) *Purpose.* The mitotic gene conversion assay in the yeast, *Saccharomyces cerevisiae,* measures the conversion of differentially inactive alleles to wild-type alleles by mutagenic agents. Heteroallelic diploid yeast strains carry two different inactive alleles of the same gene locus. The presence of these alleles causes a nutritional requirement, e.g., these heteroallelic diploids grow only in medium supplemented with a specific nutrient such as tryptophan. When gene conversion occurs, a fully active wild-type phenotype is produced from these inactive alleles through intragenic recombination. These wild-type colonies grow on a medium lacking the specific nutritional requirement (selective medium).

(b) *Definitions.* (1) Mitotic gene conversion is detected by the change of inactive alleles of the same gene to wild-type alleles through intragenic recombination in mitotic cells.

(2) Heteroallelic diploids are diploid strains of yeast carrying two different, inactive alleles of the same gene locus causing a nutritional requirement.

(c) *Reference substances.* These may include but need not be limited to, hydrazine sulfate or 2-acetylaminofluorene.

(d) *Test method*—(1) *Principle.* The method is based on the fact that heteroallelic diploid yeast strains carry two inactive alleles of the same gene locus making them dependent on a specific nutritional requirement (e.g., tryptophan) for their survival. Treatment of such strains with mutagenic agents can cause conversion of these alleles back to the wild-type condition which allows growth on a medium lacking the required nutrient (selective medium).

(2) *Description.* Heteroallelic diploid strains such as D7, requiring a specific nutrient in the medium, are treated with test chemical with and without metabolic activation and plated on a selective medium lacking the required nutrient. The wild-type colonies that grow on the selective medium as a result of gene conversion are scored.

(3) *Strain selection*—(i) *Designation.* At the present time, *S. cerevisiae* strain D7 is recommended for use in this assay. The use of other strains may also be appropriate.

(ii) *Preparation and storage.* Stock culture preparation and storage, growth requirements, method of strain identification and demonstration of appropriate phenotypic requirements should be performed using good microbiological techniques and should be documented.

(iii) *Media.* YEP glucose medium enriched with the appropriate growth factors may be used for cell growth and maintenance. Other media may also be appropriate.

(4) *Selection of cultures.* Cells should be grown with aeration in liquid medium enriched with growth factors to early stationary phase. Cells should then be seeded on selective medium to determine the rate of spontaneous conversion. Cultures with a high rate of spontaneous conversion should be discarded.

(5) *Metabolic activation.* Cells should be exposed to test chemical both in the presence and absence of an appropriate metabolic activation system.

(6) *Control groups.* Concurrent positive and negative (untreated and/or vehicle) controls both with and without metabolic activation should be included in each experiment.

(7) *Test chemicals*—(i) *Vehicle.* Test chemicals and positive control reference substances should be dissolved in an appropriate vehicle and then further diluted in vehicle for use in the assay. Dimethylsulfoxide should be avoided as a vehicle.

(ii) *Exposure concentrations.* (A) The test should initially be performed over a broad range of concentrations. Among the criteria to be taken into consideration for determining the upper limits of test chemical concentration are cytotoxicity and solubility. Cytotoxicity of the test chemical may be altered in the presence of metabolic activation systems. For cytotoxic chemicals, the highest dose tested should not reduce survival to less than 10 percent of that seen in the untreated control cultures. Relatively insoluble chemicals should be tested up to the limits of solubility. For freely soluble nontoxic chemicals, the upper test chemical concentration should be determined on a case by case basis.

(B) When appropriate, a positive response should be confirmed by using a narrow range of concentrations.

(e) *Test performance*—(1) *Treatment.* Cultures should be treated in liquid suspension. Resting cells should be treated in buffer; growing cells should be treated in a synthetic medium. Cultures with low spontaneous convertant frequencies should be centrifuged, washed and resuspended in liquid at the appropriate density. Cells should be exposed to test chemical both in the presence and absence of a metabolic activation system. Independent tubes should be treated for each concentration. At the end of the treatment period, cells should be centrifuged, washed and resuspended in distilled water prior to plating on selective medium for convertant selection and on complete medium to determine survival. At the end of the incubation period, plates should be scored for survival and the presence of convertant colonies.

(2) *Number of cultures.* At least six individual plates per treatment concentration and control should be used.

(3) *Incubation conditions.* All plates in a given experiment should be incubated for the same time period. This incubation period may be from 4 to 8 days at 28 °C.

(f) *Data and report*—(1) *Treatment of results.* Individual plate counts for test substance and control should be presented for both convertants and survivors. The mean number of colonies per plate and standard deviation should also be presented. Data should be presented in tabular form indicating numbers of viable and convertant colonies scored, survival frequency and convertant frequencies for each treatment and control culture. Conversion frequencies should be expressed as number of convertants per number of survivors. Sufficient detail should be provided for verification of survival and convertant frequencies.

(2) *Statistical evaluation.* Data should be evaluated by appropriate statistical methods.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of gene convertants. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test points.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of gene conversions or a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* (i) Positive results in this assay indicate that under the test conditions the test chemical causes mitotic gene conversion in the yeast *S. cerevisiae.*

(ii) Negative results indicate that under the test conditions the test chemical does not cause mitotic gene conversion in *S. cerevisiae.*

(5) *Test report.* In addition to the reporting recommendations as specified under 40 CFR Part 792, Subpart J, the following specific information should be reported:

(i) Strain of organism used in the assay.

(ii) Test chemical vehicle, doses used and rationale for dosage selection.

(iii) Method used to select cultures.

(iv) Treatment protocol including cell density at treatment and length of exposure to test substance.

(v) Details of both the protocol used to prepare the metabolic activation system and its use in the assay.

(vi) Incubation times and temperatures.

(vii) Dose-response relationship, if applicable.

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Ames, B.N., McCann, J., Yamasaki, E. "Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test," *Mutation Research,* 31:347–364 (1975).

(2) Callen, D.F., Philpot, R.M. "Cytochrome P-450 and the activation of promutagens in *Saccharomyces cerevisiae,*" *Mutation Research,* 45:309–324 (1975).

(3) Zimmermann, F.K. "Procedures used in the induction of mitotic recombination and mutation in the yeast *Saccharomyces cerevisiae.*" *Handbook of mutagenicity test procedures.* Eds. Kilby, B.J., Legator, M., Nicols, W., Ramel, C. (Amsterdam: Elsevier/North Holland Biomedical Press, (1979), pp. 119–134.

(4) Zimmermann, F.K., Kern, R., Rosenberger, H. "A yeast strain for simultaneous detection of induced mitotic crossing over, mitotic gene conversion and reverse mutation," *Mutation Research,* 28:381–388 (1975).

§ 798.5900 In vitro sister chromatid exchange assay.

(a) *Purpose.* The sister chromatid exchange (SCE) assay detects the ability of a chemical to enhance the exchange of DNA between two sister chromatids of a duplicating chromosome. The test may be performed in vitro, using, for example, rodent or human cells, or in vivo using mammals, for example, rodents such as mice, rats and hamsters.

(b) *Definition.* Sister chromatid exchanges represent reciprocal interchanges of the two chromatid arms within a single chromosome. These exchanges are visualized during the metaphase portion of the cell cycle and presumably require enzymatic incision, translocation and ligation of at least two DNA helices.

(c) *Reference substances.* Not applicable.

(d) *Test method*—(1) *Principle.* Following exposure of cell cultures to test chemicals, they are allowed to replicate in the presence of bromodeoxyuridine (BrdU), followed by treatment with colchicine or colcemid to arrest cells in a metaphase-like stage of mitosis (c-metaphase). Cells are then harvested and chromosome preparations made. Preparations are stained and metaphase cells analyzed for SCEs.

(2) *Description. In vitro* SCE assays may employ monolayer or suspension cultures of established cell lines, cell strains or primary cell cultures. Cell cultures are exposed to test chemical

and are allowed to replicate in the presence of BrdU. Prior to harvest, cells are treated with a spindle inhibitor (e.g. Colchicine or Colcemid ®) to accumulate cells in c-metaphase. Chromosome preparations from cells are made, stained and analyzed for SCEs.

(3) *Cells*—(i) *Type of cells used in the assay.* There are a variety of cell lines or primary cell cultures, including human cells, which may be used in the assay. Established cell lines and strains should be checked for *Mycoplasma* contamination and may be periodically checked for karyotype stability.

(ii) *Cell growth and maintenance.* Appropriate culture media and incubation conditions (culture vessels, temperature, and humidity CO_2) should be used.

(4) *Metabolic activation.* Cells should be exposed to test chemical both in the presence and absence of an appropriate metabolic activation system.

(5) *Control groups—Concurrent controls.* Positive and negative (untreated and/or vehicle) controls, with and without metabolic activation, should be included in each experiment.

(6) *Test chemicals*—(i) *Vehicle.* Test substances may be prepared in culture media or dissolved or suspended in appropriate vehicles prior to treatment of the cells. Final concentration of the vehicle should not reduce cell viability or growth rate.

(ii) *Exposure concentrations.* Multiple concentrations of the test substance over a range adequate to define the response should be tested. Among the criteria to be taken into consideration for determining the upper limits of test chemical concentration are cytotoxicity and solubility. Cytotoxicity of the test substance may be altered in the presence of metabolic activation systems. Cytotoxicity may be evidenced by a large (e.g., 75 percent) decrease in the number of cells that have divided twice in the presence of BrdU. Relatively insoluble substances should be tested up to the limit of solubility. For freely soluble nontoxic chemicals, the upper test chemical concentration should be determined on a case by case basis. When appropriate, a positive response should be confirmed by using a narrow range of test concentrations.

(e) *Test performance*—(1) *Established cell lines and strains.* (i) Prior to use in the assay, cells should be generated from stock cultures, seeded in culture vessels at the appropriate density and incubated at 37 °C.

(ii) Cell lines and strains should be treated with test chemical both with and without metabolic activation when they are in the exponential stage of growth. At the end of the exposure period, cells should be washed and incubated for two replication cycles in medium containing BrdU. After BrdU is added, the cultures should be handled in darkness, under "safe" (e.g., darkroom) lights, or in dim light from incandescent lamps to minimize photolysis of BrdU containing DNA. At the end of the BrdU incubation period, cells should be fixed and stained for SCE determination. Cultures should be treated with a spindle inhibitor (e.g., colchicine or Colcemid®) 2 hr prior to harvesting.

(2) *Human lymphocyte cultures.* (i) For preparation of human lymphocyte cell cultures, heparinized or acid-citrate-dextrose treated whole blood should be added to culture medium containing a mitogen, e.g., phytohemagglutinin (PHA) and incubated at 37 °C. White cells sedimented by gravity (buffy coat) or lymphocytes which have been purified on a density gradient such as Ficoll-Hypaque may also be utilized.

(ii) Cells should be exposed to the test chemical during at last two time intervals, e.g. G_0 and S. Exposure during the G_0 phase of the cell cycle should be accomplished by adding the test substance prior to addition of mitogen. Exposure during or after the first S phase may be accomplished by exposing cells 24–30 hrs after mitosis, under "safe" (e.g. darkroom) lights, or in dim light from incandescent lamps to minimize photolysis of BrdU containing DNA. At the end of the BrdU incubation period, cells should be fixed and stained for SCE determination. Cultures should be treated with a spindle inhibitor (e.g. colchicine or Colcemid®) 2 hr prior to harvesting.

(3) *Human lymphocyte cultures.* (i) For preparation of human lymphocyte cell cultures, heparinized or acid-citrate-dextrose treated whole blood should be added to culture medium containing a mitogen, e.g., phytohemagglutinin (PHA) and incubated at 37 °C. White cells sedimented by gravity (buffy coat) or lymphocytes which have been purified on a density gradient such as Ficoll-Hypaque may also be utilized.

(ii) Cells should be exposed to the test chemical during at least two time intervals, e.g., G_0 and S. Exposure during the G_0 phase of the cell cycle should be accomplished by adding the test substance prior to addition of mitogen. Exposure during or after the first S phase may be accomplished by exposing cells 24–30 hrs after mitogen stimulation. After exposure, cells should be washed and then cultured in the absence of the chemical.

(4) *Culture harvest time.* A single harvest time, one that yields an optimal percentage of second division metaphases, is recommended. If there is reason to suspect that this is not a representative sampling time (which may occur for short-lived, cycle specific chemicals), then additional harvest times should be selected.

(5) *Staining method.* Staining of slides to reveal SCEs can be performed according to any of several protocols. However, the fluorescence plus Giemsa method is recommended.

(6) *Number of cultures.* At least two independent cultures should be used for each experimental point.

(7) *Analysis.* Slides should be coded before analysis. The number of cells to be analyzed should be based upon the spontaneous control frequency and defined sensitivity and the power of the test chosen before analysis. In human lymphocytes, only cells containing 46 centromeres should be analyzed. In established cell lines and strains, only metaphases containing ±2 centromeres of the modal number should be analyzed. Uniform criteria for scoring SCEs should be used.

(f) *Data and report*—(1) *Treatment of results.* Data should be presented in tabular form, providing scores for both the number of SCEs for each metaphase and the number of SCEs per chromosome for each metaphase.

(2) *Statistical evaluation.* Data should be evaluated by appropriate statistical methods.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of sister chromatid exchanges. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test substance concentrations.

(ii) A test substance which produces neither a statistically significant dose-related increase in the number of sister chromatid exchanges nor a statistically significant and reproducible positive response at any one of the test points is considered not to induce rearrangements of segments of DNA in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* (i) Positive results in the in vitro SCE assay indicate that under the test conditions the test substance induces reciprocal chromatid interchanges in cultured mammalian somatic cells.

(ii) Negative results indicate that under the test conditions the test substance does not induce reciprocal chromatid interchanges in cultured mammalian somatic cells.

(5) *Test report.* In addition to the reporting recommendations as specified under 40 CFR Part 792, Subpart J, the following specific information should be reported:

(i) Cells used, density at time of treatment, number of cell cultures.

(ii) Methods used for maintenance of cell cultures including medium, temperature and CO_2 concentration.

(iii) Test chemical vehicle, concentration and rationale for the selection of the concentrations of test chemical used in the assay, duration of treatment.

(iv) Details of both the protocol used preparation of the metabolic activation system and its use in the assay.

(v) Growth period in BrdU; identity of spindle inhibitor, its concentration and duration of treatment.

(vi) Time of cell harvest.

(vii) Positive and negative controls.

(viii) Method used to prepare slides for SCE determination.

(ix) Criteria for scoring SCEs.

(x) Details of the protocol used for growth and treatment of human cells if used in the assay.

(xi) Dose-response relationship, if applicable.

(g) *References.* For additional background information on this test guideline the following references shoud be consulted:

(1) Latt, .S.A., Allen, J., Bloom, S.E., Carrano, A., Falke, E., Kram, D., Schneider, E., Schreck, R., Tice, R., Whitfield, B., Wolff, S. "Sister chromatid exchanges: a report of the U.S. EPA's Gene-Tox Program" *Mutation Research,* 87:17–62 (1981).

(2) [Reserved]

§ 798.5915 In vivo sister chromatid exchange assay.

(a) *Purpose.* The sister chromatid exchange (SCE) assay detects the ability of a chemical to enhance the exchange of DNA between two sister chromatids of a duplicating chromosome. The test may be perfomed in vitro using cultured mammalian cells or in vivo using nonmammalian or mammalian tissues. The most commonly used assays employ bone marrow or lymphocytes from mammalian species such as mice, rats or hamsters. Human lymphocytes may also be used.

(b) *Definition.* Sister chromatid exchanges represent reciprocal interchanges of the two chromatid arms within a single chromosome. These exchanges are visualized during the metaphase portion of the cell cycle and presumably require enzymatic incision, translocation and ligation of at least two DNA helices.

(c) *Reference substances.* Not applicable.

(d) *Test method*— (1) *Principle.* Animals are exposed to test substance by appropriate routes followed by administration of bromodeoxyuridine (BrdU). A spindle inhibitor (e.g., colchicine or Colecimid ®) is administered prior to sacrifice. After sacrifice, tissue is obtained and metaphase preparations made, stained and scored for SCE.

(2) *Description.* The method described here employs bone marrow of laboratory rodents exposed to test chemicals. After treatment with test chemical, animals are futher treated with BrdU and, prior to sacrifice, with a spindle inhibitor (e.g., colchicine or Colcemid ®) to arrest cells in c-metaphase. After sacrifice, chromosome preparations from bone marrow cells are made, stained and scored for SCE.

(3) *Animal selection*— (i) *Species and strain.* Any appropriate mammalian species may be used. Examples of commonly used rodent species include mice, rats, and hamsters.

(ii) *Age.* Healthy, young adult animals should be used.

(iii) *Number and sex.* At least five female and five male animals per experimental and control group should be used. The use of a single sex or different number of animals should be justified.

(iv) *Assignment to groups.* Animals should be randomized and assigned to treatment and control groups.

(4) *Control groups*—(i) *Concurrent controls.* Current positive and negative (vehicle) controls should be included in the assay.

(ii) *Positive controls.* A compound know to produce SCE *in vivo* should be employed as the positive control.

(5) *Test chemicals*—(i) *Vehicle.* When possible, test chemicals should be dissolved in isotonic saline or distilled water. Water insoluble chemicals may be dissolved or suspended in appropriate vehicles. The vehicle used should neither interfere with the test compound nor produce toxic effects. Fresh preparations of the test compound should be employed.

(ii) *Dose levels.* For an initial assessment, one dose of the test substance may be used, the dose being the maximum tolerated dose or that producing some indication of toxicity as evidenced by animal morbidity (including death) or target cell toxicity. The LD_{50} is a suitable guide. Additional dose levels may be used. For determination of dose-response, at least three dose levels should be used.

(iii) *Route of administration.* The usual routes of administration are IP or oral. Other routes may be appropriate.

(iv) *Treatment schedule.* In general, test substances should be administered only once. However, based upon toxicological information a repeated treatment schedule may be employed.

(e) *Test performance*—(1) *Treatment.* Animals should be treated with test chemical followed by administration of BrdU. BrdU may be administered by multiple IP injections, by continuous tail vein infusion or by subcutaneous implantation of tablets. Animals should be treated with a spindle inhibitor (e.g. colchicine or Colcemid®) 2 hr prior to sacrifice. After sacrifice, bone marrow should be extracted and slides made and prepared for SCE evaluation.

(2) *Staining method.* Staining of slides to reveal SCEs can be performed according to any of several protocols. However, the fluorescence plus Giemsa method is recommended.

(3) *Number of cells scored.* The number of cells to be analyzed per animal should be based upon the number of animals used, the negative control frequency, the predetermined sensitivity and the power chosen for the test. Slides should be coded before microscopic analysis.

(f) *Data and report*—(1) *Treatment of results.* Data should be presented in tabular form, providing scores for both the number of SCE for each metaphase and the number of SCE per chromosome for each metaphase. Differences among animals within each group should be considered before making comparisons between treated and control groups.

(2) *Statistical evaluation.* Data should be evaluated by appropriate statistical methods.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of SCE. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test points.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of SCE or a statistically significant and reproducible positive response at any one of the test points is considered not to induce rearrangements of DNA segments in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* (i) Positive results in the *in vivo* SCE assay indicate that under the test conditions the test

substance induces reciprocal interchanges in the bone marrow of the test species.

(ii) Negative results indicate that under the test conditions the test substance does not induce reciprocal interchanges in the bone marrow of the test species.

(5) *Test report.* In addition to the reporting recommendations as specified under 40 CFR Part 792, Subpart J, the following specific information should be reported:

(i) Species, strain, age, weight, number and sex of animals in each treatment and control group.

(ii) Test chemical vehicle, dose level used, rationale for dose selection, toxicity data, negative and positive controls.

(iii) Route and schedule of administration of both test chemical and BrdU.

(iv) Identity of spindle inhibitor, its concentration and duration of treatment.

(v) Time of sacrifice after administration of BrdU.

(vi) Details of the protocol used for slide preparation.

(vii) Criteria for scoring SCE.

(viii) Dose-response relationship, if applicable.

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Allen, J.W., Shuler, C.F., Latt, S.A. "Bromodeoxyuridine tablet methodology for in vivo studies of DNA synthesis," *Somatic Cell Genetics*, 4:393–405 (1978).

(2) Allen, J.W., Shuler, C.F., Mendes, R.W., Latt, S.A. "Simplified technique for in vivo analysis of sister chromatid exchanges using 5-bromodeoxyuridine tablets" *Cytogenetics Cell Genetics*, 18:231–237 (1977).

(3) Latt, S.A., Allen, J., Bloom, S.E., Carrano, A., Falke, E., Kram, D., Schneider, E., Schreck, R., Tice, R., Whitfield, B., Wolff, S. "Sister chromatid exchanges: a report of the U.S. EPA Gene-Tox Program," *Mutation Research*, 87:17–62 (1981).

§ 798.5955 Heritable translocation test in drosophila melanogaster.

(a) *Purpose.* The heritable translocation test in *Drosophila* measures the induction of chromosomal translocations in germ cells of insects. Stocks carrying genetic markers on two or more chromosomes are used to follow the assortment of chromosomes in meiosis. The F_1 male progeny of treated parents are individually mated to females and the F_2 progeny phenotypes are scored. The observed spectrum of phenotypes is used to determine the presence or absence of a translocation. This is usually indicated by a lack of independent assortment of genes on different chromosomes.

(b) *Definitions*—(1) Chromosome mutations are chromosomal changes resulting from breakage and reunion of chromosomes. Chromosomal mutations are also produced through nondisjunction of chromosomes during cell division.

(2) Reciprocal translocations are chromosomal translocations resulting from reciprocal exchanges between two or more chromosomes.

(3) Heritable translocations are reciprocal translocations transmitted from parent to the succeeding progeny.

(c) *Reference substances.* These may include, but need not be limited to, ethyl methanesulfonate or N-dimethylnitrosamine.

(d) *Test method*— (1) *Principle.* The method is based on the principle that balanced reciprocal chromosomal translocations can be induced by chemicals in the germ cells of treated flies and that these translocations are detected in the F_2 progeny using genetic markers (mutations). Different mutations may be used as genetic markers and two or more of the four chromosomes may be genetically marked for inclusion in this test.

(2) *Description.* Wild-type males are treated with chemical and bred with females of known genetic markers. The F_1 males are collected and individually bred with virgin females of the female parental stock. The resulting F_2 progeny are scored. Putative translocation carriers are confirmed with an F_3 cross.

(i) *Illustrative example.* The following example serves to illustrate the method. Males carrying genes for red eye color on chromosomes II and III are bred with females of white eye color carrying alleles for brown (bw) on the second chromosome and scarlet (st) and pink (pp) on the third chromosome. The F_1 male progeny are bred with virgin females of the female parental stock and the resulting F_2 progeny are examined for eye color phenotypes. If there is no translocation in the F_1 male, then the resulting F_2 progeny will have four eye color phenotypes: red, white, orange, and brown. If the F_1 male carries a translocation between chromosomes II and III, only red and white eye phenotypes are obtained in the F_2 generation. This happens because the F_1 translocation heterozygote produces two balanced (carrying either the parental or the translocated configuration of markers) and two unbalanced gametes. The unbalanced gametes (carrying one normal and one translocated chromosome) are unable to develop into normal individuals in the F_2 generation.

(ii) [Reserved]

(3) *Drosophila stocks.* Wild-type males and females of the genotype bw;st;pp (white eyes) may be used in the heritable translocation test. Other appropriately marked *Drosophila* stocks may also be used.

(4) *Control groups.* (i) Concurrent positive and negative (vehicle) controls should be included in each experiment.

(ii) Negative (vehicle) controls should be included. The size of the negative (vehicle) control group should be determined by the availability of appropriate laboratory historical control data.

(iii) If the historical control data are of sufficient numbers, concurrent controls may not be necessary.

(5) *Test chemicals*—(i) *Vehicle.* Test chemicals should be dissolved in water. Compounds which are insoluble in water may be dissolved or suspended in appropriate vehicles (e.g., a mixture of ethanol and Tween-60 or 80), and then diluted in water or saline prior to administration. Dimethylsulfoxide should be avoided as a vehicle.

(ii) *Dose levels.* For the initial assessment of mutagenicity, it may be sufficient to test a single dose of the test substance. This dose should be the maximum tolerated dose or that which produces some indication of toxicity. If the test is being used to verify mutagenic activity, at least two additional exposure levels should be used.

(iii) *Route of administration.* Exposure may be oral, by injection or by exposure to gases or vapours. Feeding of the test compound may be done in sugar solution. When necessary, substances may be dissolved in 0.7 percent NaCl solution and injected into the thorax or abdomen.

(e) *Test performance*—(1) *P1 mating.* (i) In the primary screen of a chemical, it is enough to sample one germ cell stage, either mature sperm or spermatids (for indirect acting mutagens). Other stages may be sampled if needed, i.e., when mature germ cells give a positive result and data from earlier germ cells are needed for the purpose of risk assessment. Thus, the treated males may be mated only once for a period of 3 days to sample sperm or transferred every 2 to 3 days to cover the entire germ cell cycle.

(ii) Mass matings may be performed because the control rate for translocations in the available literature is very low (near 0) and clustered events are extremely rare. Mated females may be aged for 2 weeks in order to recover an enhanced incidence of translocation due to the storage effect. The females are then allowed to lay eggs and F_1 males are collected for test mating.

(2) *F_1 mating.* F_1 males should be bred with virgin females of the parental female stock. Since each F_1 male represents one treated gamete of the male parent, the F_1 males have to be mated individually to virgin females. Each F_1 male should be mated to three females to ensure sufficient progeny.

(3) *Scoring the F_2 generation.* F_2 cultures (each representing 1 F_1 male tested) should be scored for the presence or absence of phenotype variations (linkage of markers) from the expected types. The test should be designed with a predetermined sensitivity and power. The number of flies in each group should reflect these defined parameters. The spontaneous mutant frequency observed in the appropriate control group will strongly influence the number of treated chromosomes that must be analyzed to detect substances which show mutation rates close to those of the controls. A positive test should be confirmed by F_3 mating trials.

(4) *Number of replicate experiments.* Replicate experiments are usually performed for each dose of the compound tested. If a chemical is a potent inducer of translocations, one experiment may be sufficient. Otherwise two or three replicate experiments should be done.

(f) *Data and report*—(1) *Treatment of results.* Data should be tabulated to show the number of translocations and the number of fertile F_1 males at each exposure for each germ cell stage sampled.

(2) *Statistical evaluation.* Data should be evaluated by appropriate statistical methods.

(3) *Interpretation of results.* (i) There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of heritable translocations. Another criterion may be based upon detection of a reproducible and statistically significant positive response for at least one of the test points.

(ii) A test substance which does not produce either a statistically significant dose-related increase in the number of heritable translocations or a statistically significant and reproducible positive response at any one of the test points is considered nonmutagenic in this system.

(iii) Both biological and statistical significance should be considered together in the evaluation.

(4) *Test evaluation.* (i) Positive results in the heritable translocation test in *Drosophila* indicate that under the test conditions the test substance causes chromosome damage in germ cells of this insect.

(ii) Negative results indicate that under the test conditions the test substance does not cause chromosomal damage in *D. melanogaster.*

(5) *Test report.* In addition to the reporting recommendations as specified under 40 CFR Part 792, Subpart J, the following specific information should be reported:

(i) *Drosophila* stock used in the assay, age of insects, number of males treated, number of F_2 cultures established, number of replicate experiments.

(ii) Test chemical vehicle, treatment and mating schedule, exposure levels, toxicity data, dose and route of exposure.

(iii) Positive and negative (vehicle) controls.

(iv) Historical control data, if available.

(v) Number of chromosomes scored.

(vi) Criteria for scoring mutant chromosomes.

(vii) Dose-response relationship, if applicable.

(g) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Wurgler, F.E., Sobels, F.H., Vogel, E. "*Drosophila* as assay system for detecting genetic changes," *Handbook of mutagenicity test procedures.* Eds. Kilby, B.J., Legator, M., Nichols, W., Ramel, C. (Amsterdam: Elsevier/North Holland Biomedical Press, 1979) pp. 335–374.

(2) [Reserved]

Subpart G—Neurotoxicity

§ 798.6050 Functional observational battery.

(a) *Purpose.* In the assessment and evaluation of the potential human health effects of substances, it may be necessary to test for neurotoxic effects. Substances that have been observed to cause neurotoxic signs (e.g., convulsions, tremors, ataxia) in other toxicity tests, as well as those having a structural similarity to known neurotoxicants, should be evaluated for neurotoxicity. The functional observational battery is a noninvasive procedure designed to detect gross functional deficits in young adults resulting from exposure to chemicals and to better quantify neurotoxic effects detected in other studies. This battery of tests is not intended to provide a detailed evaluation of neurotoxicity. It is designed to be used in conjunction with neuropathologic evaluation and/or general toxicity testing. Additional functional tests may be necessary to assess completely the neurotoxic potential of a chemical.

(b) *Definitions.* (1) Neurotoxicity is any adverse effect on the structure or function of the central and/or peripheral nervous system related to exposure to a chemical substance.

(2) A toxic effect is an adverse change in the structure or function of an experimental animal as a result of exposure to a chemical substance.

(c) *Principle of the test method.* The material is administered by an appropriate route to laboratory rodents. The animals are observed under carefully standardized conditions with sufficient frequency to ensure the detection of behavioral and/or neurologic abnormalities, if present. Various functions that could be affected by neurotoxicants are assessed during each observation period.

(d) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* The laboratory rat or mouse is recommended. Although information will generally be lacking, whenever possible the choice of species should take into consideration such factors as the comparative metabolism of the chemical and species sensitivity to the toxic effects of the test substance, as evidenced by the results of other studies. The potential for combined studies should also be considered. Standard strains should be used.

(ii) *Age.* Young adult animals (at least 42 days old for the rat or mouse) should be used.

(iii) *Sex.* (A) Equal numbers of animals of each sex are required for each dose level.

(B) The females should be nulliparous and nonpregnant.

(2) *Number of animals.* All exposed animals should be tested. At least 10 animals of each sex should be used at each dose level. If interim sacrifices are planned, the number should be increased by the number of animals scheduled to be sacrificed before the end of the study. Animals should be randomly assigned to treatment and control groups.

(3) *Control groups.* (i) A concurrent ("sham" exposure or vehicle) control group is required. Subjects should be treated in the same way as for an exposure group except that administration of the test substance is omitted.0, RULES AND REGS) A35AD0 Barrett, Douglas 04942 7-29-85 J. 54-999 File a35ad0.422 Folio 1110-11

(ii) Concurrent or historic data from the laboratory performing the testing should provide evidence of the ability of the procedures used to detect major neurotoxic endpoints such as limb weakness or paralysis (e.g., acrylamide), CNS stimulation (e.g., β, β'-iminodiproprionitrile) autonomatic signs (e.g., physostigmine).

(iii) A satellite group may be treated with the high dose level for the duration of exposure and observed for reversibility, persistence, or delayed occurrence of toxic effects for a post-treatment period of appropriate duration, normally not less than 28 days.

(4) *Dose levels and dose selection.* At least 3 doses, equally spaced on a log scale (e.g., ½ log units) over a range of at least 1 log unit shall be used in addition to a zero dose or vehicle administration. The data should be sufficient to produce a dose-effect curve.

(i) The highest dose shall produce (A) clear behavioral effects or (B) life-threatening toxicity.

(ii) The data from the lower doses must show either (A) graded dose-dependent effects at 2 dose levels or (B) no effects at 2 dose levels, respectively.

(5) *Duration and frequency of exposure.* The duration and frequency of exposure will be specified in the test rule.

(6) *Route of exposure.* The test substance shall be administered by the route specified in the test rule. This route will usually be the one most closely approximating the expected route of human exposure. The exposure potocol shall conform to that outlined in the appropriate acute or subchronic toxicity study guideline under Subpart B or Subpart C of this Part.

(7) *Combined protocol.* Subjects used for other toxicity studies may be used if none of the requirements of either study are violated by the combination.

(8) *Study conduct.* (i) All animals in a given study should be observed carefully by the same trained technician who is blind with respect to the animals' treatments. All animals should be observed prior to initiation of exposure. Subsequent observations should be made with sufficient frequency to ensure the detection of behavioral and/or neurologic abnormalities, if present. At minimum, observations at 1 hour, 6 hours, 24 hours, 7 days, and 14 days and monthly thereafter are recommended. In a subchronic study, subsequent to the first exposure, all observations should be made before the daily exposue. The animals should be removed from the home cage to a standard arena for observation. Effort should be made to ensure that variations in the test conditions are minimal and are not systematically related to treatment. Among the variables that can affect behavior are sound level, temperature, humidity, lighting, odors, time of day, and environmental distractions. Explicit, operationally defined, scales for each function should be used. The development of objective quantitative measures of the observational endpoints specified is encouraged.

(ii) The following is a minimal list of observations that should be noted:

(A) Any unusual responses with respect to body position, activity level, coordination of movement, and gait.

(B) Any unusual or bizarre behavior including, but not limited to, headflicking, head searching, compulsive biting or licking, self-mutilation, circling, and walking backwards.

(C) The presence of:

(*1*) Convulsions.

(*2*) Tremors.

(*3*) Increased levels of lacrimation and/or red-colored tears.

(*4*) Increased levels of salivation.

(*5*) Piloerection.

(*6*) Pupillary dilation or constriction.

(*7*) Unusual respiration (shallow, labored, dyspneic, gasping, and retching) and/or mouth breathing.

(*8*) Diarrhea.

(*9*) Excessive or diminished urination.

(*10*) Vocalization.

(D) Forelimb/hindlimb grip strength. The procedure described by Meyer et al. (1979), under paragraph (f)(9) of this section is recommended.

(E) Sensory function. A simple assessment of sensory function (vision, audition, pain perception) should be made. Marshall et al. (1971) under paragraph (f)(8) of this section have described a neurologic exam for this purpose; these procedures are also discussed by Deuel (1977), under paragraph (f)(4) of this section. Irwin (1968) under paragraph (f)(7) of this section described a number of reflex tests intended to detect gross sensory deficits, including the visual placing response, Preyer reflex, and tail pinch. Many procedures have been developed for assessing pain perception (e.g., Ankier, 1974 under paragraph (f)(1) of this section; D'Amour and Smith 1941 under paragraph (f)(3) of this section; Evans 1971 under paragraph (f)(6) of this section).

(e) *Data reporting and evaluation.* In addition to the reporting requirements specified under 40 CFR Part 792 Subpart J the final test report must include the following information.

(1) *Description of system and test methods.* (i) A detailed description of the procedures used to standardize observation, including the arena and operational definitions for scoring observations.

(ii) Positive control data from the laboratory performing the test that demonstrate the sensitivity of the procedures being used. Historic data may be used if all aspects of the experimental protocol are the same, including personnel.

(2) *Results.* The following information must be arranged by test group dose level.

(i) In tabular form, data for each animal must be provided showing:

(A) Its identification number.

(B) Its body weight and score on each sign at each observation time, the time and cause of death (if appropriate).

(ii) Summary data for each group must include:

(A) The number of animals at the start of the test.

(B) The number of animals showing each observation score at each observation time.

(C) The percentage of animals showing each abnormal sign at each observation time.

(D) The mean and standard deviation for each continuous endpoint at each observation time.

(3) *Evaluation of data.* The findings of a functional observational battery should be evaluated in the context of preceding and/or concurrent toxicity studies and any correlative histopathological findings. The evaluation should include the relationship between the doses of the test substance and the presence or absence, incidence and severity, of any neurotoxic effects. The evaluation should include appropriate statistical analyses. Choice of analyses should consider tests appropriate to the experimental design and needed adjustments for multiple comparisons.

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Ankier, S.I. "New hot plate tests to quantify antinociceptic and narcotic antagonist activities," *European Journal of Pharmacology,* 27: 1–4 (1974).

(2) Coughenour, L.L., McLean, J.R. and Parker, R.B. "A new device for the rapid measurement of impaired motor function in mice," *Pharmacology, Biochemistry and Behavior,* 6: 351–353 (1977).

(3) D'Amour, F.E., Smith, D.L. "A method for determining loss of pain sensation," *Journal of Pharmacology and Experimental Therapeutics,* 72: 74–79 (1941).

(4) Deuel, R.K. "Determining sensory deficits in animals," *Methods in Psychobiology* Ed. Myers R.D. (New York: Academic Press, 1977) pp. 99–125.

(5) Edwards, P.M., Parker, V.H. "A simple, sensitive and objective method for early assessment of acrylamide neuropathy in rats," *Toxicology and*

Applied Pharmacology, 40: 589–591 (1977).

(6) Evans, W.O. "A new technique for the investigation of some analgesic drugs on reflexive behavior in the rat," *Psychopharmacologia, 2:* 318–325 (1961).

(7) Irwin, S. "Comprehensive observational assessment: Ia. A systematic quantitative procedure for assessing the behavioral and physiologic state of the mouse," *Psychopharmacologia, 13:* 222–257 (1968).

(8) Marshall, J.F., Turner, B.H., Teitlbaum, P. "Sensory neglect produced by lateral hypothalamic damage," *Science, 174:* 523–525 (1971).

(9) Meyer, O.A., Tilson, H.A., Byrd, W.C., Riley, M.T. "A method for the routine assessment of fore- and hindlimb grip strength of rats and mice," *Neurobehavioral Toxicology, 1:* 233–236 (1979).

§ 798.6200 Motor activity.

(a) *Purpose*—(1) *General.* In the assessment and evaluation of the toxic characteristics of a substance, determination of the effects of administration of the substance on motor activity is useful when neurotoxicity is suspected.

(2) *Acute Motor Activity Test.* The purpose of the acute motor activity test is to examine changes in motor activity occurring over a range of acute exposure levels. These changes may then be evaluated in the context of changes occurring in other organ systems. This test is an initial step in determining the potential of a substance to produce acute neurotoxicity and may be used to screen members of a class of substances for known neurotoxicity, and/or to establish a dosage regimen prior to the initiation of subchronic neurotoxicity testing.

(3) *Subchronic Motor Activity Test.* The purpose of the subchronic motor activity test is to determine whether the repeated administration of a suspected neurotoxicant results in changes in motor activity. These changes may be evaluated in the context of changes occurring in other organ systems. This test is an initial step in determining the potential of a substance to produce subchronic neurotoxicity.

(b) *Definitions.* (1) Neurotoxicity is the adverse effect on the structure or function of the central and/or peripheral nervous system related to exposure to a chemical substance.

(2) Motor activity is any movement of the experimental animal.

(3) A toxic effect is an adverse change in the structure or function of an experimental animal as a result of exposure to a chemical substance.

(c) *Principle of the test method.* The test substance is administered to several groups of experimental animals, one dose being used per group. Measurements of motor activity are made. The exposure levels at which significant changes in motor activity are produced are compared to those levels which produce toxic effects not originating in the central and/or peripheral nervous system.

(d) *Test procedures*—(1) *Animal selection*—(i) *Species and strain.* Testing shall be performed in a laboratory rat or mouse. The choice of species should take into consideration such factors as the comparative metabolism of the chemical and species sensitivity to the toxic effects of the test substance, as evidenced by the results of other studies, the potential for combined studies, and the availability of other toxicity data for the species.

(ii) *Age.* Young adult animals (at least 42 days old for rat or mouse) should be used.

(iii) *Sex.* (A) Equal numbers of animals of each sex are required for each dose level for the motor activity test.

(B) The females should be nulliparous and nonpregnant.

(2) *Number of animals.* Animals shall be randomly assigned to test and control groups. Each test or control group must be designed to contain a sufficient number of animals at the completion of the study to detect a 40 percent change in activity of the test groups relative to the control group with 90 percent power at the 5 percent level. For most designs, calculations can be made according to Dixon and Massey (1957) under paragraph (f)(1) of this section, Neter and Wasserman (1974) under paragraph (f)(5) of this section, Sokal and Rohlf (1969) under paragraph (f)(9) of this section, or Jensen (1972) under paragraph (f)(3) of this section.

(3) *Control groups.* (i) A concurrent control group is required. This group must be an untreated group, or, if a vehicle is used in administering the test substance, a vehicle control group. If the toxic properties of the vehicle are not known or cannot be made available, both untreated and vehicle control group are required.

(ii) Positive control data are required to demonstrate the sensitivity and reliability of the activity measuring device and testing procedure. These data should demonstrate the ability to detect increases or decreases in activity and to generate a dose-effect curve or its equivalent using three values of the dose or equivalent independent variable. A single administration of the dose (or equivalent) is sufficient. It is recommended that chemical exposure be used to collect positive control data. Positive control data shall be collected at the time of the test study unless the laboratory can demonstrate the adequacy of historical data for this purpose.

(iii) A satellite group may be treated with the high dose level for 90 days and observed for reversibility, persistence or delayed occurrence of toxic effects for a post-treatment period of appropriate length, normally not less than 28 days.

(4) *Dose levels and dose selection.* At least 3 doses, equally spaced on a log scale (e.g., ½ log units) over a range of at least 1 log unit shall be used in addition to a zero dose or vehicle administration. The data should be sufficient to produce a dose-effect curve.

(i) The highest dose shall produce (A) clear effects on motor activity or (B) life-threatening toxicity.

(ii) The data from the lower doses must show either (A) graded dose-dependent effects at 2 dose levels or (B) no effects at 2 dose levels, respectively.

(5) *Duration of testing.* The duration of exposure will be specified in the test rule.

(6) *Route of administration.* The test substance shall be administered by the method specified in the test rule. This will usually be the route most closely approximating the route of human exposure. The exposure protocol shall conform to that outlined in the appropriate acute or subchronic toxicity study guideline.

(7) *Combined protocol.* The tests described herein may be combined with any other toxicity study, as long as none of the requirements of either are violated by the combination.

(8) *Study conduct*—(i) *General.* Motor activity must be monitored by an automated activity recording apparatus. The device used must be capable of detecting both increases and decreases in activity, i.e. baseline activity as measured by the device must not be so low as to preclude decreases nor so high as to preclude increases. Each device shall be tested by standard procedure to ensure, to the extent possible, reliability of operation across devices and across days for any one device. In addition, treatment groups must be balanced across devices. Each animal shall be tested individually. The test session shall be long enough for motor activity to approach asymptotic levels by the last 20 percent of the session for most treatments and animals. All sessions should have the same duration. Treatment groups shall be counter-balanced across test times. Effort should be made to ensure that variations in the

test conditions are minimal and are not systematically related to treatment. Among the variables which can affect motor activity are sound level, size and shape of the test cage, temperature, relative humidity, lighting conditions, odors, use of home cage or novel test cage and environmental distractions. Tests shall be executed by an appropriately trained individual.

(ii) *Acute.* Testing shall be timed to include the time of peak signs.

(iii) *Subchronic.* All animals shall be tested prior to initiation of exposure and at 30 ±2, 60 ±2 and 90 ±2 days during the exposure period. Testing shall occur prior to the daily exposure. Animals shall be weighed on each test day and at least once weekly during the exposure period.

(e) *Data reporting and evaluation.* In addition to the reporting requirements specified under 40 CFR Part 792, Subpart J the final test report must include the following information:

(1) *Description of system and test methods.* (i) Positive control data from the laboratory performing the test which demonstrate the sensitivity of the procedure being used.

(ii) Procedures for calibrating and assuring the equivalence of devices and balancing treatment groups.

(2) *Results.* The following information must be arranged by test group (dose level).

(i) In tabular form, data must be provided showing for each animal:

(A) Its identification number.

(B) Body weight, total session activity counts, and intrasession subtotals for each date measured.

(ii) Group summary data should also be reported.

(3) *Evaluation of data.* An evaluation of the test results (including statistical analysis comparing total activity counts at the end of exposure of treatment vs control animals must be made and supplied. This submission must include dose-effect curves for motor activity expressed as activity counts.

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Dixon, W.J., Massey, E.J. *Introduction to Statistical Analysis* 2nd Ed. (New York: McGraw-Hill, 1957).

(2) Finger, F.W. "Measuring behavioral activity," *Methods in Psychobiology* Vol. 2. Ed. R.D. Myers (New York: Academic, 1972) pp. 1–19.

(3) Jensen, D.R. "Some simultaneous multivariate procedures using Hotelling's T^2 Statistics," *Biometrics*, 28:39–53 (1972).

(4) Kinnard, E.J. and Watzman, N. "Techniques utilized in the evaluation of psychotropic drugs on animals activity," Journal of Pharmaceutical Sciences, 55:995–1012 (1966).

(5) Neter, J. and Wasserman, W. *Applied Linear Statistical Models.* Homewood, Richard D. Irwin, Inc., 1974.

(6) Reiter, L.E. "Use of activity measures in behavioral toxicology," *Environmental Health Perspectives*, 26:9–20 (1978).

(7) Reiter, L.W. and MacPhail, R.C. "Motor Activity: A survey of methods with potential use in toxicity testing," *Neurobehavioral Toxicology*, 1: Suppl. 1, 53–66 (1979).

(8) Robbins, T.W. "A critique of the methods available for the measurement of spontaneous motor activity," *Handbook of Psychopharmacology.* Vol. 7. Eds. Iversen, L.L., Iversen, D.S., Snyder, S.H. (New York: Plenum, 1977) pp. 37–82.

(9) Sokal, R.P. and Rohlf, E.J. *Biometry.* (San Francisco: W.H. Freeman and Co., 1969).

§ 798.6400 Neuropathology.

(a) *Purpose.* The techniques in this guideline are designed to develop data on morphologic changes in the nervous system for chemical substances and mixtures subject to such testing under the Toxic Substances Control Act. The data will detect and characterize morphologic changes, if and when they occur, and determine a no-effect level for such changes. Neuropathological evaluation should be complemented by other neurotoxicity studies, e.g. behavioral and neurophysiological studies. Neuropathological evaluation may be done following acute, subchronic or chronic exposure.

(b) *Definition.* Neurotoxicity or a neurotoxic effect is an adverse change in the structure or function of the nervous system following exposure to a chemical agent.

(c) *Principle of the test method.* The test substance is administered to several groups of experimental animals, one dose being used per group. The animals are sacrificed and tissues in the nervous system are examined grossly and prepared for microscopic examination. Starting with the highest dosage level, tissues are examined under the light microscope for morphologic changes, until a no effect level is determined. In cases where light microscopy has revealed neuropathology, the no effect level may be confirmed by electron microscopy.

(d) *Test procedure*—(1) *Animal selection*—(i) *Species and strain.* Testing should be performed in the species being used in other tests for neurotoxicity. This will generally be the laboratory rat. The choice of species shall take into consideration such factors as the comparative metabolism of the chemical and species sensitivity to the toxic effects of the test substance, as evidenced by the results of other studies, the potential for combined studies, and the availability of other toxicity data for the species.

(ii) *Age.* Animals shall be young adults (150–200 gm for rats) at the start of exposure.

(iii) *Sex.* Both sexes should be used unless it is demonstrated that one sex is refractory to the effects.

(2) *Number of Animals.* A minimum of six animals per group shall be used. The tissues from each animal shall be examined separately. It is recommended that ten animals per group be used.

(3) *Control Groups.* (i) A concurrent control group(s) is (are) required. This group must be an untreated control group or, if a vehicle is used in administering the test substance, a vehicle control group. If the vehicle used has a known or potential toxic property, both untreated and vehicle control groups are required.

(ii) A satellite group of animals may be treated with the high level for 90 days and observed for reversibility, persistence, or delayed occurrence of toxic effects for a post-treatment period of appropriate length; normally not less than 28 days.

(4) *Dose Levels and Dose Selection.* At least 3 doses, equally spaced on a log scale (e.g., ½ log units) over a range of at least 1 log unit shall be used in addition to a zero dose or vehicle administration. The data should be sufficient to produce a dose-effect curve.

(i) The highest dose shall produce (A) clear behavioral effects or (B) life-threatening toxicity.

(ii) The data from the lower doses must show either (A) graded dose-dependent effects at two dose levels or (B) no effects at two dose levels, respectively.

(5) *Duration of testing.* The exposure duration will be specified in the test rule. This will generally be 90 days exposure.

(6) *Route of administration.* The test substance shall be administered by a route specified in the test rule. This will generally be the route most closely approximating the route of human exposure. The exposure protocol shall conform to that outlined in the appropriate acute or subchronic toxicity guideline.

(7) *Combined protocol.* The tests described herein may be combined with any other toxicity study, as long as none of the requirements of either are violated by the combination.

(8) *Study conduct*—(i) *Observation of animals.* All toxicological (e.g., weight loss) and neurological signs (e.g., motor disturbance) shall be recorded frequently enough to observe any abnormality, and not less than weekly.

(ii) *Sacrifice of animals*—(A) *General.* The goal of the techniques outlined for sacrifice of animals and preparation of tissues is preservation of tissues morphology to simulate the living state of the cell.

(B) *Perfusion technique.* Animals shall be perfused *in situ* by a generally recognized technique. For fixation suitable for light or electronic microscopy, saline solution followed by buffered 2.5 percent glutaraldehyde or buffered 4.0 percent paraformaldehyde, is recommended. While some minor modifications or variations in procedures are used in different laboratories, a detailed and standard procedure for vascular perfusion may be found in the text by Zeman and Innes (1963) under paragraph (F)(7) of this section, Hayat (1970) under paragraph (F)(3) of this section, and by Spencer and Schaumburg (1980) under paragraph (F)(6) of this section. A more sophisticated technique is described by Palay and Chan-Palay (1974) under paragraph (F)(4) of this section.

(C) *Removal of brain and cord.* After perfusion, the bonystructure (cranium and vertebral column) should be exposed. Animals should then be stored in fixative-filled bags at 4°C for 8–12 hours. The cranium and vertebral column shall, be removed carefully by trained technicians without physical damage of the brain and cord. Detailed dissection procedures may be found in the text by Palay and Chan-Palay (1974) under paragraph (F)(4) of this section. After removal, simple measurement of the size (length and width) and weight of the whole brain (cerebrum, cerebellum, pons-medulla) should be made. Any abnormal coloration or discoloration of the brain and cord should also be noted and recorded.

(D) *Sampling.* Unless a given test rule specifies otherwise, cross-sections of the following areas shall be examined: the forebrain, the center of the cerebrum, the midbrain, the cerebellum and pons, and the medulla oblongata; the spinal cord at cervical and lumbar swelling (C_3–C_6 and L_1–L_4); Gasserian ganglia, dorsal root ganglia (C_3–C_6, L_1–L_4), dorsal and ventral root fibers (C_3–C_6, L^1–L_4), proximal sciatic nerve (mid-thigh and sciatic notch), sural nerve (at knee), and tibial nerve (at knee). Other sites and tissue elements (e.g., gastrocnemius muscle) should be examined if deemed necessary. Any observable gross changes shall be recorded.

(iii) *Specimen storage.* Tissue samples from both the central and peripheral nervous system shall be further immersion fixed and stored in appropriate fixative (e.g., 10 percent buffered formalin for light microscopy; 2.5 percent buffered gluteraldehyde or 4.0 percent buffered paraformaldehyde for electron microscopy) for future examination. The volume of fixative versus the volume of tissues in a specimen jar shall be no less than 25:1. All stored tissues should be washed with buffer for at least 2 hours prior to further tissue processing.

(iv) *Histopathology examination.* (A) *Fixation.* Tissue specimens stored in 10 percent buffered formalin may be used for this purpose. All tissues must be immersion fixed in fixative for at least 48 hours prior to further tissue processing.

(B) *Dehydration.* All tissue specimens should be washed for at least 1 hour with water or buffer, prior to dehydration. (A longer washing time is needed if the specimens have been stored in fixative for a prolonged period of time.) Dehydration can be performed with increasing concentration of graded ethanols up to absolute alcohol.

(C) *Clearing and embedding.* After dehydration, tissue specimens shall be cleared with xylene and embedded in paraffin or paraplast. Multiple tissue specimens (e.g. brain, cord, ganglia) may be embedded together in one single block for sectioning. All tissue blocks shall be labelled showing at least the experiment number, animal number, and specimens embedded.

(D) *Sectioning.* Tissue sections, 5 to 6 microns in thickness, shall be prepared from the tissue blocks and mounted on standard glass slides. It is recommended that several additional sections be made from each block at this time for possible future needs for special stainings. All tissue blocks and slides shall be filed and stored in properly labeled files or boxes.

(E) *Histopathological techniques.* Although the information available for a given chemical substance may dictate test-rule specific changes, the following general testing sequence is proposed for gathering histopathological data:

(*1*) *General staining.* A general staining procedure shall be performed on all tissue specimens in the highest treatment group. Hematoxylin and eosin (H&E) shall be used for this purpose. The staining shall be differentiated properly to achieve bluish nuclei with pinkish background.

(*2*) *Special stains.* Based on the results of the general staining, selected sites and cellular components shall be further evaluated by the use of specific techniques. If H&E screening does not provide such information, a battery of stains shall be used to assess the following components in all appropriate required samples: neuronal body (e.g., Einarson's gallocyanin), axon (e.g., Bodian), myelin sheath (e.g., Kluver's Luxol Fast Blue) and neurofibrils (e.g., Bielchosky). In addition, peripheral nerve fiber teasing shall be used. Detailed staining methodology is available in standard histotechnological manuals such as AFIP (1968) under paragraph (f)(1) of this section, Ralis et al. (1973) under paragraph (f)(5) of this section, and Chang (1979) under paragraph (f)(2) of this section. The nerve fiber teasing technique is discussed in Spencer and Schaumberg (1980) under paragraph (f)(6) of this section. A section of normal tissue shall be included in each staining to assure that adequate staining has occurred. Any changes shall be noted and representative photographs shall be taken. If a lesion(s) is observed, the special techniques shall be repeated in the next lower treatment group until no further lesion is detectable.

(*3*) *Alternative technique.* If the anatomical locus of expected neuropathology is well-defined, epoxy-embedded sections stained with toluidine blue may be used for small sized tissue samples. This technique obviates the need for special stains for cellular components. Detailed methodology is available in Spencer and Schaumberg (1980) under paragraph (f)(6) of this section.

(*4*) *Electron microscopy.* Based on the results of light microscopic evaluation, specific tissue sites which reveal a lesion(s) shall be further evaluated by electron microscopy in the highest treatment group which does not reveal any light microscopic lesion. If a lesion is observed, the next lower treatment group shall be evaluated until no significant lesion is found. Detailed methodology is available in Hayat (1970) under paragraph (f)(3) of this section.

(F) *Examination*—(*1*) *General.* All stained microscopic slides shall be examined with a standard research microscope. Examples of cellular alterations (e.g., neuronal vacuolation, degeneration, and necrosis) and tissue changes (e.g., gliosis, leukocytic infiltration, and cystic formation) shall be recorded and photographed.

(*2*) *Electron microscopy.* Since the size of the tissue samples that can be examined is very small, at least 3 to 4 tissue blocks from each sampling site must be examined. Tissue sections must be examined with a transmission electron microscope. Three main categories of structural changes must be considered:

(*i*) *Neuronal body.* The shape and position of the nucleus and nucleolus as well as any change in the chromatin patterns shall be noted. Within the neuronal cytoplasm, cytoplasmic organelles such as mitochondria, lysosomes, neurotubules, neurofilaments, microfilaments, endoplasmic reticulum and polyribosomes (Nissl substance), Golgi complex, and secretory granules shall be examined.

(*ii*) *Neuronal processes.* The structural integrity or alterations of dendrites, axons (myelinated and unmyelinated), myelin sheaths, and synapses shall be noted.

(*iii*) *Supporting cells.* Attention must also be paid to the number and structural integrity of the neuroglial elements (oligodendrocytes, astrocytes, and microglia) of the central nervous system, and the Schwann cells, satellite cells, and capsule cells of the peripheral nervous system. Any changes in the endothelial cells and ependymal lining cells shall also be noted whenever possible. The nature, severity, and frequency of each type of lesion in each specimen must be recorded. Representative lesions must be photographed and labeled appropriately.

(e) *Data collection, reporting, and evaluation.* In addition to information meeting the requirements stated under 40 CFR Part 792 Subpart J, the following specific information should be reported:

(1) *Description of test system and test methods.* A description of the general design of the experiment should be provided. This should include a short justification explaining any decisions where professional judgment is involved such as fixation technique and choice of stains.

(2) *Results.* All observations shall be recorded and arranged by test groups. This data may be presented in the following recommended format:

(i) *Description of signs and lesions for each animal.* For each animal, data must be submitted showing its identification (animal number, treatment, dose, duration), neurologic signs, location(s) nature of, frequency, and severity of lesion(s). A commonly-used scale such as 1+, 2+, 3+, and 4+ for degree of severity ranging from very slight to extensive may be used. Any diagnoses derived from neurologic signs and lesions including naturally occurring diseases or conditions, should also be recorded.

(ii) *Counts and incidence of lesions, by test group.* Data shall be tabulated to show: (A) The number of animals used in each group, the number of animals displaying specific neurologic signs, and the number of animals in which any lesion was found; (B) The number of animals affected by each different type of lesion, the average grade of each type of lesion, and the frequency of each different type and/or location of lesion.

(iii) *Evaluation of data.* (A) An evaluation of the data based on gross necropsy findings and microscopic pathology observations shall be made and supplied. The evaluation shall include the relationship, if any, between the animal's exposure to the test substance and the frequency and severity of the lesions observed.

(B) The evaluation of dose-response, if existent, for various groups shall be given, and a description of statistical method must be presented. The evaluation of neuropathology data should include, where applicable, an assessment in conjunction with other neurotoxicity studies performed (eg. electrophysiological, behavioral, neurochemical).

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) AFIP. *Manual of Histologic Staining Methods.* (New York: McGraw-Hill (1968).

(2) Chang, L.W. *A Color Atlas and Manual for Applied Histochemistry.* (Springfield, IL: Charles C. Thomas, 1979).

(3) Hayat, M.A. "Vol. 1. Biological applications," *Principles and techniques of electron microscopy.* (New York: Van Nostrand Reinhold, 1970)

(4) Palay S.L., Chan-Palay, V. *Cerebellar Cortex: Cytology and Organization.* (New York: Springer-Verlag, 1974.

(5) Ralis, H.M., Beesley, R.A., Ralis, Z.A. *Techniques in Neurohistology.* (London: Butterworths, 1973).

(6) Spencer, P.S., Schaumburg, H.H. (eds). *Experimental and Clinical Neurotoxicology.* (Baltimore: Williams and Wilkins, 1980).

(7) Zeman, W., JRM Innes, J.R.M. *Craigie's Neuroanatomy of the Rat.* (New York: Academic, 1963).

§ 798.6450 NTE neurotox assay.

(a) *Purpose.* (1) A variety of organophosphorus (O–P) compounds cause a type of delayed neurotoxicity (OPIDN) in which inhibition of the esterase activity of a protein called neurotoxic esterase (NTE) in neural tissue is the primary biochemical correlate and predictor. This guideline will describe the conduct of an assay for measurement of the inhibition of NTE in the brain or spinal cord of animals exposed to O–P. It is based on the method described by Johnson (1975, 1977, 1982) under paragraph (f) (2),(3), and (4) of this section and quotes those sources directly. Other methods for performing the assay have been described by Sprague et al. (1981) under paragraph (f)(6) of this section, Soliman et al. (1982) under paragraph (f)(7) of this section and Caroldi and Lotti (1982) under paragraph (f)(1) of this section for peripheral nerve. These and other assays are reviewed by Johnson (1982) under paragraph (f)(4) of this section and Johnson and Richardson (1983) under paragraph (f)(5) of this section. This biochemical test is intended as an adjunct to a behavioral and pathological study in hens.

(2) NTE measurements provide objective quantitative data on the first step in initiation of OPIDN. Thus, they can be a great help in evaluating studies where the more poorly quantified behavioral or pathological data may be equivocal, or where the adequacy of a negative study is at issue. The second step necessary is "aging" of the phosphorylated NTE (the loss of an R group resulting in a negatively charged substituent bound to NTE). Not all O–P that inhibit NTE, then, cause OPIDN, but all neuropathic O–P inhibit NTE.

(3) Thus, the NTE assay is a valuable adjunct to but not a complete replacement for in-vivo testing. Johnson (1982) (under paragraph (f)(4) of this section has proposed a detailed testing scheme for combining the NTE assay with behavioral and histopathological studies in hens. Such combined testing can be faster, more accurate, and less expensive than the current EPA approach. OTS encourages testing schemes that combine this assay with in-vivo studies.

(b) *Definitions.* (1) Organophosphorus induced delayed neurotoxicity (OPIDN) is a neurological syndrome in which limb weakness and upper motor neuron spasticity are the predominant clinical signs and distal axonopathy of peripheral nerve and spinal cord are the correlative pathological signs. Clinical signs and pathology first appear between 1 and 2 weeks following exposure which normally inhibits greater than 80 percent of NTE (For O–Ps that "age").

(2) Neurotoxic target esterase (NTE) is a membrane-bound neural protein that hydrolyzes phenyl valerate and is highly correlated with the initiation of OPIDN. NTE activity is operationally defined as the phenyl valerate hydrolytic activity resistant to paraoxon but sensitive to mipafox or neuropathic O–P ester inhibition.

(c) *Principle of the test method.* The test method is a differential assay of the ability of neural tissue, following O–P exposure, to selectively hydrolyze a phenyl valerate substrate. The principle of the assay is first, to determine the amount of hydrolysis that occurs in the presence of a non-neurotoxic inhibitor, paraoxon, (a), which is intended to occupy irrelevant sites. Second, we determine the activity in the presence of paraoxon and a known neuropathic inhibitor, mipafox, (b). NTE activity is the difference between (a) and (b), that is, the proportion of activity inhibited only by mipafox. Thus, the "mipafox site" is already occupied following exposure to a neuropathic O–P ester and the activity of (b) is therefore reduced.

(d) *Testing procedure.* Because this guideline is intended as an adjunct to an in-vivo study, only those aspects of study conduct specific to the assay will be described.

(1) *Animal selection.* The adult domestic laying hen is recommended. Standard size breeds and strains should be employed.

(2) *Number of animals.* (i) At least four hens/dose or control group should be used.

(ii) It is recommended that at least two hens be dosed with a known neuropathic O–P as a positive control. Tri-o-tolyl phosphate (TOCP) or diisopropyl phosphorofluoridate (DFP) can be used but TOCP is recommended because of its low acute toxicity.

(3) *Materials.* To conduct this assay, you will need paraoxon (diethyl 4-nitrophenyl phosphate), mipafox (N, N'-diisopropylphosphorodiamido fluoridate), and phenyl valerate. Johnson (1977) under paragraph (f)(3) of this section includes a detailed description of the steps necessary for the synthesis, purification, and storage of these chemicals. Paraoxon can be obtained commercially.

(4) *Study conduct.* (i) The assay should be performed on the whole brain and spinal cord of subjects sacrificed 24 hours following acute exposure, and at regular intervals, e.g., weekly, during repeated exposure. The last measurement should follow the last exposure by 24 or 48 hours. Each assay should be performed in duplicate.

(ii) The assay has four stages: preparation of tissue; differential preincubation; hydrolysis of substrate; and measurement of product. The quotations that follow are from Johnson (1977) as corrected or modified in Johnson (1982). His is the best known method for conduct of this assay. Other acceptable methods have been used. They primarily involve minor technical modification (Sprague et al. 1981 under paragraph (f)(7) of this section and Soliman et al. 1982 under paragraph (f)(6) of this section). Testers are encouraged to discuss planned methods with EPA prior to conducting testing for approval.

(A) *Preparation of tissue.* "Normal or dosed birds are killed by cervical dislocation. The head is cut off and the whole brain removed and cooled in ice-cold buffer (50 mM Tris/0.2 mM EDTA adjusted to pH 8.0 at 25° with HCL). Meninges and blood vessels are rapidly removed and the brain is blotted dry, weighed, and homogenized thoroughly in ice-cold buffer (6.5 ml/g) using a high-speed rotating perspex pestle with not more than 0.25 mm difference in diameter between pestle and tube. The homogenate is then diluted to 1 g/65 ml for assay."

(B) *Differential preincubation.* "Paired samples of homogenate (equivalent to about 6.0 mg tissue) are pre-incubated in Tris/EDTA buffer pH 8 at 37° for exactly 20 minutes with paraoxon (40 to 100 uM) plus either (a) buffer or (b) mipafox (50uM) in a final volume of 2 ml." This step is essential.

(C) *Hydrolysis of substrate.* "After preincubation, dispersion (2ml) of phenyl valerate is added and the incubation is continued for exactly 15 minutes. The dispersion is prepared by adding a solution of Triton X–100 (0.03 percent in water) (30 vol) to a solution of phenyl valerate (15 or 30 mg/ml) in redistilled dimethylformamide (1 vol) and mixing thoroughly (by swirling): other solvents give less satisfactory dispersions. Reaction is stopped by adding 2 ml of sodium dodecyl sulphate (1% w/v) in buffer containing 4-aminoantipyrine (otherwise known as 4-aminophenazone) (0.25 percent)."

(D) *Measurement of product.* This assay is based on the colorimetric detrimination of liberated phenol.

(*1*) "The coupling of phenol liberated in the assay with the aminoantipyrine may be performed at any convenient time after quenching the enzyme: 1 ml of $K_3Fe(CN)_6$ (0.4 percent in water) is added and the stable red colour is read at 510 nm."

(*2*) "A non-tissue blank, kept to 10 percent of the "B" (paraoxon tube) value by maintaining the substrate phenol fee, should be included in each group of assay tubes. Typical control absorbance values would be 0.8 for paraoxon, 0.35 for paraoxon and mipafox and 0.07 for the blank. Colour development takes (1–2 min) in solutions stopped with sodium dodecyl sulphate. The extinction coefficient of phenol under these conditions is 13,900. NTE activity is represented by the difference in absorbance obtained from samples incubated under conditions (a) and (b) respectively."

(*3*) "To clarify the incubation media when NTE activity of spinal cord homogenates is assayed, (up to 16 mg wet weight/tube), increase sodium dodecyl sulphate concentration to 2 percent. Under standard conditions NTE hydrolyzes about 2400 umol of substrate/min/g of cortex, 550 for spinal cord, and 100 for sciatic nerve."

(e) *Data reporting and evaluation*—(1) *Test report.* In addition to the reporting requirements specified in the EPA Good Laboratory Practice Standards under 40 CFR Part 792, Subpart J. The final test report must include the following information:

(i) Neurotoxic esterase data including absorbance values for each subject tested.

(ii) Indication of whether each subject survived to sacrifice or time of death.

(iii) Data from control animals and blank samples.

(iv) Statistical evaluation of results.

(2) *Evaluation of results.* (i) Results should be evaluated in terms the extent of inhibition as a function of treatment and dose, comparison to inhibition of acetylcholinesterase (Ach E) where available, lethal potency, and data on blank samples and control group results.

(ii) Results on NTE should be compared to and evaluated with behavioral and histopathological data. Sequential data from repeated exposures studies should also be evaluated for evidence that the NTE level has plateaued.

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Caroldi, S., Lotti, M. "Neurotoxic Esterose in Peripheral Nerve: Assay, Inhibition, and Rate of Resynthesis," *Toxicology and Applied Pharmacology*, 62, 498–501 (1982).

(2) Johnson, M.K. "The Delayed Neuropathy Caused By Some Organophosphorus Esters: Mechanism and Challenge," *Critical Reviews In Toxicology*, 3, 289–316 CRC Press, Inc. (1975).

(3) Johnson, M.K. "Improved Assay of Neurotoxic Esterase for Screening Organophosphates for Delayed Neurotoxicity Potential," *Archives of Toxicology*, 37, 113–115 (1977).

(4) Johnson, M.K. "The Target of Initiation of Delayed Neurotoxicity by Organophosphorus Esters: Biochemical Studies and Toxicological Applications," Reviews in *Biochemical Toxicology*, 4. Eds. Hodgson, E., Bend, Jr., Philpot, R.M., (Elsevier: New York, 1982); pp. 141–212.

(5) Johnson, M.K., Richardson, R.J. "Biochemical Endpoints: Neurotoxic Esterase Assay," *Neurotoxicology*, 4(2): 311–320 (1983).

(6) Soliman, S.A., Linder, R., Farmer, J., Curley, A. "Species Susceptibility to Delayed Toxic Neuropathy in relation to in vivo inhibition of Neurotoxic Esterase by Neurotoxic Organophosphorus Esters," *Journal of Toxicology and Environmental Health*, 9, 189–197 (1982).

(7) Sprague, G.L., Sandvik, L.L., Bickford, A.A. "Time course for neurotoxic esterase activity in hens given multiple diisopropyl fluorophosphate injections," *Neurotoxicology*, 2, 523–532 (1981).

§ 798.6500 Schedule-controlled operant behavior.

(a) *Purpose.* (1) In the assessment and evaluation of the potential human health effects of substances, it may be necessary to test for functional neurotoxic effects. Substances that have been observed to produce neurotoxic signs in other toxicity studies (e.g. CNS depression or stimulation), as well as substances with a structural similarity to known neurotoxicants should be evaluated for these effects.

(2) This guideline defines procedures for conducting studies of schedule-controlled operant behavior, one way of evaluating functional neurotoxic effects (Dews, 1972 under paragraph(f)(1) of this section; NAS 1975, 1977, 1982 under paragraph (f)(4), (5) and (6) of this section). Our purpose is to evaluate the effects of acute and repeated exposures on the rate and pattern of responding under schedules of reinforcement. Operant behavior tests may be used to evaluate many other aspects of behavior (Laties, 1978 under paragraph (f)(3) of this section). Additional tests may be necessary to completely assess the behavioral effects of any substance. Behavioral evaluation should be used in conjunction with neuropathologic evaluation and the evaluation of other toxic effects.

(b) *Definitions*—(1) *Neurotoxicity.* Neurotoxicity or a neurotoxic effect is an adverse change in the structure or function of the nervous system following exposure to a chemical agent. Behavioral toxicity is an adverse change in the functioning of the organism with respect to its environment following exposure to a chemical agent.

(2) *Operant, operant behavior, operant conditioning.* An operant is a class of behavioral responses which change or operates on the environment in the same way. Operant behavior is further distinguished as behavior which is modified by its consequences. Operant conditioning is the experimental procedure used to modify some class of behavior by reinforcement or punishment.

(3) *Schedule of reinforcement.* A schedule of reinforcement specifies the relation between behavioral responses and the delivery of reinforcers, such as food or water (Ferster and Skinner, 1957 under paragraph (f)(2) of this section). For example, a fixed ratio (FR) schedule requires a fixed number of responses to produce a reinforcer (e.g. FR 30). On a fixed interval (FI) schedule, the first response after a fixed period of time is reinforced (e.g. FI 5 minutes).

(c) *Principle of the test method.* Experimental animals are trained to perform under a schedule of reinforcement and measurements of their operant behavior are made. Several doses of the test substance are then administered according to the experimental design (between groups or within subjects) and the duration of exposure (acute or repeated). Measurements of the operant behavior are repeated. A descriptive and statistical evaluation of the data is made to evaluate the nature and extent of any changes in behavior in relation to exposures to the test substance. Comparisons are made between any exposures that influence the behavior and exposures that have neuropathological effects or effects on other targets of the chemical.

(d) *Test procedures*—(1) *Experimental design.* These test procedures may be used to evaluate the behavior of experimental animals receiving either acute or repeated exposures. For acute exposure studies, either within-subject or between groups, experimental designs may be used. For repeated exposure studies, between groups designs should be used, but within subject comparisons (pre-exposure and post-exposure) are recommended and encouraged.

(2) *Animal selection*—(i) *Species.* (A) For most studies, the laboratory mouse or rat is recommended. Standard strains should be used.

(B) Under some circumstances other species may be recommended.

(ii) *Age.* Experimental animals should be young adults. Rats or mice should be at least 14 and 6 weeks old, respectively, prior to exposure.

(iii) *Sex.* (A) Approximately equal numbers of male and female animals are required for each dose level and control group.

(B) Virgin females should be used.

(iv) *Experimental history.* Animals should be experimentally and chemically naive.

(3) *Number of animals.* Six to twelve animals should be exposed to each level of the test substance and/or control procedure. If post exposure effects are examined, a separate group, 6 to 12 additional animals not sacrificed for pathology, will required in subchronic studies.

(4) *Control groups*—(i) *Untreated controls.* A concurrent "sham" exposure or vehicle control group or session (according to the design of the study) is required. The subjects should be treated similarly except that administration of the test substance is omitted.

(ii) *Positive controls.* Positive control data is required to demonstrate that the experimental procedures, under the specific conditions in the testing laboratory, are sensitive to substances known to affect operant behavior. Both increases and decreases in response rate should be demonstrated. Data based on acute exposures will be adequate. Data should be collected according to the same experimental design as that proposed for the test substance. Historical data on the procedure collected in the same species and under the same conditions in the testing laboratory may be acceptable, but the presentation of concurrent control data is strongly encouraged since it provides evidence that the test has remained sensitive.

(5) *Dose levels and dose selection.* At least 3 doses, equally spaced over a log scale (e.g., 10, 30, 100), over a range of at least 1 log unit shall be used in addition to a zero dose or vehicle administration. The data should be sufficient to produce a dose-effect curve.

(i) The highest dose shall produce: (A) Clear behavioral effects; or (B) life-threatening toxicity.

(ii) The data from the lower doses must show either: (A) Graded dose-dependent effects at 2 dose levels; or (B) no effects at 2 dose levels, respectively.

(6) *Duration of exposure.* The duration and frequency of exposure will be specified in the test rule.

(7) *Route of Administration.* The route of administration will also be specified in the test rule and will usually be identical to one of the anticipated or actual routes of human exposure. For some chemicals, another route (e.g. parenteral) may be justified. The exposure protocol should conform to that outlined in the appropriate acute or subchronic toxicity study guideline under Subpart B or Subpart C of this Part.

(8) *Study conduct*—(i) *Apparatus.* Behavioral responses and the delivery of reinforcers shall be controlled and monitored by automated equipment located so that its operation does not provide unintended cues or otherwise

interfere with the ongoing behavior. Individual chambers should be sound attenuated to prevent disruptions of behavior by external noise. The response manipulanda, feeders, and any stimulus devices should be tested before each session; these devices should periodically be calibrated.

(ii) *Chamber assignment.* Concurrent treatment groups should be balanced across chambers. Each subject should be tested in the chamber to which it is initially assigned.

(iii) *Deprivation and training.* (A) If a nonpreferred positive reinforcer is used, all subjects should be deprived of food until they reach a fixed percentage (e.g. 80 to 90 percent, commonly) of their ad libitum body weight or for a fixed period (e.g., 18 hours) prior to training. Deprivation should be kept constant throughout the study.

(B) Subjects must be trained until they display demonstrable stability in performance across days prior to exposure. One simple and useful criterion is a minimum number of sessions on the schedule and no systematic trend during the 5 days before exposure.

(C) Cumulative records of cumulative responding over time for each animal should be presented to demonstrate that the pattern of responding is representative of that generated by the schedule of reinforcement.

(iv) *Time, frequency, and duration of testing*—(A) *Time of testing.* All experimental animals should be tested at the same time of day and with respect to the time of exposure. For acute studies, testing should be performed when effects are estimated to peak, usually shortly after exposure. For subchronic studies, subjects should be tested prior to daily exposure in order to assess cumulative effects.

(B) *Frequency of testing.* The maintenance of stable operant behavior normally will require regular and frequent (e.g., 5 days a week) testing sessions. Animals should be weighed on each test day.

(C) *Duration of testing.* (*1*) Experimental sessions should be long enough to reasonably see the effects of exposure, but brief enough to be practical. Under most circumstances, a session length of 30–40 minutes should be adequate.

(*2*) If the nature or duration of effects following cessation of repeated exposure are a concern, animals from the high dose group should be tested following exposure for a suitable period of time.

(v) *Schedule selection.* The schedule of reinforcement chosen should generate response rates that may increase or decrease as a function of exposure. Many schedules of reinforcement can do this: a single schedule maintaining a moderate response rate; fixed-interval schedules, which engender a variety of response rates in each interval; or multiple schedules, where different components may maintain high and low response rates.

(e) *Data reporting and evaluation.* In addition to the reporting requirements specified under 40 CFR Part 792, Subpart J the final test report should contain the following information:

(1) *Description of system, test methods, experimental design, and control data.* (i) A description of the experimental chamber, programming equipment, data collection devices, and environmental conditions.

(ii) A description of the experimental design including counterbalancing procedures, and the stability criterion.

(iii) A description and statistical evaluation of positive control and other control data, including standard measures of central tendency, variability, coefficient of variation of response rates, and the slope of the dose-effect curve.

(2) *Results.* (i) Data for each animal should be arranged by test group in tabular form including the animal identification number, body weight, pre-exposure rate of responding, changes in response rate produced by the chemical, and group data for the same variables, including standard measures of central tendency, variability and coefficient of variation.

(ii) A description and statistical evaluation of the test results: With particular reference to the overall statistical procedures (e.g., parametric or nonparametric) dose-effect curve, and calculation of slope. Presentation of calculations is encouraged.

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Dews, P.B. "Assessing the Effects of Drugs," *Methods in Psychobiology,* Vol. 2, Ed., R.D. Myers (New York: Academic Press, 1972) 83–124.

(2) Ferster, C.B. Skinner, B.F. Schedules of Reinforcement. (New York: Appleton-Century-Crofts, 1957).

(3) Laties, V.G. "How Operant Conditioning can Contribute to Behavioral Toxicology," Environmental Health Perspectives, 26: 29–35 (1978).

(4) National Academy of Science. *Principles for Evaluating Chemicals in the Environment.* (Washington, DC: National Academy of Sciences, 1975).

(5) National Academy of Science. *Principles and Procedures for Evaluating the Toxicity of Household Substances.* (Washington, DC: National Academy of Sciences, 1977).

(6) National Academy of Science. "Strategies to determine needs and priorities for toxicity testing," Appendix 3B. *Reference Protocol Guidelines For Neurobehavioral Toxicity Tests.* 2: 123–129 (1982).

§ 798.6540 Acute delayed neurotoxicity of organophosphorus substances.

(a) *Purpose.* Organophosophorus substances should be considered as candidates for delayed neurotoxicity studies using the adult hen as the test animal. This test has certain limitations, e.g., in predicting effects from repeated exposures. These limitations may be minimized by conducting an adjunct test in which inhibition and aging of neurotoxic esterase of hen neural tissue are measured.

(b) *Definitions.* Acute delayed neurotoxicity is a prolonged, delayed-onset locomotor ataxia resulting from single administration of the test substance, repeated once if necessary.

(c) *Principle of the test method.* The test substance is administered orally in a single dose to domestic hens (Gallus gallus domesticus) which have been protected from acute cholinergic effects, when appropriate. The animals are observed for at least 21 days for delayed neurotoxicity, with redosing and observation for another 21 days if no effects or equivocal responses are seen. The animals are observed daily for behavioral abnormalities, locomotor ataxia and paralysis. Histopathological examination of selected neural tissues is undertaken on all animals surviving the initial cholinergic phases.

(d) *Test procedures*—(1) *Animal selection.* The adult domestic laying hen, aged 8 to 14 months, is recommended. Standard size breeds and strains should be employed.

(2) *Number of animals.* A sufficient number of hens should be utilized so that at least six survive the observation period.

(3) *Control groups*—(i) *General.* Appropriate control groups should be used. These should include a positive control group of at least two hens treated with a known delayed neurotoxicant and a concurrent control group of at least six hens treated in a manner identical to the treated group, except that administration of the test substance and any protective agents is omitted.

(ii) *Reference substances.* A substance which is known to produce acute delayed neurotoxicity should be used as a positive control. Examples of such substances are triorthocresyl phosphate (TOCP) and leptophos.

(4) *Housing and feeding conditions.* Cages or enclosures which are large enough to permit free mobility of the hens and easy observation of gait should be used. Where the lighting is artificial, the sequence should be 12 hours light, 12 hours dark. Appropriate diets should be administered as well as an unlimited supply of drinking water.

(5) *Dose level.* The selected dose level of the test substance should not be less than the unprotected LD_{50} dose. Atropine or another noninterfering protective agent may be used to prevent death due to acute cholinergic effects. Doses of test substances higher than 5000 mg/kg of body weight need not be tested.

(6) *Dose selection.* A preliminary LD_{50} test using an appropriate number of animals, dosages and dose groups, as recommended in § 798.1175, should be performed in unprotected hens to establish the dose level to be used in this test. Healthy young adult hens free from interfering viral diseases and medication and without abnormalities of gait should be acclimatized to the laboratory conditions for at least 5 days prior to randomization and assignment to treatment and control groups.

(7) *Route of administration.* Dosing with the test substance should normally be by the oral route using gavage, gelatine capsules, or a comparable method.

(8) *Study conduct*—(i) *General.* The test or control substance should be administered and observations begun. All hens should be carefully observed at least once daily for a period of at least 21 days and signs of toxicity recorded, including the time of onset, degree and duration. Observations should include, but not be limited to, behavioral abnormality, locomotor ataxia and paralysis. At least twice a week the hens should be taken outside the cages and subjected to a period of forced motor activity, such as ladder climbing, in order to enhance the observation of minimal responses. If neurotoxic responses are not observed or if equivocal responses are seen, then the dose should be administered again and the animals observed for an additional 21 days. The hens should be weighed weekly. Any moribund hens should be removed and sacrificed.

(ii) *Pathology*—(A) *Gross necropsy.* In the presence of clinical signs of delayed neurotoxicity useful information may be provided by gross necropsy.

(B) *Histopathology.* All animals should be subjected to microscopic examination. Tissues should be fixed *in situ*, preferably using perfusion techniques. Sections should include medulla oblongata, spinal cord and peripheral nerves. The spinal cord sections should be taken from the upper cervical bulb, the midthoracic and the lumbo-sacral regions. Section of the proximal region of the tibial nerve and its branches should be taken. Sections should be stained with appropriate myelin and axon-specific stains.

(e) *Data reporting and evaluation*—(1) *Test report.* In addition to the reporting requirements specified under 40 CFR Part 792, Subpart J the final test report must include the following information:

(i) Toxic response data by group with a description of clinical manifestations of nervous system damage; where a grading system is used the criteria should be defined.

(ii) For each animal, time of death during the study or whether it survived to termination.

(iii) The day of observation of each abnormal sign and its subsequent course.

(iv) Body weight data.

(v) Necropsy findings for each animal, when performed.

(vi) A detailed description of all histopathological findings.

(vii) Statistical treatment of results, where appropriate.

(2) *Treatment of results.* Data may be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals showing lesions or effects, the types of lesions or effects and the percentage of animals displaying each type of lesion or effect.

(3) *Evaluation of results.* The findings of an acute delayed neurotoxicity study should be evaluated in terms of the incidence and severity of neurotoxic effects and of any other observed effects and histopathological findings in the treated and control groups.

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Ankier, S.I. "New hot plate tests to quantify antinociceptic and narcotic antagonist activities," European Journal of Pharmacology *27:* 1–4 (1974).

(2) Coughenour, L.L. McLean, J.R., and Parker R.B. "A new device for the rapid measurement of impaired motor function in mice," Pharmacology, Biochemistry and Behavior *6:* 351–353 (1977).

(3) D'Amour, F.E. Smith, D.L. "A method for determining loss of pain sensation," *Journal of Pharmacology and Experimental Therapeutics, 72:* 74–79 (1941).

(4) Deuel, R.K." Determining sensory deficits in animals," *Methods in Psychobiology Ed.* R.D. Myers (New York: Academic Press, 1977) pp. 99–125.

(5) Edwards, P.M., Parker, V.H. "A simple sensitive and objective method for early assessment of acrylamide neuropathy in rats," *Toxicology and Applied Pharmacology 40:* 589–591 (1977).

(6) Evans, W.O. "A new technique for the investigation of some analgesic drugs on reflexive behavior in the rat," *Psychopharmacologia, 2:* 318–325 (1961).

(7) Irwin, S. "Comprehensive observational assessment: Ia. A systematic quantitative procedure for assessing the behavioral and physiologic state of the mouse," *Psychopharmacologia, 13:* 222–257 (1968).

(8) Marshall, J.F., Turner, B.H., Teitlbaum, P. "Sensory neglect produced by lateral hypothalamic damage," *Science, 174:* 523–525 (1971).

(9) Meyer, O.A. Tilson, H.A., Byrd, W.C., Riley, M.T. "A method for the routine assessment of fore- and hindlimb grip strength of rats and mice," Neurobehavioral Toxicology *1:* 233–236 (1979).

§ 798.6560 Subchronic delayed neurotoxicity of organophosphorus substances.

(a) *Purpose.* In the assessment and evaluation of the toxic characteristics of organophosphorus substances the determination of subchronic delayed neurotoxicity may be carried out, usually after initial information on delayed neurotoxicity has been obtained by acute testing or by the demonstration of inhibition and aging of neurotoxic esterase in hen neural tissue. The subchronic delayed neurotoxicity test provides information on possible health hazards likely to arise from repeated exposures over a limited period of time. It will provide information on dose response and can provide an estimate of a non-effect level which can be of use for establishing safety criteria for exposure.

(b) *Definitions.* Subchronic delayed neurotoxicity is a prolonged, delayed-onset locomoter ataxia resulting from repeated daily administration of the test substance.

(c) *Principle of the test method.* Multiple dose levels of the test substance are administered orally to domestic hens *(Gallus gallus domesticus)* for 90 days. The animals are observed at least daily for behavioral abnormalities, locomotor ataxia and paralysis. Histopathological examination of selected neural tissues is undertaken at the termination of the test period.

(d) *Test procedures*—(1) *Animal selection.* The adult domestic laying hen, aged 8 to 14 months, is recommended. Standard size breeds and strains should be employed.

(2) *Number of animals.* Ten hens should be used for each treatment and control group.

(3) *Control group*—(i) *General.* A concurrent control group should be used. This group should be treated in a manner identical to the treated group, except that administration of the test substance is omitted.

(ii) *Reference substances.* If a positive control is used, a substance which is known to produce delayed neurotoxicity should be employed. Examples of such substances are triorthocresyl phosphate (TOCP) and leptophos.

(4) *Housing and feeding conditions.* Cages or enclosures which are large enough to permit free mobility of the hens and easy observation of gait should be used. Where the lighting is artificial, the sequence should be 12 hours light, 12 hours dark. Appropriate diets should be administered as well as an unlimited supply of drinking water.

(5) *Dose levels.* At least three dose levels should be used in addition to the control group(s). The highest dose level should result in toxic effects, preferably delayed neurotoxicity, but not produce an incidence of fatalities which would prevent a meaningful evaluation. The lowest dose level should not produce any evidence of toxicity.

(6) *Route of administration.* Oral dosing each day for at least 5 days per week should be carried out, preferably by gavage or administration of gelatine capsules.

(7) *Study conduct*—(i) *General.* Healthy young adult hens free from interfering viral diseases and medication and without abnormalities of gait should be acclimatized to the laboratory conditions for at least 5 days prior to randomization and assignment to treatment and control groups. The test or control substance should be administered and observations begun. All hens should be carefully observed at least once daily throughout the test period. Signs of toxicity should be recorded, including the time of onset, degree and duration. Observations should include, but not be limited to, behavioral abnormality, locomotor ataxia and paralysis. At least once a week the hens should be taken outside the cages and subjected to a period of forced motor activity, such as ladder climbing, in order to enhance the observation of minimal responses. The hens should be weighed weekly. Any moribund hens should be removed and sacrificed.

(ii) *Pathology*—(A) *Gross necropsy.* In the presence of clinical signs of delayed neurotoxicity useful information may be provided by gross necropsy.

(B) *Histopathology.* Tissues from all animals should be fixed *in situ*, using perfusion techniques. Sections should include medulla oblongata, spinal cord and peripheral nerves. The spinal cord sections should be taken from the upper cervical bulb, the mid-thoracic and lumbosacral regions. Sections of the proximal region of the tibial nerve and its branches and of the sciatic nerve should be taken. Sections should be stained with appropriate myelin and axon-specific stains. Microscopic examination should be carried out on all hens in the control and high-dose groups. Microscopic examination should also be carried out on hens in the low and intermediate dose groups when there is evidence of effects in the high-dose group.

(e) *Data reporting and evaluation*—(1) *Test report.* In addition to the reporting requirements specified under 40 CFR Part 792, Subpart J the final test report must include the following information:

(i) Toxic response data by group with a description of clinical manifestations of nervous system damage; where a grading system is used the criteria should be defined.

(ii) For each animal, time of death during the study or whether it survived to termination.

(iii) The day of observation of each abnormal sign and its subsequent course.

(iv) Body weight data.

(v) Necropsy findings for each animal, when performed.

(vi) A detailed description of all histopathological findings.

(vii) Statistical treatment of results, where appropriate.

(2) *Treatment of results.* (i) Data may be summarized in tabular form, showing for each test group the number of animals at the start of the test, the number of animals showing lesions or effects, the types of lesions or effects and the percentage of animals displaying each type of lesion or effect.

(ii) All observed results should be evaluated by an appropriate statistical method. Any generally accepted statistical method may be used; the statistical methods should be selected during the design of the study.

(3) *Evaluation of results.* The findings of a subchronic delayed neurotoxicity study should be evaluated in conjunction with the findings of preceding studies and considered in terms of the incidence and severity of observed neurotoxic effects and any other observed effects and histopathological findings in the treated and control groups. A properly conducted subchronic test should provide a satisfactory estimation of a no-effect level based on lack of clinical signs and histopathological changes.

(f) *References.* For additional background information on this test guideline the following references should be consulted:

(1) Abou-Donia, M.B. "Organophosphorus ester-induced delayed neurotoxicity" Annual Review of Pharmacology and Toxicology, 21:511–548 (1981).

(2) Abou-Donia, M.B., Pressing, S.H. "Delayed neurotoxicity from continuous low-dose oral administration of leptophos to hens." *Toxicology and Applied Pharmacology*, 38:595–608 (1976).

(3) Baron, R.L. (ed). "Pesticide Induced Delayed Neurotoxicity," Proceedings of a Conference, February 19–20, 1976, Washington, D.C. U.S. Environmental Protection Agency. EPA Report No. 600/1–76–025, Washington, DC (1976).

(4) Cavanaugh, J.B. "Peripheral neuropathy caused by chemical agents" *Critical Reviews of Toxicity*, 2:365–417 CRC Press, Inc. (1973).

(5) Johannsen, F.R., Wright, P.L., Gordon, D.E., Levinskas, G.L., Radue, R.W., Graham, P.R. "Evaluation of delayed neurotoxicity and dose-response relationship of phosphate esters in the adult hen," *Toxicology and Applied Pharmacology*, 41:291–304 (1977).

(6) Johnson, M.K. "Organophosphorus esters causing delayed neurotoxic effects: mechanism of action and structure/activity studies," *Archives of Toxicology*, 34:259–288 (1975).

§ 798.6850 Peripheral nerve function.

(a) *Purpose.* The techniques in this guideline are designed to develop data on neurophysiological changes in the nervous system for chemical substances and mixtures subject to such testing under the Toxic Substances Control Act. The data will characterize the neurophysiological changes produced by substances known to be peripheral neurotoxicants and determine dose-effect. The EPA will use these data to assess the risk of neurotoxic effects these chemicals may present to human health.

(b) *Definitions.* (1) Neurotoxicity or a neurotoxic effect is an adverse change in the structure or function of the nervous system following exposure to a chemical agent.

(2) Conduction velocity is the speed at which the compound nerve action potential traverses a nerve.

(3) Amplitude is the voltage excursion recorded during the process of recording the compound nerve action potential. It is an indirect measure of the number of axons firing.

(c) *Principle of the test method.* The test substance is administered to several groups of experimental animals, one dose being used per group. The peripheral nerve conduction velocity and amplitude are assessed using electrophysiological techniques. A dose-effect function is determined.

(d) *Test procedure*—(1) *Animal selection*—(i) *Species and strain.* Testing should be performed on a laboratory rodent unless such factors as the comparative metabolism of the chemical or species sensitivity to the toxic effects of the test substance, as evidenced by the results of other studies, dictate otherwise. All animals should have been laboratory-reared to ensure consistency of diet and environmental conditions across groups and should be of the same strain and from the same supplier. If this is not possible, groups shall be balanced to ensure that differences are not systematically related to treatment.

(ii) *Age and weight.* Young adult animals (at least 60 days for rats) must be used. Age (± 15 days for rats) must not vary across groups. Weights should be within ± 10 percent of the mean.

(iii) *Sex.* Either (or both) sex(es) may be used. Sex must not vary across groups.

(2) *Number of animals.* Sufficient numbers of animals shall be used to detect a 10 percent change from normal conduction velocity at the 5 percent level with 90 percent power. Generally, 20 animals/group will satisfy this requirement.

(3) *Control groups.* (i) A concurrent control group is required. This group must be an untreated group, or, if a vehicle is used in administering the test substance, a vehicle control group. If the toxic properties of the vehicle are not known or cannot be made available, both untreated and vehicle control groups are required.

(ii) A satellite group may be treated with the high dose level for 90 days and observed for reversibility, persistence, or delayed occurrence of toxic effects for a post-treatment period of appropriate length, normally not less than 28 days.

(4) *Dose levels and dose selection.* At least 3 doses, equally spaced on a log scale (e.g., ½ log units) over a range of at least 1 log unit shall be used in addition to a zero dose or vehicle administration. The data should be sufficient to produce a dose-effect curve.

(i) The highest dose shall produce (A) clear effects on nerve conduction velocity and/or amplitude or (B) life-threatening toxicity.

(ii) The data from the lower doses must show either (A) graded dose-dependent effects at two dose levels or (B) no effects at two dose levels, respectively.

(5) *Duration of testing.* The exposure duration will be specified in the test rule. This will generally be 90 days of exposure.

(6) *Route of administration.* The test substance shall be administered by a route specified in the test rule. This will usually be the route most closely approximating the route of human exposure. The exposure protocol shall conform to that outlined in the appropriate acute or subchronic toxicity guideline.

(7) *Combined protocol.* The test described herein may be combined with any other toxicity study, as long as none of the requirements of either are violated by the combination.

(8) *Study conduct*—(i) *Choice of nerve(s).* The nerve conduction velocity test must separately assess the properties of both sensory and motor nerve axons. Either a hind limb (e.g., tibial) or tail (e.g., ventral caudal) nerve must be chosen. Response amplitude may be measured in a mixed nerve.

(ii) *Preparation.* (A) *In vivo* testing of anesthetized animals is required. A barbiturate anesthetic is appropriate. Care should be taken to ensure that all animals are administered an equivalent dosage and that the dosage is not excessive. If dissection is used, extreme caution must be observed to avoid damage to either the nerve or the immediate vascular supply.

(B) Both core and nerve temperature must be monitored and kept constant (±0.5 °C) during the study. Monitoring of skin temperature is adequate if it can be demonstrated that the skin temperature reflects the nerve temperature in the preparation under use. Skin temperature should be monitored with a needle thermistor at a constant site, the midpoint of the nerve segment to be tested.

(C) *Electrodes*—(*1*) *Choice of Electrodes.* Electrodes stimulation and recording may be made of any conventional electrode material, such as stainless steel, although electrodes for non-polarizing materials are preferable. If surface electrodes are used, care must be taken to ensure that good electrical contact is achieved between the electrode and the tissue surface. Following each application, any electrode must be throughly cleaned.

(*2*) *Electrode placement.* Electrode placement must be constant with respect to anatomical landmarks across animals (e.g., a fixed number of mm from the base of the tail). Distances between electrodes used to calculate conduction velocity must be measurable to ±0.5 mm. The recording electrodes should be as far from the stimulating electrodes as possible. A 40 mm separation is adequate in the caudal tail nerve of the rat.

(*3*) *Recording conditions.* The animal should be grounded at about the midpoint between the nearest stimulating and recording electrodes. The recording conditions must be such that the stimulus artifact has returned to baseline before any neural response is recorded which is used in the analysis, under condition of maximal band width of the preamplifier.

(D) The electrical stimulator must be isolated from ground. Biphasic or balanced pair stimuli to reduce polarization effects are acceptable. A constant current stimulator is preferred (and required for polarizable electrodes) and should operate from about 10 uA to about 10 mA. If a constant voltage stimulator is used, it should operate to 250V. All equipment shall be calibrated with respect to time, voltage, and temperature.

(E) The recording environment should be enclosed in a Faraday cage unless electromagnetic field pick-up can be shown to be more than 1.5 times the amplifier baseline noise, under recording conditions. The recording output should be amplified sufficiently to render the compound action potential easily measurable with an oscilloscope. The amplifier should pass signals between 2.0 Hz and 4 kHz without more than a 3dB decrement. The preamplifier must be capacitatively coupled or, if direct coupled to the first stages, must be able to tolerate any DC potentials which the electrode-preparation interface produces, and to operate without significant current leakage through the recording electrodes.

(F) A hard copy must be available for all waveforms or averaged waveforms from which measurements are derived, and for all control recording required by this standard. Hard copies must include a time and voltage calibration signal.

(iii) *Procedure*—(A) *General.* Stimulation should occur at inter-stimulus interval significantly below the relative refractory period for the nerve under study. Stimulus intensity should be increased gradually until the response amplitude no longer increases. At this point the "maximal" stimulus current is determined. An intensity 25–

50 percent (a fixed value in a given study) above the maximal intensity so determined should be used for determining response peak latency and response amplitude. Response peak latency may be read off the oscilloscope following single sweeps or determined by an average of a fixed number of responses. The baseline-to-peak height technique (Daube, 1980) is acceptable for determination of the nerve compound action potential amplitude, but in this case, at least 16 responses must be averaged.

(B) *Motor nerve.* Motor conduction velocity may be measured from a mixed nerve by recording the muscle action potential which follows the compound action potential of the nerve. The stimulus intensity is adjusted so that the amplitude of the muscle action potential is supramaximal. Measurement of the latency from stimulation to the onset of the compound muscle action potential gives a measure of the conduction time of the motor nerve fibers. To calculate the conduction velocity, the nerve must be stimulated sequentially in two places each with the same cathode-anode distance, and with the cathode located toward the recording electrode. The cathode to cathode distance between the two sets of stimulating electrodes is divided by the difference between the two latencies of muscle action potential in order to obtain conduction velocity. Placement of electrodes shall be described-site of nerve stimulation may differ from point of entry through skin.

(C) *Sensory nerve.* The somatosensory evoked potential may be used to determine the sensory nerve conduction velocity in a mixed nerve. The cathode is placed proximally at the two stimulation locations with the same cathode-anode distances. The recording electrodes are placed on the skull. The conduction velocity is calculated by dividing the distance between the two stimulating cathodes by the difference between the two latencies of the largest primary peak of the somatosensory evoked potential. Between 64 and 128 responses should be averaged. The stimulation frequency should be about 0.5 Hz. Stimulus intensity should be the same as that used for determining the motor conduction velocity. Should the peak of the somatosensory response be so broad that it cannot be replicated with an accuracy of less than 5 percent of the latency difference observed, then a point on the rising phase of the potential should be chosen, e.g. at a voltage 50 percent of the peak voltage. Alternatively, the sensory nerve conduction velocity can be obtained from a purely sensory nerve or from stimulation of the dorsal rootlets of a mixed nerve, using two recording electrode pairs.

(e) *Data collection, reporting and evaluation.* In addition to information meeting the requirements stated under 40 CFR Part 792, Subpart J, the following specific information should be reported:

(1) *Description of test system and test methods.* (i) Positive control data from the laboratory performing the test which demonstrate the sensitivity of the procedure being used.

(ii) Hard copies of waveforms from which measurements were made as well as control recordings.

(iii) Voltage and time calibration referable to the standards of the Bureau of Standards or to other standards of accuracy sufficient for the measurements used.

(iv) Data demonstrating that nerve temperature was maintained constant throughout the recording period.

(2) *Results.* The following information must be arranged by test group (dose level):

(i) In tabular form, data must be provided showing for each animal:

(A) Its identification number.

(B) Body weight, nerve conduction velocity, and amplitude.

(ii) Group summary data should also be reported.

(3) *Evaluation of data.* An evaluation of the test results (including their statistical analysis) must be made and supplied. This submission must include dose-effect curves for conduction velocity and amplitude and a description of statistical methods. Deviation from conventional parametric techniques must be justified.

(f) *References.* For additional background information on this test guidline the following references should be consulted:

(1) Aminoff, M.J. (Ed). *Electrodiagnosis in Clinical Neurology.* (New York: Churchill Livingstone, 1980).

(2) Daube, J. "Nerve Conduction Studies," *Electrodiagnosis in Clinical Neurology.* Ed. M.J. Aminoff (New York: Churchill Livingstone, 1980). pp. 229–264.

(3) Glatt, A.F., H.N. Talaat and W.P. Koella "Testing of peripheral nerve function in chronic experiments in rats," *Pharmacology and Therapeutics,* 5:539–534 (1979).

(4) Johnson, E.W. *Practical Electromyography.* (Baltimore: Williams and Wilkins, 1980).

Subpart H—Special Studies

§ 798.7100 Metabolism.

(a) *Purpose.* (1) Data from studies on the absorption, distribution, excretion and metabolism of a test chemical are desirable to aid in the evaluation of test results from other toxicology studies and in the extrapolation of data from animals to man. Such studies should be done on each chemical of toxicological concern. The concern may be predicated on the level and type of toxicity observed (or anticipated) and by the magnitude of potential human exposure to the chemical. The main purpose of metabolism studies is to produce data which fortify the understanding of the safety of the chemical in consideration of its intended uses and anticipated human exposure. In addition to the general reasons stated above, a metabolism study may be performed for the following purposes:

(i) To determine the amount and rate of absorption of the test chemical at different dose levels.

(ii) To determine the pattern of distribution of the test chemical among tissues, organs and fluid compartments at different dose levels, after single and repeated doses.

(iii) To identify and, to the extent possible, quantify significant metabolites.

(iv) To characterize route(s) and rate(s) of excretion.

(v) To determine any possible bioaccumulation (bioretention) of the test substance and/or metabolites.

(vi) To determine absorption, metabolism, excretion and distribution as a function of single or repeated doses. For certain chemicals, metabolism studies may not adequately define all of these.

(b) *Definitions.* Bioaccumulation (bioretention) is the uptake and, at least temporary, storage of a chemical by an exposed animal. The chemical can be retained in its original form and/or as modified by enzymatic and non-enzymatic reactions in the body.

(c) *Test procedures*—(1) *Animal selection*—(i) *Species.* The preferred species is the rat. If another mammalian species is used, the tester should provide justification/reasoning for its selection. Commonly used laboratory strains should be employed. Preliminary studies may be performed in several species to develop information on comparative metabolism. Information derived from preliminary studies may help in the selection of species for subsequent toxicity tests.

(ii) *Age.* Young adult animals should be used. For specific purposes, a comparative study using very young animals may provide information about the effects of age on metabolism.

(iii) *Sex.* (A) Equal numbers of animals of each sex should be used at each dose level.

(B) Females should be nulliparous and nonpregnant.

(iv) *Numbers.* At least eight animals (four females and four males) should be used at each dose level.

(2) *Dose levels and dose selection.* (i) At least two dose levels should be used.

(ii) The low dose should correspond to a no-effect-level.

(iii) The upper dose should produce toxic or pharmacologic signs, but not severe effects or a high incidence of mortality which would prevent a meaningful evaluation.

(iv) The determination of absorption, tissue distribution and elimination should be studied as a function of single or repeated doses.

(v) The conclusive identification of a chemical, and its metabolites, requires the use of suitable analytical methods.

(3) *Observation period.* Animals should be kept in individual metabolism cages for 7 days after the radioactive dose or until 95 percent of the administered dose is excreted (whichever occurs first), at which time all of the animals should be killed.

(4) *Administration of the test substance.* (i) The study should be done using the oral route (capsule or gavage). If another route of administration is used, the tester should provide justification/reasoning for its selection. When vehicles are used, attention should be given to the possibility that they may interfere with the kinetics of the test chemical.

(ii) *Labeled test material:* (A) Single dose testing should be performed with an analytically pure grade of the active ingredient, usually in an isotopically labeled form.

(B) Labeled compound may not be required if sufficiently selective and sensitive physical-chemical tests for identifying the compound and its metabolites are used. The label may be radioactive such as ^{14}C, ^{35}S, and ^{36}Cl or stable such as ^{15}N and ^{18}O. In some cases, more than one label per molecule may be advantageous. Labels should be placed in positions that may be expected to follow the "core" of the molecule or significant portions thereof. If possible, one should avoid placing labels such as ^{14}C in positions from which it may be expected to enter the carbon pool of the test animal. Use of readily exchangeable labeling, should be avoided.

(iii) The following four groups of animals should be studied:

(A) Group A animals shall each receive a single intravenous dose of the labeled test substance at the low dose. If it is not possible to dissolve the test substance in physiological saline or water, this group should be omitted.

(B) Group B animals should each receive a single oral dose of the labeled test substance at the low dose.

(C) Group C animals should each receive a series of single daily oral doses of the nonlabeled test substance (by capsule or intubation) over a period of at least 14 days, followed at 24 hours after the last dose by a single oral dose (by capsule or intubation) of the labeled test substance. Each dose should be at the low dose level.

(D) Group D animals should each receive a single oral dose (by capsule or intubation) of the labeled test substance at the high dose level.

(5) *Observation of animals*—(i) *Distribution.* Concentration and quantity of test chemicals in the tissues and organs should be measured at the time of sacrifice.

(ii) *Metabolism.* For determining the extent of biotransformation, urine samples and fecal extracts should be analyzed by suitable techniques. Major metabolites of the chemical should be identified by appropriate methods. It is also important to determine the metabolite pattern of the test chemical after repeated doses.

(iii) *Excretion.* When determining excretion of the test chemical by laboratory animals, the use of individual metabolism cages is recommended for collection of urine and fecal samples. The quantities of test chemical and major metabolites in urine, feces and in expired air should be measured at several time points after exposure (i.e., 4, 8, 12 and 24 hours) and daily thereafter, until approximately 95 percent of the administered dose has been excreted or until 7 days after dosing.

(iv) (A) In the rat, quantities of label in urine, Feces and expired air should be measured at appropriate intervals (i.e. 4, 8, 12, and 24 hours, 1.5, 2, 3, 4, 5, 6, and 7 days) throughout the study for all animals. However if a preliminary study shows no volitile labeled materials are exhaled during the period of zero to 24 hours after dosing such evidence may be submitted in lieu of measuring label in the expired air for this study. In the dog, quantities of label in urine and feces should be measured at appropriate intervals (i.e., every 6 hours for the first 48 hours after dosing and every 12 hours for the remaining 5 days) throughout the study for all animals.

(B) For all animals in groups B, C, and D, the quantity of label in tissues and organs should be measured at sacrifice by suitable methods with particular attention to bone, brain, fat, gonads, heart, kidney, liver, lungs, muscle, spleen, tissues which displayed pathology (in this or prior studies), and residual carcass.

(v) Urine and feces from all groups should be analyzed by suitable methods in order to determine the extent of absorption and biotransformation and to identify the metabolites. An assay method for detection of each major metabolite may be requested by the Agency.

(d) *Data and reporting*—(1) *Treatment of results.* Data should be summarized in tabular form.

(2) *Evaluation of results.* All observed results, quantitative or incidental, should be evaluated by an appropriate statistical method.

(3) *Test report.* In addition to the reporting requirements as specified under 40 CFR Part 792, Subpart J the following specific information should be reported:

(i) Quantity of isotope, together with percent recovery of the administered dose, in feces, urine, and the following tissues and organs of animals in all groups: bone, brain, fat, gonads, heart, kidney, liver, lungs, blood, muscle, spleen, tissues which displayed pathology (in this or prior studies), and residual carcass.

(ii) Percent absorption. If possible by the oral route in groups B, C, and D.

(iii) A full description of the sensitivity and precision of all procedures used to produce the data.

(iv) Information on the degree (i.e., specific activity for a radiolabel) and site(s) of labeling of the test substance.

(v) Counting efficiency data should be made available to the Agency upon request.

(vi) Species and strain.

(e) *Additional metabolism studies.* Additional, more specific studies may be required to clarify important points. Some areas for possible further study include: identification of tissue residues; binding by macromolecules in the blood, liver, gonads and other tissues; placental transfer; entrance into breast milk; biotransformation by specific organs, tissues and cell fractions; and absorption by dermal or inhalation routes of exposure. Plasma binding studies may be conducted, usually *in vitro* with plasma. Placental transfer of a chemical substance may be determined by dosing pregnant rodents with chemicals and assaying their fetuses for the chemical. Additional species may be utilized as the rat and dog differ significantly in metabolic pattern.

[FR Doc. 85-23076 Filed 9-26-85; 8:45 am]

BILLING CODE 6560-50-M

40 CFR Part 796

[OPTS-46015; FRL 2896-2]

Toxic Substances Control Act Test Guidelines

AGENCY: Environmental Protection Agency (EPA).

ACTION: Final rule.

SUMMARY: This rule presents certain Toxic Substances Control Act (TSCA) Test Guidelines. These guidelines have been published as Organization for Economic Cooperation and Development (OECD) Guidelines for the Testing of Chemicals. Elsewhere in this issue of the Federal Register, additional TSCA test guidelines previously published or prepared by publication by EPA are also codified.

EFFECTIVE DATE: September 27, 1985.

FOR FURTHER INFORMATION CONTACT: Edward A. Klein, Director, TSCA Assistance Office (TS-799), Office of Toxic Substances, Rm. E-543, 401 M St., SW., Washington, D.C. 20460, Toll free: (800-424-9065), in Washington, D.C.: (554-1404). Outside the USA: (Operator-202-554-1404).

SUPPLEMENTARY INFORMATION: This notice codifies certain TSCA test guidelines which have been published by OECD. Elsewhere in this issue of the **Federal Register**, additional test guidelines, previously published by NTIS, are also codified into the TSCA test guidelines.

Section 4(b)(1) of the Toxic Substances Control Act (TSCA) specifies that test rules shall include standards for the development of test data. This action codifies guidelines which will be used to establish standards in future TSCA section 4 test rules.

These guidelines have been published in the Organization for Economic Cooperation and Development (OECD) Guidelines for the Testing of Chemicals. The OECD test guidelines contain generally formulated internationally accepted procedures for the laboratory testing of a property or effect deemed important for the evaluation of health or environmental hazards of a chemical.

These guidelines will be used in developing test standards in future TSCA section 4 test rules; therefore, the Agency finds it necessary to codify these guidelines. This process will make chemical specific rules under Part 799 more usable and understandable. Elsewhere in this issue of the **Federal Register** additional OTS guidelines, previously published by NTIS, are also codified into TSCA test guidelines.

Codification of these guidelines does not impose any regulatory obligation on any person who may be subject to a TSCA section 4 test rule. Specific guidelines will not become mandatory test standards until they are promulgated as such in individual section 4 rulemakings. When promulgated in such test rules, the pertinent TSCA guidelines will become test standards for only that particular section 4 rule and will not serve as generic test standards. EPA may propose modifications to the various guidelines as they are utilized for chemical-specific test rules. In each chemical-specific rule, the proposed test standards and any modifications will be subject to public comment.

List of Subjects in 40 CFR Part 796

Testing, Environmental protection, Chemical fate, Chemicals.

Dated: September 23, 1985.

John A. Moore,

Assistant Administrator for Pesticides and Toxic Substances.

PART 796—[AMENDED]

Therefore, 40 CFR Part 796 is amended as follows:

1. The authority citation for Part 796 continues to read:

Authority: 15 U.S.C. 2603.

2. Subpart B is amended by adding §§ 796.1050, 796.1220, 796.1370 and 796.1520 to read as follows:

§ 796.1050 Absorption in aqueous solution: Ultraviolet/visible spectra.

(a) *Introductory information.*

(1) *Guidance information.* (i) Molecular formula. (ii) Structural formula.

(2) *Standard documents.* The spectrophotometric method is based on national standards and consensus methods which are applied to measure the absorption spectra.

(b) *Method*—(1)(i) *Introduction, purpose, scope, relevance, application and limits of test.* (A) The primary environmental purpose in determining the ultraviolet-visible (UV-VIS) absorption spectrum of a chemical compound is to have some indication of the wavelengths at which the compounds may be susceptible to photochemical degradation. Since photochemical degradation is likely to occur in both the atmosphere and the aquatic environment, spectra appropriate to these media will be informative concerning the need for further persistence testing.

(B) Degradation will depend upon the total energy absorbed in specific wavelength regions. Such energy absorption is characterized by both molar absorption coefficient (molar extinction coefficient) and band width. However, the absence of measurable absorption does not preclude the possibility of photodegradation.

(ii) *Definitions and units.* The *UV-VIS absorption spectrum* of a solution is a function of the concentration, c_i, expressed in mol/L, of all absorbing species present; the path length, d, of the spectrophotometer cell, expressed in cm; and the molar absorption (extinction) coefficient, ϵ_i, of each species. The absorbance (optical density) A of the solution is then given by:

$$A = d \sum_{i} \epsilon_{ci}$$

For a resolvable absorbance peak, the band width λ is the wavelength range, expressed in $nm = 10^{-9}$ m, of the peak at half the absorbance maximum.

(iii) *Reference substances.* (A) The reference substances need not be employed in all cases when investigating a new substance. They are provided primarily so that calibration of the method may be performed from time to time and to offer the chance to compare the results when another method is applied.

(B) Reference compounds appropriate for the calibration of the system are:

(*1*) Potassium dichromate (in 0.005 mol/L, H_2SO_4 solution) from J.A.A. Ketelaar, paragraph (d)(2) of this section:

log ϵ	3.56	3.63	3.16	3.50
λ in nm	235	257	313	350

(*2*) Fluoranthene (in methanol) from *C.R.C. Atlas of Spectral Data*, paragraph (d)(3) of this section:

log ϵ	4.75	4.18	4.73	3.91	3.92
λ in nm	237	236	288	339	357

(*3*) 4-nitrophenol (in methanol) from *C.R.C. Atlas of Spectral Data*, paragraph (d)(3) of this section:

log ϵ	3.88	4.04
λ in nm	288	311

See also paragraph (d)(1) of this section.

(iv) *Principle of the test method.* This method utilizes a double-beam spectrophotometer which records only the absorption differences between the blank and test solutions to give the spectrum of the chemical being tested.

(v) *Quality criteria—Reproducibility and sensitivity.* (A) Reproducibility and sensitivity, need not be measured directly. Instead, the accuracy of the system in measuring the spectra of reference compounds will be defined so as to assure appropriate reproducibility and sensitivity. It is preferable to use a recording double-beam spectrophotometer to obtain the UV-VIS spectrum of the test compound. Such an instrument should have a photometric accuracy of ±0.02 units over the absorbance range of 0 to 2 units. It should be capable of recording absorbances at wavelengths of 200 to 750 nanometers nm with a wavelength accuracy of ±0.5 nm. The cells employed with the instrument must necessarily be transparent over this wavelength range and must have a path length determined to within 1 percent. To ensure that the instrument is performing satisfactorily, spectra for test solutions of $K_2Cr_2O_7$ (for absorbance accuracy) and holmium glass (for wavelength accuracy) should be run periodically.

(B) In the event that a recording double-beam instrument is not available, it will be necessary to determine the absorbance of the test solution in a single-beam instrument at 5-nm intervals over the entire wavelength range and at 1-nm intervals where there are indicated absorbance maxima. Wavelength and absorbance tests should be done as with the double-beam instrument.

(2) *Description of the test procedure*—(i) *Preparation*—(A) *Preparation of test solutions.* (*1*) Solutions should be prepared by accurately weighing an appropriate amount of the purest form of the test substance available. This should be made up in a concentration which will result in at least one absorbance maximum in the range 0.5 to 1.5 units.

(*2*) The absorption of a compound is due to its particular chemical form. It is often the case that different forms are present, depending on whether the medium is acidic, basic, or neutral. Consequently, spectra under all three conditions are required where solubility and concentration allow. Where it is not possible to obtain sufficient concentrations in any of the aqueous media, a suitable organic solvent should be used (methanol preferred).

(*3*) The acid medium should have a pH of less than 2, and the basic medium should be at least pH 10. The solvent for the neutral solution, and for preparing the acidic and basic ones, should be distilled water, transparent to ultraviolet radiation down to 200 nm. If methanol must be used, acidic and basic solutions can be prepared by adding 10 percent by volume of HCl or NaOH in aqueous solution ([HCl], [NaOH] = 1 mol/L).

(*4*) In theory, all chemical species other than that being tested are present in both beams and would therefore not appear in the recorded spectrum of a double-beam instrument. In practice, because the solvent is usually present in great excess, there is a threshold value of wavelength below which it is not possible to record the spectrum of the test chemical. Such a wavelength will be a property of the solvent or of the test medium. In general, distilled water is useful from 200 nm (dissolved ions will often increase this), methanol from 210 nm, hexane from 210 nm, acetonitrile from 215 nm and dichloromethane from 235 nm.

(B) *Blank solutions.* A blank must be prepared which contains the solvent and all chemical species other than the test chemical. The absorption spectrum of this solution should be recorded in a manner identical to that of the test solution and preferably on the same chart. This "baseline" spectrum should never record an absorbance reading varying more than ±0.05 from the nominal zero value.

(C) *Cells.* Cell pathlengths are usually between 0.1 cm and 10 cm. Cell lengths should be selected to permit recording of at least one maximum in the absorbance range of 0.5 to 1.5 units. Which set of cells should be used will be governed by the concentration and the absorbance of the test solution as indicated by the Beer-Lambert Law. The cells should be transparent over the range of the spectrum being recorded, and the pathlengths should be known to an accuracy of at least 1 per cent. Cells should be thoroughly cleaned in an appropriate manner (chromic acid is useful for quartz cells) and rinsed several times with the test or blank solutions.

(ii) *Performance of the test.* Both cells to be employed should be rinsed with the blank solution and then filled with same. The instrument should be set to scan at a rate appropriate for the required wavelength resolution and the spectrum of the blank recorded. The sample cell should then be rinsed and filled with the test solution and the scanning repeated, preferably on the same spectrum chart, to display the baseline. The test should be carried out at 25° C.

(c) *Data and reporting*—(1) *Treatment of results.* (i) The molar absorption coefficient ε should be calculated for all absorbance maxima of the test substance. The formula for this calculation is

$$\epsilon = \frac{A}{c_o \times d},$$

where the quantities are as defined above (see Definitions and units).

(ii) For each peak which is capable of being resolved, either as recorded or by extrapolated symmetrical peaks, the bandwidth should be recorded.

(2) *Test report.* (i) The report should contain a copy of each of the three spectra (3 pH conditions). If neither water nor methanol solutions are feasible, there will be only one spectrum. Spectra should include a readable wave-length scale. Each spectrum should be clearly marked with the test conditions.

(ii) For each maximum in each spectrum, the ε value and bandwidth (when applicable) should be calculated and reported, along with the wavelength of the maximum. This should be presented in tabular form.

(iii) The various test conditions should be included, such as scan speed, the name and model of the spectrophotometer, the slit width (where available), cell type and path length, the concentrations of the test substance, and the nature and acidity of the solvent medium. A recent test spectrum on appropriate reference materials for photometric and wavelength accuracy should also be submitted (see Reproducibility and sensitivity).

(d) *Literature references.* For additional background information on this test guideline, the following references should be consulted:

(1) Milazzo, G., Caroli, S., Palumbo-Doretti, M., Violante, N., *Analytical Chemistry*, 49: 711 (1977).

(2) Katelaar, J.A.A., *Photoelectric Spectrometry Group Bulletin*, 8, (Cambridge, 1955).

(3) Chemical Rubber Company, *Atlas of Spectral Data*, (Cliffland, Ohio).

§ 796.1220 Boiling point/boiling range.

(a) *Introductory information.* (Ebulliometric Method; Dynamic Method; Distillation Method; Siwoloboff Method; Photocell Detection Method).

(1) *Qualifying statement.* The methods and devices described in this test guideline can be applied to liquids, provided that these do not undergo chemical reaction at temperatures below the boiling point (for example: autoxidation, rearrangement, degradation, etc.).

(2) *Additional comments.* The Dynamic Method was tested in the OECD Laboratory Intercomparison Testing Programme, Part I, 1979, for vapor pressure determination.

(3) *Standard documents.* In this guideline, five methods to determine the boiling point are described. The Ebulliometric Method is based on the ASTM D 1120–72. The Distillation Method is based on the standards ISO R 918 and the draft ISO DIS 4626, BS 4359/68, BS 4591/71, DIN 53171. The Siwoloboff Method is based on JIS K 0064–1966. The Photocell Detection Method is based on the manufacturer's manual (4).

(b) *Method.* (1)(i) *Introduction, purpose, scope, relevance, application, and limits of test.* (A)(*1*) The boiling point of a substance is an environmentally relevant physical chemical property because it is important for identification purposes and is one factor influencing the states in which the substance will exist in the environment.

(*2*) The emphasis in this test guideline has been placed on the description of the method using photocell detection, because this method allows the determination of melting as well as boiling points. Moreover, the measurements can be performed automatically.

(B) The Dynamic Method has the advantage that it can also be applied for the determination of vapor pressure and that it is not necessary to correct the boiling temperature to the normal pressure (101.325 kPa) because the standard pressure can be adjusted during the measurement. However, this method is not at present automated. (For a detailed description, see OECD Test Guideline 104 for the Vapor Pressure Curve.)

(C) Note: In the literature, different boiling points are sometimes quoted for the same substance. These differences are due to such variables as the dimension of the apparatus (for example, the fit of the thermometer), the type of the thermometer, the stem correction, the pressure correction, and the accuracy of the pressure measurement. Therefore, the above-mentioned international and national standardised methods contain precise requirements for these specified conditions.

(D) The influence of impurities on the determination of the boiling point depends greatly upon the kind of impurity. Thus, the effect can be considered if a highly volatile solvent is present in the sample. Impurities will usually increase/decrease the measured boiling temperature.

(ii) *Definitions and units.* (A) The standard *boiling point* is described as the temperature at which the pressure of the saturated vapor of a liquid is the same as the standard pressure.

(B) The measured boiling point is dependent on the atmospheric pressure. This dependence can be described quantitatively by the Clausius-Clapeyron equation as follows:

$$\log p = -\frac{\Delta H_v}{2.3\,RT} + \text{constant}$$

(where p is the vapor pressure of the substance, ΔH_v is its heat of vaporization, and R is the universal molar gas constant. $R = 8.31441\ \text{J mol}^{-1}\text{K}^{-1}$. The temperature T is expressed in K).

(C) The temperature at the boiling point (boiling temperature) is stated in K, with regard to the ambient pressure during the measurement. If no pressure is given, the result refers to a standard pressure of 101.325 kPa.

(D) Conversions:

Pressure—(units—kPa)

100 kPa = 1 bar = 0.1 MPa

("bar" units are still permissible but not recommended)

133 Pa = 1 mm Hg = 1 Torr

(the units mm Hg and Torr are no longer permissible)

Temperature—(units—K)

$t = T - 273.15$

t in ° C, and T in K

(*1*) At small deviations from the normal pressure (max. ± 5 kPa), the boiling point temperatures are normalized to T_n by means of the following number-value-equation by Sidney-Young:

$$T_n = T + f_T \cdot \Delta p$$

where:

$\Delta p = (101.325 - p)$ note sign

p = barometer measurement in kPa

f_T = rate of change of boiling point with pressure in K/kPa

T = measured boiling temperature in K

T_n = boiling temperature corrected to normal pressure in K

(*2*) The temperature-correction factors f_T and equations for their approximation are included in the international and national standards mentioned in paragraph (b)(2) of this section for many substances. For example, the DIN 53171 method mentions the following rough corrections for solvents included in paints under the following Table 1:

TABLE 1—TEMPERATURE-CORRECTION-FACTORS F_T

Temperature T K	Correction factor f_T K/kPa	Temperature T K	Correction factor f_T K/kPa
323.15	0.26	473.15	0.39
348.15	0.28	498.15	0.41
373.15	0.31	523.15	0.44
398.15	0.33	548.15	0.45
423.15	0.35	573.15	0.47
448.15	0.37		

A table of temperature-correction factors for organic solvents (see ISO/DIS 4626) is included in the following Table 2:

TABLE 2—TEMPERATURE-CORRECTION FACTORS FOR ORGANIC SOLVENTS (SEE ISO/DIS 4626)

Product	Thermometer	Boiling point °C at 101.325 kPa	Rate of change of boiling point with pressure	
			K' °C/0.1 kPa (°C/mbar)	K °C/mmHg
Acetone	39 C	56.1	0.029	0.039
Acetonitrile	40 C	81.6	0.032	0.043
Allyl alcohol	40 C	96.9	0.028	0.038
Allyl chloride	38 C	45.1	0.029	0.039
n-Amyl acetate	102 C	149.5	0.036	0.048
n-Amyl alcohol	41 C	138.0	0.031	0.041
Aromatic solvent naptha	42 C		0.037	0.049
Benzene	40 C	80.1	0.032	0.042
iso-Butyl acetate	41 C	117.3	0.034	0.045
n-Butyl acetate	41 C	126.1	0.034	0.045
sec-Butyl acetate	40 C	112.4	0.034	0.045
iso-Butyl-alcohol	40 C	107.9	0.027	0.036
n-Butyl-alcohol	40 C	117.7	0.028	0.037
sec-Butyl-alcohol	40 C	99.5	0.026	0.035
tert-Butyl alcohol	40 C	82.5	0.025	0.033
p-tert-Butyl toluene	104 C	192.8	0.042	0.056
Cumene	102 C	152.4	0.038	0.051
Cyclohexane	40 C	80.7	0.033	0.044
Cyclohexanone	102 C	155.7	0.038	0.051
Diacetone alcohol	102 C	169.2	0.038	0.050
Di-iso-butyl ketone	103 C	169.3	0.038	0.051
Di-iso-butylene	40 C	101.4	0.034	0.046
1,2-Dichloroethane	40 C	83.5	0.032	0.043
Dichloromethane	38 C	39.8	0.028	0.037
Diethyleneglycol	106 C	245.8	0.038	0.050
Diethylene glycol-mono-n-butyl ether	105 C	230.4	0.038	0.051
Diethylene glycol-monoethyl ether	104 C	201.9	0.036	0.048
Diethylene glycol-monomethyl ether	104 C	193.8	0.035	0.047
Dimethyl formamide	102 C	153.0	0.033	0.044
Dipropylene glycol	106 C	232.8	0.038	0.051
Di-iso-propyl ether	39 C	68.3	0.031	0.041
Ethyl acetate	39 C	77.2	0.031	0.041
Ethyl alcohol	39 C	78.3	0.025	0.033

TABLE 2—TEMPERATURE-CORRECTION FACTORS FOR ORGANIC SOLVENTS (SEE ISO/DIS 4626)—Continued

Product	Thermometer	Boiling point °C at 101.325 kPa	Rate of change of boiling point with pressure	
			K' °C/0.1 kPa (°C/mbar)	K °C/mmHg
Ethyl benzene	41 C	136.2	0.037	0.049
Ethylene glycol	104 C	197.6	0.032	0.043
Ethylene glycol-mono-n-butyl ether	103 C	171.2	0.035	0.047
Ethylene glycol-monoethyl ether	102 C	135.1	0.033	0.044
Ethylene glycol-monoethyl ether acetate	102 C	156.3	0.035	0.046
Ethylene glycol-monomethyl ether	41 C	124.5	0.031	0.041
Ethylene glycol-mono-iso-propyl ether	102 C	142.8	0.033	0.044
2-Ethyl hexanol	104 C	184.8	0.034	0.046
Ethyl-iso-amyl ketone	103 C	158.2	0.037	0.049
n-Hexyl acetate	103 C	171.6	0.038	0.050
Hexylene glycol	104 C	197.1	0.034	0.045
Isophorone	105 C	215.3	0.043	0.057
Mesityloxide	41 C	129.8	0.035	0.047
4-Metoxy-4-methyl-2-pentanone	103 C	160.6	0.037	0.049
Methanol	39 C	64.6	0.025	0.033
Methyl-iso-amyl acetate	102 C	146.2	0.036	0.048
Methyl-iso-amyl ketone	102 C	144.9	0.036	0.048
Methyl-iso-butyl carbinol	41 C	131.8	0.031	0.041
Methyl-iso-butyl ketone	41 C	116.2	0.035	0.046
Mono-ethanol amine	103 C	170.7	0.030	0.040
Perchloroethylene	41 C	121.2	0.036	0.048
n-Propyl acetate	40 C	101.6	0.032	0.042
iso-Propyl acetate	40 C	88.5	0.031	0.041
n-Propyl alcohol	40 C	97.2	0.026	0.034
iso-Propyl alcohol	40 C	82.3	0.025	0.033
Propylene glycol	104 C	187.6	0.032	0.043
Propylene oxide	38 C	34.3	0.027	0.036
Pyridine	41 C	115.4	0.035	0.046
Toluene	41 C	110.6	0.035	0.046
Triethylene glycol	107 C	287.6	0.038	0.050
Triethylene glycol-monoethyl ether	106 C	255.4	0.038	0.051
Trichloroethylene	40 C	87.1	0.032	0.043
Vinyl acetate	39 C	72.7	0.030	0.040
White spirit	103 C		0.041	0.055
Xylene (isomer mixture)	41 C		0.037	0.049
m-Xylene	41 C	139.1	0.037	0.049
o-Xylene	41 C	144.4	0.037	0.050
p-Xylene	41 C	138.3	0.037	0.049

(iii) *Reference substances.* The standard methods listed include specifications for calibration and evaluation substances. These compounds need not be employed in all cases when investigating a new substance. They should primarily serve to calibrate the method from time to time and to offer the chance to compare the results when another method is applied.

(iv) *Principle of the test methods.* All methods for the determination of the boiling point (boiling range) are based on the measurement of the boiling temperature.

(A) *Determination by use of the Ebulliometer.* See paragraph (d) (1) and (4) of this section. Ebulliometers were originally developed for the determination of molecular weight by boiling point elevation, but they are also suited for exact boiling point measurements. A very simple apparatus is described in ASTM D 1120-72. The liquid is heated in this apparatus under equilibrium conditions at atmospheric pressure until it boils. The determined temperature of the liquid, corrected to standard pressure, is the boiling point.

(B) *Dynamic method.* See paragraph (d)(2) of this section. This method measures the vapor recondensation temperature by means of a thermocouple in the reflux while boiling. The pressure can be varied in this method.

(C) *Distillation method for boiling point (and boiling range).* This method involves distillation of the liquid and measurement of the vapor recondensation temperature and determination of the amount of distillate.

(D) *Method according to Siwoloboff.* See paragraph (d)(2) of this section. A sample is heated in a sample tube which is immersed in a heat-bath liquid. A fused capillary, containing an air bubble in the lower part, is dipped in the sample tube. The temperature at which a regular string of bubbles escapes from the capillary or the temperature at which the string of bubbles stops and the fluid suddenly starts rising in the capillary (Siwoloboff under paragraph (d)(2) of this section) is determined.

(E) *Photocell detection.* See paragraph (d)(3) of this section. Using the principle according to Siwoloboff. Measurements are automatic, the rising bubbles being detected photo-electrically.

(v) *Quality criteria.* The different methods for the determination of the boiling point (boiling range) are compared with regard to their use and precision and possibility to standardize/automate in the following Table 3:

TABLE 3.—COMPARISON OF THE METHODS

Method of measurement	Approximate accuracy	Ability to standardise	Automation
Ebulliometer	±1.4 K (up to 373 K) [1] ±2.5 K (above 373 K) [1].	Existing standard ASTM D 1120-72 [1]	Difficult as yet
Dynamic method.	±0.5 K	Possible	Difficult.
Distillation process (Boiling range).	±0.5 K	Existing standards, e.g., ISO/R 918, DIN 53171, BS 4591/17.	Difficult as yet.
According to Siwoloboff.	±1K to ±2K	Possible	Automatic method exists see: photocell detection.
Photocell detection.	±0.3 K (at 373 K).	Possible	Measurement process works automatically

[1] This accuracy is only valid for pure substances and for the simple device as, for example, described in ASTM D 1120-72; it can be improved with more sophisticated ebulliometer devices.

(2) *Description of the test procedures.* The procedures of several of the test methods have been established by various international and national standards mentioned above. Reference is made here to those standards which prescribe details of preparations, test conditions, and conduct of the test.

(i) *Ebulliometer.* See: ASTM D 1120-72, Standard Test Method for Boiling Point of Engine Antifreezes, and reference under paragraph (d)(4) of this section.

(ii) *Dynamic method.* See: OECD Test Guideline 104 for Vapour Pressure Curve, Principle of the test methods. The boiling temperature observed with an applied pressure of 101.325 kPa is recorded.

(iii) *Distillation process (boiling range).*

See: ISO/R 918, Test Method for Distillation (Distillation Yield and Distillation Range).

ISO 4626/1980, Volatile Organic Liquids—Determination of Boiling Range of Organic Solvents Used as Raw Materials.

BS 4349/68, Method for Determination of Distillation of Petroleum Products.

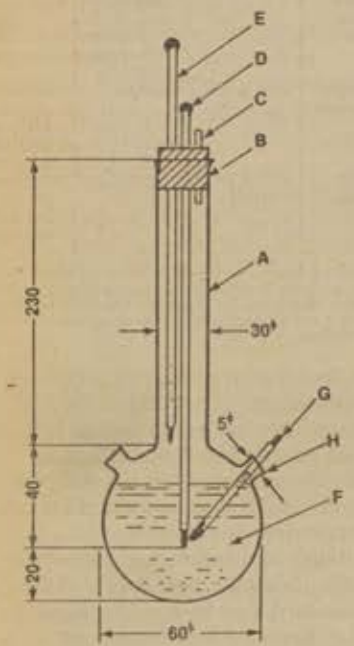
BS 4591/71, Method for the Determination of Distillation Characteristics.

DIN 53171, Lösungsmittel für Anstrichstoffe, Bestimmung des Siedeverlaufes.

DIN 51751, Prüfung flüssiger Mineralkohlenwasserstoffe—Bestimmung des Siedeverlaufes.

(iv) *Method according to Siwoloboff.* (A) The sample is heated in a melting point apparatus in a sample tube, with a diameter of approximately 5 mm in the following Figure 1:

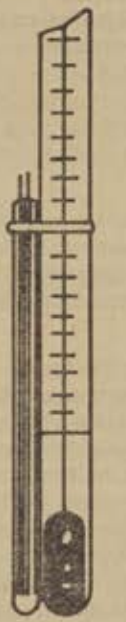
Figure 1—Apparatus for the determination of melting and boiling point (JISK 0064–1966), With Specifications in mm.



A: MEASURING TUBE
B: CORK STOPPER
C: VENT
D: THERMOMETER
E: AUXILIARY THERMOMETER
F: BATH LIQUID
G: SAMPLE TUBE; MAX. 5mm OUTER DIAMETER; CAPILLARY TUBE, APPROX. 1mm INNER DIAMETER, AND APPROX. 0.2mm TO 0.3mm WALL-THICKNESS
H: SIDE TUBE

(B) A capillary tube (boiling capillary) fused about 1 cm above the lower end is placed in the sample tube. The level to which the test substance is filled is such that the fused section of the capillary is below the surface of the liquid. The sample tube containing the boiling capillary is fastened either to the thermometer with a rubber-band or is fixed with a support from the side under the following Figure 2:

Figure 2—Principle According to Siwoloboff

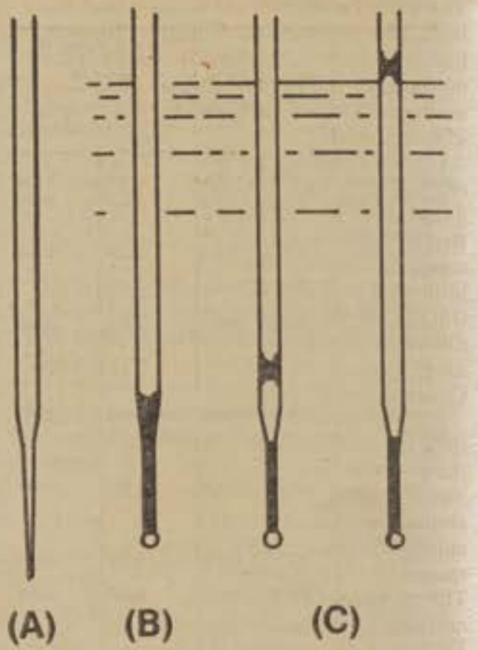


(C) The bath liquid is chosen according to boiling temperature. At temperatures of up to 573 K sulfuric acid or silicon oil can be used. Liquid paraffin may only be used up to 473 K. The heating of the bath liquid should be adjusted to a temperature rise of 3 K/min at first. The bath liquid must be stirred. At about 10 K below the expected boiling point, the heating is reduced so that the rate of temperature rise is less than 1 K/min. When the boiling temperature is approached, bubbles begin to emerge from the boiling capillary.

(D) The boiling point is reached when the string of bubbles stops and fluid suddenly starts rising in the capillary. The corresponding thermometer reading is the boiling temperature of the substance.

(E) In the modified principle, under the following Figure 3, the boiling point is determined in the melting point capillary which is stretched to a fine point about 2 cm in length (1) and a small amount of the sample is aspirated. The open end of the fine capillary is closed by melting, so that a small air bubble is located at the end. When heated in the melting point apparatus (2), the air bubble expands. The boiling point corresponds to the temperature at which the substance plug reaches the level of the surface of the bath liquid (3).

Figure 3—Modified principle.



(v) *Photocell detection.* (A) The sample is heated in a capillary tube inside a heated metal block. A light beam is directed via suitable holes in the block through the substance onto a precisely calibrated photocell. During the increase of the sample temperature, single air bubbles emerge from the boiling capillary. When the boiling temperature is reached, the amount of bubbles increases immensely.

(B) This causes a change in the intensity of light, which is recorded by a photocell and gives a stop signal to the digital indicator reading out the temperature of a platinum resistance thermometer, located in the block.

(C) This method is especially useful because it allows determinations below room temperature as low as 253.15 K (−20°C) without any changes in the apparatus. The instrument merely has to be placed in a cold room or cooling bath. The exact execution of the boiling point determination can be obtained from the instrument manual.

(3) *General remarks.* (i) The results obtained for mixtures or impure samples are to be interpreted with care. With an impure sample, for instance, the emergence of a low boiling component will be registered as the boiling point. Repeated determinations with the same impure sample can change the composition from measurement to measurement, due to the volatilization of low boiling components: continously increasing values are obtained in these circumstances.

(ii) Liquids with a tendency to superheat can yield incorrect results. The values obtained are usually too high. This happens more frequently at higher temperatures. Distillation methods or the dynamic vapor pressure method are more suitable for these types of compound.

(c) *Data and reporting*—(1) *Treatment of results.* (i) The boiling point to be determined should be a mean of at least two measurements, which are in the range of approximate accuracy indicated in Table 2 under paragraph (b)(1)(v) of this section. If determinations are not reproducible, other methods should be considered (see General remarks, above).

(ii) The measured boiling points and their mean should be stated in K. and the pressure(s) at which the measurement(s) was (were) made should be recorded in kPa. Where a test substance boils over a temperature range, this range should be provided. The measured values should also be corrected to standard pressure. Estimates of accuracy should be provided for all results.

(iii) The method used should be indicated, including any deviations from procedures described in this Test Guideline.

(2) [Reserved]

(d) *Literature references.* For additional background information on this test guideline the following references should be consulted:

(1) Kienitz, H. *Methoden der Organischen Chemie* ed. Houben-Weyl, Vol. 2, (Georg Thieme Verlag, Stuttgart, 1953, pp. 815–821.

(2) *Test Guideline 104 for Vapour Pressure Curve,* A 80/5 Umweltbundesamt, Berlin (1980).

(3) Siwoloboff, A. *Berichte-Deutsche Chemische Gesellschaft* 19: 795 (1886).

(4) Manual of Apparatus FP 5+FP 51, FP 52 and FP 53, Mettler Instrumente AG, CH-8606 Greifensee-Zürich, Switzerland.

(5) *European Pharmacopoeia,* 1:75 (1974).

§ 796.1370 Dissociation constants in water.

(a) *Introductory information.* (Titration Method; Spectrophotometric Method; Conductometric Method)

(1) *Prerequisites.* (i) Suitable analytical method. (ii) Water solubility.

(2) Guidance information. (i) Structural formula. (ii) Electrical conductivity for conductometric method.

(3) *Qualifying statements.* (i) All test methods may be carried out on pure or commercial grade substances. The possible effects of impurities on results should be considered.

(ii) The *titration method* is not suitable for low solubility compounds (see Test solutions, under paragraph (b)(2)(i)(A) of this section.

(iii) The *spectrophotometric method* is only applicable to compounds having appreciably different UV/VIS-absorption spectra for the dissociated and undissociated forms. This method may also be suitable for low solubility compounds and for non-acid/base dissociations, e.g. complex formation.

(iv) In cases where the Onsager equation holds, the *conductometric method* may be used, even at moderately low concentrations and even in cases for non-acid/base equilibria.

(4) *Standard documents.* This Test Guideline is based on methods given in the references listed in Section 4 and on the Preliminary Draft Guidance for Premanufacture Notification EPA, August 18, 1978.

(b) *Method*—(1) *Introduction, Purpose, Scope, Relevance, Application and Limits of Test.* (i) The dissociation of a chemical in water is of importance in assessing its impact upon the environment. It governs the form of the substance which in turn determines its behavior and transport. It may affect the adsorption of the chemical on soils and sediments and adsorption into biological cells.

(ii) *Definitions and units. Dissociation* is the reversible splitting into two or more chemical species which may be ionic. The process is indicated generally by

$$RX \rightleftharpoons R^+ + X^-.$$

and the concentration equilibrium constant governing the reaction is

$$K = \frac{[R^+][X^-]}{[RX]}$$

For example, in the particular case where R is hydrogen (the substance is an acid), the constant is

$$K_a = [H^+] \cdot \frac{[X^-]}{[HX]}$$

or

$$pK_a = pH - \log \frac{[X^-]}{[HX]}$$

(iii) *Reference substances.* (A) The following reference compounds need not be employed in all cases when investigating a new substance. They are provided primarily so that calibration of the method may be performed from time to time and to offer the chance to compare the results when another method is applied.

	pK_a (1)	Temp. in °C
p-Nitrophenol	7.15	25[1]
Benzoic acid	4.12	20
p-Chloroaniline	3.93	20

[1] No value for 20 °C is available, but it can be assumed that the variability of measurement results is higher than the temperature dependence to be expected.

(B) It would be useful to have a substance with several pK's as indicated in Principle of the method, under paragraph (b)(1)(iii) of this section. Such a compound could be:

	pK_a (8)	Temp. in °C
Citric acid	(1) 3.14	20
	(2) 4.77	20
	(3) 6.39	20

(iv) *Principle of the test method.* The chemical process described is generally only slightly temperature dependent in the environmentally relevant temperature range. The determination of the dissociation constant requires a measure of the concentrations of the dissociated and undissociated forms of the chemical substance. From a knowledge of the stoichiometry of the dissociation reaction indicated in Definitions and units, above, the appropriate constant can be determined. In the particular case described in this guideline the substance is behaving as an acid or a base, and the determination is most conveniently done by determining the relative concentrations of ionized and un-ionized forms of the substance and the pH of the solution. The relationship between these terms is given in the equation for pK_a in Definitions and units, above. Some compounds exhibit more than one dissociation constant and similar equations can be developed. Some of the methods described herein are also suitable for non-acid/base dissociation.

(v) *Quality criteria.* (A) *Repeatability.* The dissociation constant should be replicated (a minimum of three determinations) to within ±0.1 log units.

(B) [Reserved]

(2) *Description of the test procedures.* (i) There are two basic approaches to the determination of pK_a. One involves titrating a known amount of substance with standard acid or base, as appropriate; the other involves determining the relative concentrations of the ionized and un-ionized forms and their pH dependence.

(ii) *Preparations.* Methods based on those principles may be classified as titration, spectrophotometric and conductometric procedures.

(A) *Test solutions.* (*1*) For the titration method and the conductometric method the chemical substance should be dissolved in distilled water. For spectrophotometric and other methods buffer solutions are used. The concentrations of the test substances should not exceed the lesser of 0.01 M or half the saturation concentration, and the purest available form of the substance should be employed in making up the solutions. If the substance is only sparingly soluble, it may be dissolved in a small amount of a water-miscible solvent prior to adding to the concentrations indicated above.

(*2*) Solutions should be checked for the presence of emulsions using a Tyndall beam, especially if a co-solvent has been used to enhance solubility. Where buffer solutions are used, the buffer concentration should not exceed 0.05 M.

(B) [Reserved]

(iii) *Test conditions.* (A) *Temperature.* (*1*) The temperature should be controlled to at least ±1 °C. The determination should preferably be carried out at 20°C.

(*2*) If a significant temperature dependence is suspected, the determination should be carried out at at least two other temperatures. The temperature intervals should be 10 °C in this case and the temperature control ±0.1 °C.

(B) *Analyses.* The method will be determined by the nature of the substance being tested. It must be sufficiently sensitive to allow determination of the different species at the test solution concentrations.

(iv) *Performance of the test.* (A) *Titration method.* The test solution is determined by titration with the standard base or acid solution as appropriate, measuring the pH after each addition of titrant. At least 10 incremental additions should be made before the equivalence point. If equilibrium is reached sufficiently rapidly, a recording potentiometer may be used. For this method both the total quantity of substance and its concentration need to be accurately known. Precautions must be taken to exclude carbon dioxide. Details of procedure, precautions, and calculation are given in standard tests, e.g., references under paragraph (d)(1), (2), and (4) of this section.

(B) *Spectrophotometric methods.* A wavelength is found where the ionized and un-ionized forms of the compound have appreciably different extinction coefficients. The UV/VIS absorption spectrum is obtained from solutions of constant concentration under a pH condition where the substance is essentially un-ionized and fully ionized and at several intermediate pH's. This may be done either by adding increments of concentrated acid (base) to a relatively large volume of a solution of the compound in a multicomponent buffer, initially at high (low) pH under paragraph (d)(5) of this section, or by adding equal volumes of a stock solution of the compound in, e.g., water, methanol, to constant volumes of various buffer solutions covering the desired pH range. From the pH and absorbance values at the chosen wavelength, a sufficient number of values for the pK_a is calculated using data from at least 5 pH's where the compound is at least 10 percent and less than 90 percent ionized. Further experimental details and method of calculation are given in reference under paragraph (d)(1) of this section.

(C) *Conductometric method.* Using a cell of small, known cell constant, the conductivity of an approximately 0.1 M solution of the compound in conductivity water is measured. The conductivities of a number of accurately made dilutions of this solution are also measured. (The concentration is halved each time, and the series should cover at least an order of magnitude in concentration.) The limiting conductivity at infinite dilution is found by carrying out a similar experiment with the Na salt and extrapolating. The degree of dissociation may then be calculated from the conductivity of each solution using the Onsager equation, and hence using the Ostwald Dilution Law the dissociation constant may be calculated as

$K=\alpha_2 C/1-\alpha$ where C is the concentration in moles per litre and α is the fraction dissociated. Precautions must be taken to exclude CO_2. Further experimental details and method of calculation are given in standard texts and references under paragraphs (d)(1), and (7) of this section.

(c) *Data and reporting*—(1) *Treatment of results*—(i) *Titration method.* The pK_a is calculated for 10 measured points on the titration curve. The mean and standard deviation of such pK_a values are calculated. A plot of pH versus volume of standard base or acid should be included along with a tabular presentation.

(ii) *Spectrophotometric methods.* The absorbance and pH are tabulated from each spectrum. At least five values for the pK_a are calculated from the intermediate spectra data points, and the mean and standard deviation of these results are also calculated.

(iii) *Conductometric method.* The equivalent conductance, Λ, is calculated for each acid concentration and for each concentration of a mixture of one equivalent of acid, plus 0.98 equivalent of carbonate-free sodium hydroxide. (The excess acid is to prevent an excess of OH^- due to hydrolysis.) $1/\Lambda$ is plotted against $\sqrt{C}$ and Λ_o of the salt can be found by extrapolation to zero concentration. Λ_o of the acid can be calculated using literature values for H^+ and Na^+. The pK_a can be calculated from $\alpha=\Lambda_i/\Lambda_o$ and $Ka=\alpha^2 C/1-\alpha$ for each concentration. Better values for Ka can be obtained by making corrections for mobility and activity. The mean and standard deviations of the pK_a values should be calculated.

(2) *Test report.* (i) All raw data and calculated pK_a values should be submitted together with method of calculation (preferably in a tabulated format, such as suggested in paragraph (d)(1) of this section) as should the statistical parameters described under paragraph (c)(1) of this section. For titration methods, details of the standardization of titrants should be given.

(ii) For spectrophotometric methods, all spectra should be submitted. For the conductometric method, details of the cell constant determination should be reported. Information on technique used, analytical methods and the nature of any buffers used should be given.

(iii) The test temperature(s) should be reported.

(d) *Literature references.* For additional background information on this test guideline the following references should be consulted:

(1) Albert, A. & Sergeant, E.P., *Ionization Constants of Acids and Bases.* (Wiley, Inc., New York, 1962).

(2) Nelson, N.H. & Faust, S.D., "Acidic Dissociation Constants of Selected Aquatic Herbicides," *Environment Science Technology* 3: II (1969).

(3) ASTM D 1293. American Society for Testing and Materials, 1916 Race St., Philadelphia, PA 19103.

(4) Standard Method 242. *Standard Methods for the Examination of Water and Waste Water.* 14th ed., (American Public Health Association: Washington, DC, 1976).

(5) Clark, J. & Cunliffe, A.E., "Rapid Spectrophotometric Measurement of Ionisation Constants in Aqueous Solution," Chem. Ind. (London 1973).

(6) ASTM D 1125. American Society for Testing and Materials, 1916 Race St. Philadelphia, PA 19103.

(7) Standard Method 205. *Standard Methods for the Examination of Water and Waste Water.* 14th ed. (American Public Health Association: Washington, DC, 1976).

(8) *Handbook of Chemistry and Physics*, 60th ed. (CRC-Press: Boca Raton, Florida, 1980).

§ 796.1520 Particle size distribution/fiber length and diameter distributions.

(a) *Introductory information.* Method A: Particle Size Distribution (effective hydrodynamic radius). Method B: Fiber Length and Diameter Distributions.

(1) *Prerequisites.* Method A: Water insolubility. Method B: Information on fibrous nature of product; Information on stability of fiber shape under electron-microscopic conditions.

(2) *Guidance information.* Method A: Melting point. Method B: Melting point.

(3) *Qualifying statements.* Both test methods can be applied to pure and commercial grade substances.

(i) Method A: (*A*) This method can only be applied to water-insoluble ($<10^{-6}$g/l), powdered type products.

(*B*) The equivalence of the six national and international standard methods for particle size distribution was not tested, and is currently not known. There is a particular problem in relation to sedimentation and Coulter counter measurements.

(ii) Method B: This method applies only for fibrous products. The effect of impurities on particle shape should be considered.

(4) *Recommendations.* Method A: Equivalence of the methods for determination of particle size distribution should be tested in the laboratory.

(5) *Standard documents.* The "Effective Hydrodynamic Radius Determination" is based on the following standards:

(i) ASTM—D 3360, D422.
(ii) NF-T 30044.
(iii) DIN—66115.
(iv) DIN—66116, Part 1.
(v) ASTM—C 678.
(vi) ANSI—C 690-75.

and on a test principle described in *Chem. Ing. Tech.* 46: 729 (1974).

(b) *Method*—(1) *Introduction, purpose, scope, relevance, application and limits of test.* (i)(A) Many methods are available for particle size measurements, but none of them is applicable to the entire size range. Sieving, microscopic sedimentation and elutriation techniques are most commonly employed. Moreover, in the case of airborne particles (dusts, smokes, fumes), radiation scattering and inertial methods prove particularly useful. Finally, appropriate sampling procedures should be selected in order to prepare specimens really representative of the material under test (Method A).

(B) The first method described in this guideline (Method A) is designed to provide information on the transportation and sedimentation of insoluble particles in water and air. In the special case of materials which can form fibers, an additional set of measurements (Method B) is also recommended to help identify potential health hazards arising from inhalation or ingestion.

(C) Method A is generally applicable, frequent in use and hydrodynamic in character; Method B is comparatively specialized, infrequently required and involves microscopic examination. It should be borne in mind, however, that the original particle size distribution is highly dependent on the industrial processing methods used and can also be affected by subsequent environmental or human transformations.

(D) These tests are applicable only to water insoluble (solubility $<10^{-6}$g/l) substances. Method B for fibers will be applied only if light microscopic examination, similarities to known fibrous or fiber-releasing substances or other data indicate a likelihood that fibers are present. In this context, a fiber is a water insoluble particle, of aspect ratio (length/diameter) >3 and diameter <100 μm. Fibers of length <5 μm need not be considered. Method A, which determines the effective hydrodynamic radius, R_s, will be used for both fibrous and non-fibrous particulates without prior inspection. It is useful only in the range 2 μm$<R_s<$100 μm.

(ii) *Definitions and units.* (A) For Method A the parameter of interest is the effective hydrodynamic radius, or effective Stokes radius R_s. The terminal velocity of a small sphere falling under the influence of gravity in a viscous fluid is given by:

$$v=2_g\,R^2_s(d_1-d_2)/9\eta$$

Where

v=velocity (m/sec),
g=gravitation constant (m/sec^2),
R_s=Stokes radius (m)
d_1=density of sphere (kg/m^3),
d_2=density of fluid (kg/m^3),
η=dynamic viscosity (Nsec/m^2=Pa s) of the the fluid

(B) In other situations, similar relationships apply. Particle size is usually measured in micrometers ($=10^{-6}$ m).

(C) Method B provides histograms of the length (1) and diameter (d) distributions of fibres. The ordinate is the absolute number of particles in each interval of 1 or d. Typical plots are provided in Figures 1 and 2 under paragraph (b)(1)(iv) of this section.

(*iii*) *Reference substances.* (A) Five reference substances of defined particle size covering the overall range 0.35 to 650 μm (excepting the 50 to 200 μm region) have been certified with respect to the cumulative mass distribution of particles versus equivalent settling rate diameter or equivalent volume diameter. The materials will be made available from the Community Bureau of Reference of the European Economic Community and they will be issued with certificates of measurement. The certification report under paragraph (d)(4) of this section will also be available from the Community Bureau of Reference.

(*1*) The certification report of five reference materials will be available from: Commission of the European Community, Directorate—General for Research, Science and Education, Community Bureau of Reference BCR, rue de la Loi 200, B-1049 Brussels.

(*2*) Filter equipment for sample preparations according to Method B is available commercially through the following manufacturers:

(*i*) Nuclepore Corporation, 7035 Commerce Circle, Pleasanton California 94566/USA

(*ii*) Millipore Corporation, Order Service Department, Bedford, Massachusetts 01730/USA

(*iii*) Whatman Filters, W&R Balston Limited, England

(B) *Calibration materials*—(*1*) *Method A.* A binary or ternary mixture of latex spheres (2 μm <d <100 μm) is suggested.

(*2*) *Method B.* No standard reference materials are readily available.

(C) *Evaluation materials*—(*1*) *Method A.* A ternary mixture of 2 μm, 50 μm and 100 μm latex spheres (which provides a discrete calibrated distribution) plus a sample of crushed quartz (continuous distribution).

(*2*) *Method B.* Fibrous chrysotile asbestos is recommended (specific properties not essential as long as enough of a throughly mixed sample is available for identical distribution in a ring test).

(iv) Principle of the test methods. (A) *Method A.* (*1*) There are several standard methods available which meet the sensitivity requirements:

Principle	Methods
Sedimentation	ASTM—D 3360, D 422, NF-T 30044 DIN—66-115
Centrifugation	ASTM—C 678, Chem.Ing.Tech. *46*, 729 (1974)
Coulter counter	ANSI-C 690-75.

(*2*) The comparability of these methods (especially the sedimentation) and the other methods must be determined.

(*3*) The sample should also be subjected to a simple light microscopic examination to determine the approximate nature of the particles (e.g. plates, needles, etc.).

(B) *Method B*—Since data must be collected on small diameter fibers (>0.1 μm), scanning (SEM) or transmission (TEM) electron microscopy is required. There is no standard procedure at present, and those currently under development for asbestos contamination (in which the fibrous material is already identified and in high concentration) are often more complex and expensive than necessary for the needs of this program. Extreme care must still be taken during sample preparation to avoid fiber breaking, clumping and contamination. A simple initial procedure is suggested below (Description of the test procedures). The length and diameter of the fiber images can be measured manually, semi-automatically or automatically and the results tabulated in histogram form (see the following Figures 1 and 2):

Figure 1—Sample fiber length distribution (method B).

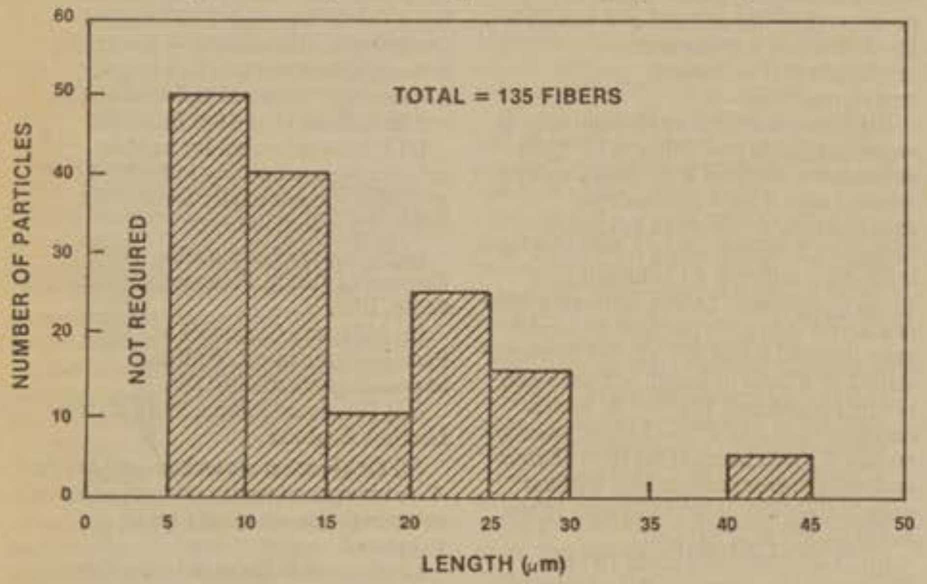
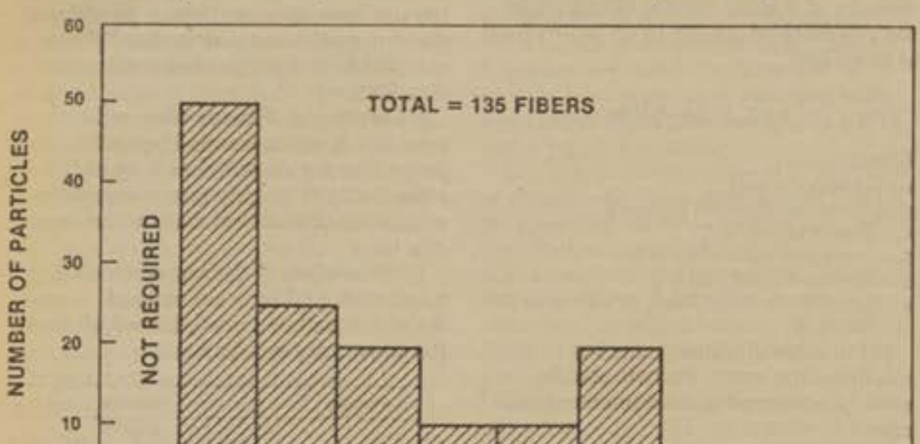


Figure 2.—Sample fiber diameter distribution (method B).



(V) *Quality criteria*—(A) *Repeatability.* (*1*) The effective hydrodynamic radius distribution (Method A) should be measured three times, with no two values differing by more than 20 percent.

(*2*) The length and diameter distributions of fibers (Method B), if required, should be measured at least twice—using separate samplings and preparations—with at least 70 fibers *per* histogram. No two values in a given histogram interval should differ by more than 50 percent or 3 fibers, whichever is larger. Such repeatability should be sufficient for the modeling and decision-making procedures currently envisaged; however, the presence of long, thin fibers—due to their potential adverse health effects—would indicate a need for further, more precise measurements.

(B) *Sensitivity.* In the general case (Method A) particles as small as 2 μm and as large as 200 μm must be measurable. The method requires that sufficient numbers of radius intervals be used to resolve the radius distribution curve. In the case of fibers (Method B), diameters as small as 0.2 μm and as large as 100 μm and lengths as small as 5 μm and as large as 300 μm, must be measurable.

(C) *Specificity.* See paragraph (b)(1) of this section.

(D) *Possibility of standardisation.* The method *procedures* can be readily standardized, if desired, but non-uniformity of sampling, preparation and prior handling may still cause considerable variation in results in Method B.

(E) *Possibility of automation.* Automation or semi-automation of these procedures if possible. Full automation of fiber 1 and d measurements and analysis is also possible.

(2) *Description of the test procedures*—(i) *Preparations*—(A) *Method A.* The small quantities used as samples must be representative of product batches comprising many kilograms; therefore, sampling and sample handling require great care. For example, small particles often form agglomerates; therefore, sample pretreatment (e.g., the addition of dispersing agents, agitation, or low-level ultrasonic treatment) may be required before the primary particle size can be determined. However, great care must be taken to avoid changing the particle size distribution. In the case of highly stable aggregates, a strict distinction between primary particles and agglomerates is not always useful. Some representative sample preparation methods will be found in the standard procedures listed in Principle of the test

methods (Method A) under paragraph (b)(1)(iv) of this section.

(B) *Method B.* Two simple sample preparation procedures (B–1, B–2) for scanning electron microscopy can be suggested.

(*1*) *Sample preparation B–1.* Suspend a given amount of sample in 10–100 ml of filtered distilled or deionized water (the suspension should be relatively light, not a slurry). Distribution of the particles in suspension may be aided by use of a surfactant, such as small amounts ($\sim$1 part/100) of absolute ethyl alcohol or a non-ionic detergent. Suspension of the powder is achieved by gentle hand agitation, vortex mixing or magnetic stirring. Filter the suspension directly onto a 47 mm diameter Nuclepore* filter overlaying a 47 mm diameter Millipore* membrane filter housed in a 47 mm diameter Millipore* filter holder (Hydrosol, stainless) using gentle vacuum. Ensure that the powder has not precipitated out of suspension. Depending on the size of particles of interest various pore-sized filters may be used. The concentration of suspended particles determines the amount filtered. A less concentrated suspension will give a more even distribution of particles on the filter surface under paragraph (d)(2) of this section. Remove the Nuclepore filter from the filter housing, being careful not to disturb the particles on the surface. Place the filter—particle-coated face upward—into a glass or plastic Petri dish containing Whatmann No. 1 filter paper; cover Petri dish and store in a dry box or under vacuum. When completely dried, the filter is cut into pieces of appropriate size and mounted, filter face up, onto copper tape which has been previously mounted onto an SEM specimen holder (using double face tape). To ensure stickiness of the tape, preheat using infrared or similar heat source for 5 to 15 minutes. Trim the edge of the filter to fit the SEM specimen holder.

(*2*) *Sample preparation B–2.* An alternate sample preparation method is the direct transfer of the dry powder onto copper tape (adhesive electrical tape) which has been mounted onto a scanning electron microscope (SEM) specimen holder. The powder may also be sprayed onto the copper tape surface by using an atomizer or pipette equipped with a large rubber bulb.

(ii) *Test conditions and apparatus*—(A) *Method A.* Ambient conditions. Measuring apparatus for all methods are readily available. Pipettes and sedimentation balances are used for the sedimentation methods.

(B) *Method B.* (*1*) Contamination by air-borne fibers can be a problem. A hood or "clean room" should be used if available.

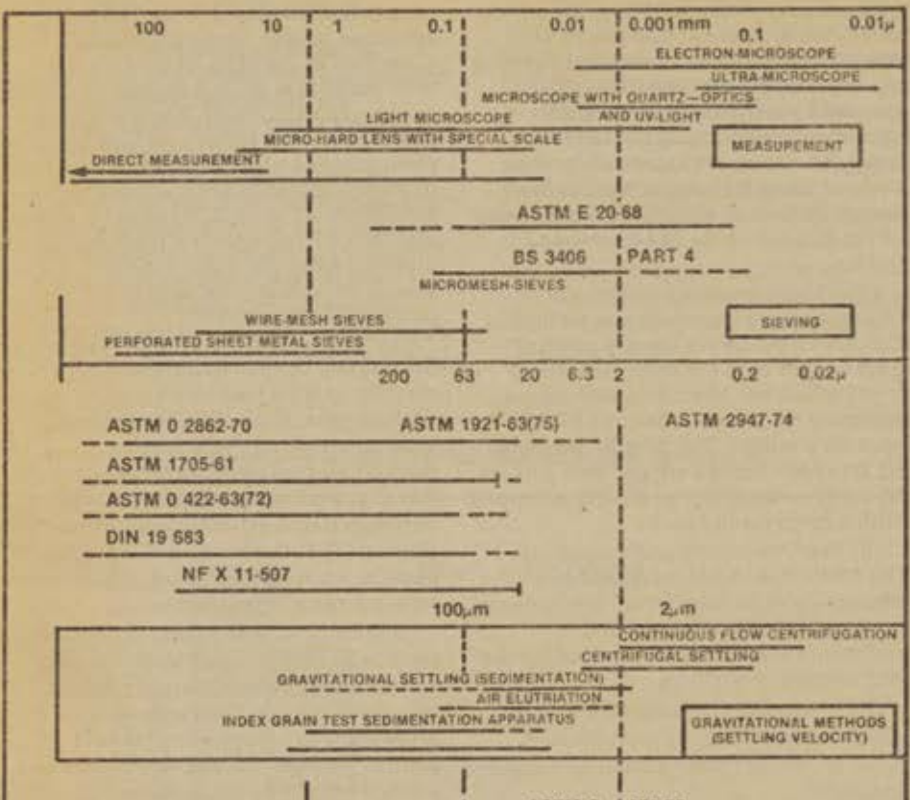
(*2*) A small electron microscope and support equipment are required.

(iii) *Performance of the tests.* (A) methods: (*1*) *Method A.* To be selected from standard procedures listed above (Principle of the test methods).

(*2*) *Method B.* Both preparation methods (B–1 and B–2) provide a particulate sample on filter paper or copper tape mounted on an SEM specimen holder. This can then be examined in the SEM, or first coated with metal film using a sputtering device or vacuum evaporator. Representative fields within the sample surface are photographed at various magnifications to yield a representative sample of the population of interest. (If desired, energy dispersive x ray analysis (EDXA) of representative particles—to check sample contamination—could be performed at this time.)

(B) Particle size distribution can be determined by measuring the screen directly or from measurements on photographs. If the SEM is equipped with an image analysis system, population statistics can be determined directly. Such measurements can be automated or semi-automated when desired (3). If the image indicates the sample is too concentrated, repeat again with a more dilute solution.

(iv) *Analysis.* Measuring the physical parameters by different methods can result in somewhat different particle size distributions; therefore, the measuring techniques used should always be reported. Representative analysis methods are discussed in reference under paragraph (d)(1) through (6) of this section, and the following "Summary of the Usual Methods for the Determination of Particle Size and the Important Granular Size Classes," (adapted from G. Müller, *Methoden der Sedimentuntersuchungen,* 1964, p. 303, Stuttgart, revised with appropriate supplements):



(c) *Data and reporting*—(1) Data. (i) Method A: Data should be obtained for 3 size ranges: >200 μm, <2 μm and the region 2 to 200 μm. Only in the latter range should the distribution curve be prepared. It should have sufficient μm increments to resolve the curve (subpopulations). A histogram presentation is required plus a statement on the weight percent of material >200 μm and <2 μm.

(ii) Method B: Full length (1) and diameter (d) data are needed on fibers of dimensions $d>0.1$ μm and $1>5$ μm. Two histogram distributions, based on examination of at least 50 fibers each, should be prepared. For diameters, the ranges should be 0.1–0.5, 0.5–1.0, 1–2, 2–3, 3–5 μm and over 5 μm. For lengths they should be 0–5, 5–10, 10–15, 15–20, (etc.) μm. This is illustrated in Figures 1 and 2, above.

(2) *Test report.* (i) Method A: The following information should be presented:

(A) Expected percent change of reported values in the future (e.g., variations between production batches).

(B) Sample preparation methods used.

(C) Analysis methods used.

(D) Approximate information on particle shape (e.g., spherical, platelike, needle shaped).

(E) Lot number, sample number.

(F) Suspending medium, temperature, pH.

(G) Concentration.

(H) Stoke's (effective hydrodynamic) radius R_s distribution for $2<R_s<200$ μm.

(I) Mean value and approximate "area" (percent) of any resolvable peaks in R_s distribution.

(J) Percent of particles with $R_s<2$ μm.

(K) Percent of particles with $R_s>200$ μm.

(ii) Method B: The following information should be presented:

(A) Sample description, method description.

(B) Number of particles *per* field.

(C) Total number of fibers measured.

(D) 1, d distributions (histograms).

(E) Mean value and approximate "area" (percent) of any resolvable peaks in the R_s distribution.

(d) *Literature references.* For additional background information on this test guideline the following references should be consulted:

(1) Allen, T., *Particle Size Measurement.* (Chapman and Hall: London, 1975).

(2) Irani, R.R., and Callis, C.F., *Particle Size Measurement.* Interpretation and Application.

(3) Orr, S., and Dallavalle, J.M., *Fine Particle Measurement.*

(4) *Certification Report on Particles of Defined Particle Size.* (Community Bureau of Reference: Brussels, 1979).

(5) McGrath, P.P., and Ewell, J.B., "Application of Electron Microscopy to Problem of Particulate Contaminants in Food, Drugs and Biologicals," *Scanning Electron Microscopy,* Part III (1976).

(6) *Symposium on Electron Microscopy of Microfibers,* ed. Asher I.M., and McGrath, P.P., Proceedings of the First FDA Office of Science Summer Symposium, (August 23–25, 1976).

3. Subpart C is amended by adding §§ 796.3180, 796.3200, 796.3220, 796.3240,

796.3260, 796.3300, 796.3340, 796.3360, 796.3400 and 796.3480 to read as follows:

§ 796.3180 Ready biodegradability: Modified AFNOR test.

(a) *Introductory information*—(1) *Prerequisites.* (i) The test material must be at least soluble at the concentration to be tested, 40 mg dissolved organic carbon/liter (DOC/l).

(ii) The organic carbon content of the test material must be established.

(2) *Guidance information.* (i) Information on the relative proportions of the major components of the test material will be useful in interpreting the results obtained, particularly in those cases where the result lies close to the "pass level."

(ii) Information on the toxicity of the chemical may be useful in the interpretation of low results and in the selection of appropriate test concentrations.

(3) *Interpretation of results.* Because of the stringency of this test a result of less than 70 percent loss of DOC (within 28 days) does not necessarily mean that the test compound is not biodegradable under environmental conditions, but indicates that more work will be necessary to establish biodegradability.

(4) *Qualifying statements.* (i) The method is only applicable to those organic test materials which, at the concentration used in the test:

(A) Are soluble in water.

(B) Have negligible vapour pressure.

(C) Are not inhibitory to bacteria.

(D) Do not significantly adsorb on glass surfaces.

(ii) This test has been found suitable by the OECD Expert Group Degradation/Accumulation for determining the ready biodegradability of organic chemicals under aerobic conditions. It has been tested in the OECD Laboratory Intercomparison Test Program (1978–1980).

(5) *Recommendations.* (i) Test Chemicals giving a result of greater than 70 per cent loss of DOC (within 28 days) should be regarded as readily biodegradable. This level must be reached within 10 days of biodegradation exceeding 10 percent.

(ii) If the limits of sensitivity of organic carbon analyzers are improved, the use of lower test concentrations may be an advantage, particularly for toxic compounds.

(6) *Standard documents.* This test is based on "Norme Experimentale AFNOR T 90-302."

(b) *Method*—(1) *Introduction, purpose, scope, relevance, application and limits of test.* (i)(A) The method described in this test guideline allows the evaluation in aqueous medium of so-called "total" biodegradability under experimental conditions which are easy to establish. These conventional conditions do not necessarily correspond in all cases to the optimal conditions which would result in the maximal value of biodegradability.

(B) The method is applicable to those organic products, alone or in mixtures, which are:

(*1*) Soluble in water at the concentration used under the conditions of the test.

(*2*) Non-volatile or at least have a negligible vapour pressure under the conditions of the test.

(*3*) At the concentration foreseen in the test, not inhibitory with respect to the bacteria responsible for the biodegradation.

(ii) *Reference substances.* (A) In some cases when investigating a new substance reference substances may be useful; however, specific reference substances cannot yet be recommended. Aniline, sodium acetate or sodium benzoate may be used for this purpose; they must exhibit DOC removal of >70 percent within 28 days, otherwise the test is regarded as invalid and should be repeated using an inoculum from a different source.

(B) In this specific test glucose is used especially for the inhibition test and may be used also to check the activity of the inoculum.

(iii) *Principle of the test method.* The biodegradation of organic products dissolved in water by chemico-organotrophic micro-organisms using the products as the sole source of carbon and energy is observed. These products are studied at a concentration such that the initial content of organic carbon is 40 mg/l. The organic carbon remaining in solution after 3, 7, 14, 28 (and 42) days is measured and the corresponding level of biodegradation calculated. The biodegradability is evaluated on the basis of this level.

(iv) *Quality criteria.* This is an evaluation method and not a method for the determination of biodegradability.

(A) *Reproducibility.* To date no OECD ring test has been performed especially for reproducibility, but some general ring tests have been made with the CEFIC and with AFNOR, which have some relevance.

(B) *Sensitivity.* This method is sensitive to an order of magnitude of ±10 percent.

(C) *Specificity.* This Test Guideline is applicable to organic products soluble in the test medium at a concentration equal at least to one containing 40 mg/l as carbon.

(D) *Possibility of standardization.* This method may be standardized in the course of work by ISO.

(E) *Possibility of automation.* Automation seems possible.

(2) *Description of the test procedure*—(i) *Preparations*—(A) *Site.* The incubation must be carried out in the absence of any intense lighting, in an incubator maintained at 25±1 °C and free from toxic vapors.

(B) *Reagents.* (*1*) The chemical products used must be of recognized analytical purity.

(*2*) Distilled water must not contain more than 2 mg/l of organic carbon. In any case the organic carbon content of the distilled water must not exceed 10 percent of the organic carbon level introduced by the test substance.

(*3*) Test medium: (*i*) Prepare the test medium as indicated, using sterile material. For one litre of solution, dissolve the following in distilled water:

Ammonium sulphate $(NH_4)_2SO_4$	0.300 g.
Ammonium nitrate NH_4NO_3	0.150 g.
Monopotassium phosphate KH_2PO_4.	0.300 g.
Disodium phosphate $Na_2HPO_4 \cdot 12H_2O$.	2.000 g.
Magnesium sulphate $MgSO_4 \cdot 7H_2O$	0.050 g.
Calcium chloride $CaCl_2 \cdot 2H_2O$	0.050 g.
Yeast extract	0.005 g.

The pH is 7.5±0.1.

(*ii*) Add 1 ml of trace element solution of the following composition:

Ferrous sulphate $FeSO_4 \cdot 7H_2O$	0.100 g.
Manganese sulphate $MnSO_4 \cdot H_2O$	0.100 g.
Potassium molybdate K_2MoO_4	0.025 g.
Sodium tetraborate $Na_2B_4O_7 \cdot 10H_2O$.	0.025 g.
Cobalt nitrate $Co(NO_3)_2 \cdot 6H_2O$	0.025 g.
Copper chloride $CuCl_2 \cdot 2H_2O$	0.025 g.
Zinc chloride $ZnCl_2$	0.025 g.
Ammonium metavanadate NH_4VO_3.	0.010 g.
Distilled water to	100 ml.

(*4*) The trace element solution can be kept for one month at a temperature between +1 and +4 °C.

(*5*) Make up to the volume stated (1 liter) and mix. The medium must be used within 12 hours.

(C) *Apparatus.* Usual laboratory items, and

(*1*) Apparatus for the assay of organic carbon.

(*2*) Spectrophotometer.

(*3*) Centrifuge, 4000 ms^{-2}.

(*4*) Shaker allowing adequate aeration and shaking.

(*5*) Apparatus for the assay of dissolved oxygen.

(*6*) pH meter.

(*7*) 500-ml wide-neck conical flasks, sterile.

(*8*) Apparatus for sterile filtration. The glassware must be thoroughly cleaned and in particular free from all traces of organic or toxic matter.

(ii) *Procedure*—(A) *Preparation of the test solutions.* (*1*) Prepare the following solutions:

(*i*) Solution of tested product in the test medium in such a way as to obtain a concentration of 40 mg/l of organic carbon.

(*ii*) Solution of glucose in the test medium in such a way as to obtain a concentration of 40 mg/l of organic carbon.

(*iii*) Solution containing in the test medium the concentrations of product and glucose used for the preparation of solutions.

(*iv*) An adequate volume of test medium should be available.

(*2*) Mix the four solutions individually and sterilize by filtration through a membrane filter of 0.22 μm porosity.

(B) *Preparation of the inoculum.* (*1*) Take an adequate volume of a mixture of three samples from polluted surface water (effluent from town sewage works) free from major specific pollutants. The bacterial count for each sample must be at least 10^8 bacteria/ml.

(*2*) The samples must be used for the inoculation within a period of 12 hours, including transportation, and must not remain for more than 6 hours without aeration.

(*3*) Filter through paper to eliminate the larger insoluble particles, collect the filtrate and pass through a membrane filter of pore size 0.22 μm. Wash with any isotonic solution. Take up the bacteria deposited on the membrane filter in a small volume of solution or with any other isotonic solution. Mix well. Measure the absorbance at 620 nm and from this deduce the concentration of bacteria in relation to a standard curve obtained previously by means of solid medium counts of *Pseudomonas fluorescens* strain ATCC 15453. Add the volume of solution required to adjust the concentration of bacteria to $5\pm3\times10^7$/ml. Use the inoculum within the next hour.

(C) *Actual test performance.* (*1*) All the necessary manipulations must be carried out by sterile methods. Divide solutions into the test flasks (previously sterilized) according to the following scheme:

Flask No. 1 (test)	150 ml solution 1.
Flask No. 2 (test)	150 ml solution 1.
Flask No. 3 (test)	150 ml solution 1.
Flask No. 4 (sterile control).	150 ml solution 1.
Flask No. 5 (glucose control).	150 ml solution 2.
Flask No. 6 (control of inhibitory action).	150 ml solution 3.
Flask No. 7 (inoculum control).	150 ml solution 4.

(*2*) Seed flasks 1, 2, 3, 5, 6 and 7 with 1.5 ml of inoculum and mix well by manual shaking.

(*3*) Take an aliquot of 3–5 ml from each flask. Centrifuge the aliquots at 4000 ms^{-2} for 15 minutes, keeping the temperature below 26 °C. In certain cases, and especially in presence of relatively low specific gravity insoluble fractions, it may be necessary to centrifuge for a longer time or with a higher acceleration. Collect the supernatants for assays of organic carbon at time 0.

(*4*) Place the flasks on the shaker and leave them there throughout the test period; the shaking must be such that the validity clause is fulfilled.

(*5*) In the same way as for the assay of organic carbon at time 0, carry out this assay on flasks 1, 2, 3, 5, 6 and 7 after 3, 7, 14, 28 (and 42) days of incubation. However, if the reduction in carbon content reaches 95 percent of the initial content in flasks 1, 2 and 3, consider the test as ended.

(*6*) The test can be finished before the 28th day in the case where a plateau is observed before the 28th day. In the case where a degradation has obviously started on day 28 but has not reached a plateau on day 28, it is considered good practice to extend the experiment for 1 of 2 weeks longer.

(*7*) At the end of the test carry out an assay of organic carbon in flask 4 in the same manner as at time 0.

(*8*) If the assays of organic carbon contents have to be deferred, keep the supernatant at 4 °C in the dark in hermetically sealed glass flasks; the maximum acceptable duration of preservation is 24 hours. If the analysis cannot be carried out within 24 hours, then freeze at a temperature below −18 °C.

(*9*) To compensate for loss of water due to evaporation, before each sampling verify the volume of medium in the flask and, if necessary, fill up with distilled water sterilized by filtration through a membrane of 0.22 μm pore size to restore the volume measured after the previous sampling.

(c) *Data and reporting*—(1) *Treatment of the results.* (i) Calculation:

(A) Determine the percentage elimination of soluble organic carbon for each sampling, using the following formula:

$$\%\ \text{elimination of carbon at time } t = \frac{(C_o-C_{oi})(C_t-C_{ti})}{(C_o-C_{oi})}\times 100$$

where

C_o = the mean level of organic carbon at time 0 in flasks 1, 2 and 3 (or the organic carbon content at time 0 in one of the flasks 4, 5 or 6).

C_{oi} = the organic carbon level in flask 7 at time 0.

C_t = the mean level of organic carbon at time t in flasks 1, 2 and 3 (or the organic carbon level at time t in one of the flasks 4, 5 or 6).

C_{ti} = the organic carbon level in flask 7 at time t.

(B) The level of biodegradation is the percentage elimination of the organic carbon.

(C) Set up a table of biodegradation levels as a function of time. Draw the curve of the change in biodegradation level as a function of time when a large enough number of significant points is available.

(ii) Note: The comparison of the percentage eliminations of carbon in flasks 1, 2 and 3, on the one hand, and in flask 4, on the other hand, allows the causes of the degradation observed to be differentiated: the physical-chemical mechanisms in flask 4 and the physical-chemical plus biological mechanisms in flasks 1, 2 and 3.

(2) *Validity of the results.* If one of the following conditions (i) to (iii) is not fulfilled, begin the test anew. In the case of condition (i), the level of shaking must be increased in the new test.

(i) The concentration of dissolved oxygen on day 3 in flask 5 must be at least 5 mg/l.

(ii) The level of glucose degradation in flask 5 must be at least 80 percent by day 7.

(iii) At the end of the test, flask 4 must still be sterile. Check this by seeding into a tube of liquid culture medium (Dehydrated yeast extract, 3 g; Pancreatic caseine peptone, 6 g; Water, 1000 ml—Dissolve the components or the dehydrated complete medium in boiling water. If necessary, adjust the pH in such a way that after sterilisation it is 7.2±0.2 at 20 °C) and incubating at 25 °C for 5 days. The level of glucose biodegradation in flask 6 must, by day 7, be at least 75 percent of that observed in flask 5. If this limit is not reached, consider that the product subjected to the test presents an inhibitory effect towards the bacteria present and that the method is not applicable to it at the concentration laid down by this standard.

(3) *Interpretation of results.* See paragraph (a) of this section.

(4) *Test report.* (i) Mention the following points in particular:

(A) Motivation for any rejection of the test.

(B) Extent of the disappearance of the product in flask 4 at the end of the test.

(C) Any inhibition phenomena observed.

(ii) *Biodegradability:* Express the biodegradability as the highest level of biodegradation noted during the period of 28 days. The course of the degradation should be displayed graphically in a diagram.

§ 796.3200 Ready biodegradability: Closed bottle test.

(a) *Introductory information*—(1) *Prerequisites.* The empirical formula of the test material is required so that the theoretical oxygen demand (TOD) may be calculated. If this is unknown, the chemical oxygen demand (COD) of the test material may serve as the reference point (6).

(2) *Guidance information.* (i) Information on the relative proportions of the major components of the test material will be useful in interpreting the results obtained, particularly in those cases where the result lies close to the "pass level."

(ii) Information on the toxicity of the chemical may be useful in the interpretation of low results and in the selection of appropriate test concentrations.

(3) *Interpretation of results.* Because of the stringency of this test, a measured BOD (biochemical oxygen demand) which is less than 60 percent of TOD (within 28 days) does not necessarily mean that the test compound is not biodegradable under environmental conditions, but indicates that more work will be necessary to establish biodegradability. The possibility that nitrogen-containing compounds may affect the results should be considered.

(4) *Qualifying statements.* (i) The method is only applicable to those organic test materials which, at the concentration used in the test, are not inhibitory to bacteria.

(ii) This test has been found suitable by the OECD Expert Group Degradation/Accumulation for determining the ready biodegradability of organic chemicals under aerobic conditions. It has been tested in the OECD Laboratory Intercomparison Test Program (1978–1980).

(5) *Recommendations.* (i) Test chemicals giving a measured BOD which is greater than 60 percent of the TOD (within 28 days) should be regarded as readily biodegradable. This level must be reached within 10 days of biodegradation exceeding 10 percent.

(ii) If the test material is not soluble at the test concentration, special measures, such as the use of ultrasound dispersion, may have to be employed to achieve a good dispersion of test material.

(6) *Standard documents.* This test guideline is based on a paper found under paragraph (d)(8) of this section.

(b) *Method*—(1) *Introduction, purpose, scope, relevance, application and limits of test.* (i) The purpose of the method is the measurement of the biodegradability of organic compounds in an aerobic, aqueous medium at a test concentration of 2 (standard concentration) to 10 mg/l of active material. Most data elaborated with this test pertain to water-soluble compounds; however, volatile compounds and those of low water solubility may also be tested, at least in principle.

(ii) *Definitions and units.* The degradation is stated as the biochemical oxygen demand (BOD) within 28 days as a percentage of either the theoretical oxygen demand (TOD) or the chemical oxygen demand (COD).

For example:

$$\text{Percent biodegradability} = \frac{\text{mg } O_2/\text{mg active substance}}{\text{TOD}} \times 100$$

or

$$\text{Percent biodegradability} = \frac{\text{mg } O_2/\text{mg active substance}}{\text{COD}} \times 100$$

where

TOD = theoretical oxygen demand (calculation, see Data and reporting, below)

COD = chemical oxygen demand determined experimentally.

(iii) *Reference substances.* In some cases when investigating a new substance, reference substances may be useful; however, specific reference substances cannot yet be recommended. In order to check the activity of the inoculum, the use of control substances is desirable. Aniline, sodium acetate, sodium benzoate, sodium n-dodecylsulfate and the sodium salt of linear alkylbenzenesulfonic acid (8, 9) may be used for this purpose. They must exhibit a BOD which is >60 percent of their TOD within 28 days, otherwise the test is regarded as invalid and should be repeated using an inoculum from a different source.

(iv) *Principle of the test method.* A predetermined amount of the compound is dissolved in an inorganic medium (mineral nutrient solution), providing a usual concentration of 2 mg active substance per liter (AS/l). The solution is inoculated with a small number of micro-organisms from a mixed population and kept in closed bottles in the dark in a constant temperature bath at 20±1 °C. The degradation is followed by oxygen analyses over a 28-day period. A control with inoculum, but without test material, is run parallel for the determination of oxygen blanks.

(v) *Quality criteria*—(A) *Reproducibility.* The reproducibility of the method is appropriate for a screening test for "ready biodegradability," which has solely an acceptance function, but is not sufficient for a final decision regarding biodegradibility.

(B) *Sensitivity.* A starting concentration of 2 mg AS/l usually allows the determination of 95 percent degradation. Compounds with a low TOD may require higher starting concentrations.

(C) *Specificity.* The test is applicable for the biodegradability evaluation of organic compounds. Most experience has been gathered with water soluble compounds; however, the test lends itself, at least in principle, to the biodegradability evaluation of volatile and insoluble compounds.

(D) *Possibility of standardization.* The test is suited for standardization.

(E) *Possibility of automation.* Although the whole test cannot be automated, parts of it, e.g., the analysis, may. The procedure is well suited, however, for being operated with whole series of test materials.

(2) *Description of the test procedure*—(i) *Preparations*—(A) *Reagents*—(*1*) *Distilled or ion exchange water.* Containing not more that 0.01 mg Cu/l, air saturated. Volume according to the need of the day, e.g., 50 l, at room temperature, as close as possible to 20 °C, aerated strongly for 20 minutes with compressed air. Generally, the water is ready for use after standing for 20 hours at 20 °C. Oxygen is determined for control purposes. The concentration at 20 °C should be about 9 mg O_2/l. All transfer and filling operations of the air saturated water must be conducted bubble-free by siphon.

(*2*) *Standard dilution water for BOD determinations according to H5 of DEV* (see paragraph (d)(1) of this section).

(*i*) Solution 1: 8.5g of KH_2PO, 21.75g of K_2HPO_4, 33.3g of $Na_2HPO_4 \cdot 2H_2O$, and 1.7g of NH_4Cl are dissolved in 500 ml of distilled water. The solution is made up to 1 l. The pH ought to be 7.2.

(*ii*) Solution 2: 22.5 g of $MgSO_4 \cdot 7\,H_2O$/l.

(*iii*) Solution 3: 27.5 g of $CaCl_2$/l.

(*iv*) Solution 4: 0.25 g of $FeCl_3 \cdot 6\,H_2O$/l.

1 ml each of solutions 1 to 4 are added to 1 litre of aerated distilled water.

(B) *Materials.* (*1*) Calibrated 250–300 ml BOD bottles with glass stoppers (Uncalibrated narrow neck 250 ml bottles with glass stoppers whose volumes have been determined may be used instead without causing any appreciable error.) All glassware is cleaned according to the H23 procedure under paragraph (d)(2) of this section.

(*2*) Several 2, 3 and 5-l bottles with self-made litre marks for the preparation of the experiments and for the filling of the BOD bottles.

(*3*) Pipettes of 1 to 10 ml volume.

(*4*) Funnels and coarse filter paper.

(*5*) Baby bottles for the preparation and shaking of the inoculum.

(*6*) Water bath for keeping the bottles at constant temperature under the exclusion of light.

(C) *Inoculation.* The following inocula may be used:

(*1*) An aqueous suspension of unfertilized garden soil. 100g of unfertilized garden soil (soil from a greenhouse which is at constant temperature throughout the year is especially advantageous) are dispersed in chlorine-free tap water (1 liter). After 30 minutes the suspension is filtered through a coarse filter and the first 200 ml of the filtrate are discarded. The main part of the filtrate serves for the inoculation (1 drop from a pointed pipette per liter of final volume, see Procedure, below). The inoculum is prepared immediately before the experiment. It must be aerated if it is to be kept for several hours. The number of bacteria may be determined according to the pour plate method or with nutrient pad sets. There should be no more than 10^3 to 10^5 bacteria per milliliter of final volume.

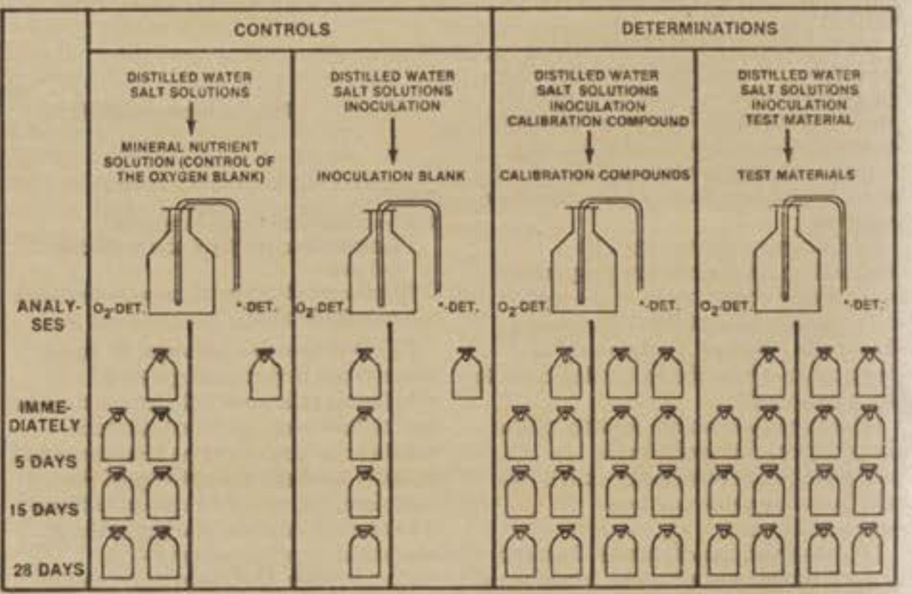
(*2*) Effluent from an activated sludge plant or trickling filter. Inoculation should preferably be made using a secondary effluent of good quality collected from a treatment plant dealing with predominantly domestic sewage. The effluent must be kept under aerobic conditions in the period between sampling and application. To prepare the inoculum the sample is filtered through a coarse filter, the first 200 ml being discarded. The rest of the filtrate is kept aerobic until used. The inoculum must be used on the day of collection.

(*3*) Effluent from a strongly aerated laboratory activated sludge plant. The inoculum is prepared as described in previous section.

(*4*) Composite inoculum. Equal volumes of the three inoculum samples are combined, mixed well and the final inoculum drawn from this mixture. The suitability of the inoculum is checked by means of a control substance.

(ii) *Procedure.* (A) (*1*) Direct comparisons are always necessary in biological experiments. Therefore groups of parallel bottles are prepared for the determination of the BOD of the test and control substances in simultaneous experimental series, see the following Figure 1:

Figure 1—Scheme for the bottle arrangement for the closed bottle test



(* = SPECIFIC ANALYSIS IF AVAILABLE)

If chemical analyses are performed simultaneously a sufficient number of bottles—including the controls for the inoculum and the blank—have to be prepared [e.g., 7 or 15 parallel bottles are prepared for one test material for the 0, 5, 15 and 28-day tests after a sufficient volume of water has been prepared in large bottles (see Reagents above)].

(*2*) These large bottles are first filled to about one third of their volume with distilled water by hose. Then the individual salt stock solutions are pipetted into these bottles according to the final volume, and the respective test or control materials are added in such amounts that final concentrations of 2 and sometimes 5 or 10 mg/l (higher concentrations only in case of poorly degradable compounds and those with a low TOD) are attained (see General considerations below). Subsequently the experimental solution is inoculated with 1 drop (from a pointed pipette) per liter of final volume, and the blank is inoculated similarly. Finally, the solutions are made up to volume with a hose which reaches down to the bottom of the flask, which achieves adequate mixing.

(*3*) Subsequently each prepared solution is filled immediately into the respective group of bottles by hose from the lower quarter (not from the bottom!)

of the bottle. Furthermore, the zero controls are analyzed or preserved for the later analyses (for the O_2-determination by precipitation with $MnCl_2$ and NaOH; for the surfactant determination by preservation with 50 mg/l of $HgCl_2$). The remaining parallels are placed in a water bath at 20 °C, kept in the dark, and removed after 5, 15 and 28 days, respectively, from the bath, and analyzed. The result is recorded as percentage biodegradability and should be visualised as a diagram.

(*4*) The test can be finished before the 28th day in the case where a plateau is observed before the 28th day. In the case where a degradation has obviously started on day 28 but has not reached a plateau on day 28, it is considered good practice to extend the experiment for one or two weeks longer.

(B) *General considerations.* (*1*) Each series is accompanied by complete parallel series for the determination of the blank, and the oxygen depletion without inoculation.

(*2*) The oxygen depletion without inoculation should not exceed 0.2 to 0.3 mg O_2/l after 5 days and 0.4 mg O_2/l after 28 days, respectively; the blank with inoculation should not exceed 0.4 to 0.5 mg O_2/l after 5 days and 0.5 to 0.6 mg O_2/l after 15 to 28 days. Typical values for the reference substances can be found in the literature references under paragraph (d)(8) and (9) of this section.

(*3*) Degradability is calculated by (*i*) subtracting the oxygen depletion result of the blank from that of the test substance and (*ii*) dividing this number by the concentration (W/V) of the test material. This calculation gives the oxygen depletion in mgO_2/mg of active material; this value is transformed to the "% biodegradability" according to Data and reporting under paragraph (c) of this section for the day under consideration.

(*4*) If the structure or elemental composition of the test material is unknown, or it is a mixture of unknown organic compounds, the chemical oxygen demand (COD) may serve as a reference instead of the TOD.

(*5*) The concentration of about 9 mg of dissolved oxygen per liter of dilution water at 20 °C limits the possible starting concentration of the test material to about 2 mg/l in order to guarantee that an oxygen concentration of 4–5 mg/l remains after the oxidation of the test substrate. Substances which are only partially degraded or those which have a low TOD are advantageously tested in parallel experiments at 5 or even 10 mg/l starting concentrations.

(C) *Inhibition test.* (*1*) Organic and inorganic materials may easily and simply be tested for inhibitory effects in the closed bottle test:

(*i*) Series 1: 2 mg/l of a well-degraded compound (e.g., fatty alcohol + 10 moles ethylene oxide).

(*ii*) Series 2: × mg/l of test material (× is usually 2).

(*iii*) Series 3: 2 mg/l of the control compound plus × mg/l of test material.

(*2*) If the BOD values of series 3 correspond to the sum of those of series 1 and 2, the test material does not display inhibitory effects. This control experiment is always necessary if a negative or poor degradability result seems illogical in view of the structure of the test material, i.e., if there are indications that it may be caused by inhibition.

(iii) *Analytical means.* (A) Oxygen determination according to Winkler under paragraph (d)(3) of this section or electrometrically, if suitability is assured.

(B) In case of anionic surfactants: MBAS-determination according to H23 of DEV under paragraph (d)(2) of this section.

(C) In case of nonionic surfactants: BiAS(bismuth active substance)-determination according to Wickbold under paragraph (d)(4) of this section.

(c) *Data and reporting*—(1) *Treatment of results*—(i) *Calculation of the theoretical oxygen demand (TOD).* (A) The TOD of the compound $C_cH_hCl_{cl}N_nNa_{na}O_oP_pS_s$ of the molecular weight is calculated according to:

$$TOD_{NH3} = \frac{16\left[2c + \frac{1}{2}(h - cl - 3n) + 3s + \frac{5}{2}p + \frac{1}{2}na - o\right]}{\text{mol. wt.}}$$

(B) This calculation implies that C is mineralised to CO_2, H to H_2O, P to P_2O_5 and Na to Na_2O. Halogen is eliminated as hydrogen halide and nitrogen as ammonia.

Example: Glucose $C_6H_{12}O_6$, mol. wt. = 180

$$TOD = \frac{16\left(2\cdot 6 + \frac{1}{2}\cdot 12 - 6\right)}{180} = 1.07\ \text{mg } O_2\text{/mg active substance}$$

(C) Molecular weights of salts other than those of the alkali metals are calculated on the assumption that the salts have been hydrolyzed.

(D) Sulfur is schematically oxidized to the state of +6. Example: Alkylbenzenesulfonate, LAS,$C_{18}H_{29}SO_3Na$, mol. wt. = 348

$$TOD = \frac{16\left(36 + \frac{29}{2} + 3 + \frac{1}{2} - 3\right)}{348} = 2.34\ \text{mg } O_2\text{/mg active substance}$$

(E) In the case of nitrogen-containing compounds the nitrogen may be eliminated as ammonia, nitrite, or nitrate corresponding to different theoretical oxygen demands:

$$TOD_{NO_2} = \frac{16\left[2c + \frac{1}{2}(h - cl) + 3s + \frac{3}{2}n + \frac{5}{2}p + \frac{1}{2}na - o\right]}{\text{mol. wt.}}$$

$$TOD_{NO_3} = \frac{16\left[2c + \frac{1}{2}(h - cl) + 3s + \frac{5}{2}n + \frac{5}{2}p + \frac{1}{2}na - o\right]}{\text{mol. wt.}}$$

(F) Suppose full nitrate formation would have been observed by analysis in case of a secondary amine:

$(C_{12}H_{25})_2$ NH; mol. wt. = 353

$$TOD_{NO_3} = \frac{16\left(48 + \frac{51}{2} + \frac{5}{2}\right)}{353} = 3.44\ \text{mg } O_2\text{/mg active substance}$$

(ii) *Calculation of results.* For the calculation of the percentage biodegradability after n days the determined oxygen depletions are divided by the concentration (W/V) of active substance.

Then—

$$\text{Percent biodegradability} = \frac{\text{mgO}_2/\text{mgAS}}{\text{TOD}} \times 100$$

or

$$\text{Percent biodegradability} = \frac{\text{mgO}_2/\text{mgAS}}{\text{mgCOD}/\text{mgAS}} \times 100$$

(2) *Test report.* The raw data may be listed in and evaluated according to the following form sheet:

(3) *Interpretation of results.* See paragraph (a) of this section.

Sample Form Sheet for the Closed Bottle Test

Exp. No.......... ______
Date of start of test.......... ______
Test/standard material.......... ______
Theoretical test concentration. mgAS/l.
Inoculum.......... ______

Sample Form Sheet for the Closed Bottle Test—Continued

Analysis: (Winkler method or oxygen electrode). ______
TOD or COD of test material... mgO_2/mgAS.
Temp. of the dilution water after aeration. K (O_C).
O_2-conc. of the distilled (deionised) water after aeration and standing before start of test. mg O_2/l.

PART I: O_2 Determinations:

Culture medium	Flask No.	Analyses	mgO₂/l after x days			
			0	5	15	28
Mineral nutrient solution *without* test material and *without* inoculum.	O_2	c_1				
	Control	c_2				
	Mean	$m_o = \frac{c_1+c_2}{2}$				
Mineral nutrient solution *without* test material but *with* inoculum.	1	c_3				
	2	c_4				
	Mean blank	$m_b = \frac{c_3+c_4}{2}$				
Mineral nutrient solution *with* test material and *with* inoculum.	1	a_1				
	2	a_2				
	Mean test material	$m_t = \frac{a_1+a_2}{2}$				

PART II: O_2 Depletions (mg BOD/l) after *x* days:

$$BOD_x = (m_0 - m_{tx}) - (m_0 - m_{bx})$$

(This difference is important as a check for the validity of the test.)

mg BOD/l after x days		
5	15	28

PART III: Evaluation:

$$\text{Percent biodegradability (I)} = \frac{\text{mg BOD}_x/\text{l}}{\text{mg AS/l.TOD}} \cdot 100$$

or

$$\text{Percent biodegradability (II)} = \frac{\text{mg BOD}_x/\text{l}}{\text{mg AS/l.COD}} \cdot 100$$

	After x days		
	5	15	28
(I)			
(II)			

(d) *Literature references.* For additional background information on this test guideline the following references should be consulted:

(1) "Methode H5," *Deutsche Einheitsverfahren zur Wasseruntersuchung,* (Verlag Chemie: Weinheim, 1960).

(2) "Methode H23," *Deutsche Einheitsverfahren zur Wasseruntersuchung,* (Verlag Chemie: Weinheim, 1960).

(3) "Methode G2," *Deutsche Einheitsverfahren zur Wasseruntersuchung,* (Verlag Chemie: Weinheim, 1960).

(4) Wickbold, R. *Wasser,* 33: 229, (1966).

(5) Wickbold, R. *Tenside Detergents* 8: 61 (1971).

(6) "Oxygen Demand," *Standard Methods for the Examination of Water and Wastewater,* 12 Ed. American Public Health Association, American Water Works Association Water Pollution Control Federation, (1965) p. 510.

(7) Fisher, W.K., Fette, Seifen, *Anstrichmittel,* 65: 37–32 (1963).

(8) Fischer, W.K., Gerike, P. and Schmid, R., *Wasser- und Abwasserforschung* 7: 99–118 (1974).

(9) Gerike, P. and Fischer, W.K., *Ecotoxicology & Environmental Safety,* Vol. *3,* No. 2, 159–173 (1979).

§ 796.3220 Ready biodegradability: Modified MITI test (I).

(a) *Introductory information*—(1) *Prerequisites.* (i) An analytical method must be available for determining the concentration of the test material in the test solution.

(ii) The empirical formula of the test material is required so that the theoretical oxygen demand (TOD) may be calculated.

(2) *Guidance information.* (i) Information on the relative proportions of the major components of the test material will be useful in interpreting the results obtained, particularly in those cases where the result lies close to the "pass level."

(ii) Information on the toxicity of the chemical may be useful to the interpretation of low results and in the selection of appropriate test concentrations.

(3) *Interpretation of results.* Because of the stringency of this test, a result of less than 60 percent of BOD (biochemical oxygen demand) (70

percent LPC (loss of parent compound)) (within 28 days) does not necessarily mean that the test compound is not biodegradable under environmental conditions, but indicates that more work will be necessary to establish biodegradability. The possibility that nitrogen-containing compounds may affect the results should be considered.

(4) *Qualifying statements.* (i) The method is only applicable to those organic test materials which, at the concentration used in the test:

(A) Have negligible vapor pressure.

(B) Are not inhibitory to bacteria.

(C) Do not reach and react with the CO_2 adsorbant.

(ii) This test has been found suitable by the OECD Expert Group "Degradation/Accumulation" for determining the "ready biodegradability of organic chemicals under aerobic conditions." It has been tested in the OECD Laboratory Intercomparison Test Program (1978–1980).

(5) *Recommendations.* (i) Test chemicals giving a result of greater than 60 percent BOD, 70 percent LPC, (within 28 days) should be regarded as readily biodegradable.This level must be reached within 10 days of biodegradation exceeding 10 percent.

(ii) If the test material is not soluble at the test concentration, special measures, such as the use of ultrasound dispersion, may have to be employed to achieve a good dispersion of the test material.

(6) *Standard documents.* This Test guideline was based upon the order prescribing the items of test, relating to new chemical substances of the Chemical Substance Control Law (Japanese Law No. 117, 1973). Order of the Japanese Prime Minister, the Minister of Health and Welfare, and the Minister of International Trade and Industry, No. 1 (July 13, 1974).

(b) *Method*—(1) *Introduction, purpose, scope, relevance, application and limits of test.* (i)(A) Purpose: Measurement of biochemical oxygen demand and the analysis of residual chemicals, intermediates necessary in the evaluation of the biodegradability of chemical substances.

(B) Appropriate chemicals: non-volatile and water soluble (more than 100 ppm) organic chemicals.

(C) Applicable chemicals: slightly water soluble chemicals; volatile chemicals (modified BOD meter should be used) ($C_{water}/C_{air}>1$ (C=concentration))

(D) Non-applicable chemicals: volatile chemical substances ($C_{water}/C_{air}<1$).

(ii) Definitions and units—(A) *Definitions. (1)*

$$\text{(}i\text{) Percentage degradation} = \frac{\text{BOD–B}}{\text{TOD}} \times 100\%$$

or

$$\text{(}ii\text{) Percentage degradation} = \frac{\text{Sb-Sa}}{\text{Sb}} \times 100(\%)$$

(2) BOD: Biochemical oxygen demand (experimental) (mg) of the test compound measured on the BOD curve.

(3) B: Oxygen consumption (experimental) (mg) of basal culture medium to which the inoculum is added measured on the BOD curve.

(4) TOD: Theoretical oxygen demand (theoretical) (mg) required when the test compound is completely oxidised.

(5) Sa: Residual amount (experimental) (mg) of the test compound after completion of the biodegradability test.

(6) Sb: Residual amount (experimental) (mg) of the test compound in the blank test with water to which only the test compound has been added.

(B) [Reserved]

(iii) *Reference substances.* In some cases when investigating a new substance reference substances may be useful; however, specific reference substances cannot yet be recommended. In order to check the activity of the inoculum, the use of control substances is desirable. Aniline, sodium acetate or sodium benzoate may be used for this purpose.

(A) *Aniline.* If the percentage of degradation of aniline calculated from the oxygen consumption does not exceed 40 percent after 7 days or 65 percent after 14 days, the test is regarded as invalid.

(B) [Reserved]

(iv) *Principle of the test method.* This test method is based on the following conditions:

(A) Test chemicals as sole organic carbon sources, and

(B) No adaptation of micro-organisms to test chemicals.

An automated closed system oxygen consumption measuring apparatus (BOD meter) is used. Chemicals to be tested are inoculated with micro-organisms in the testing vessels. During the test period, biochemical oxygen demand is measured continuously by the BOD meter. Biodegradability is calculated on the basis of BOD and supplemental chemical analysis is undertaken, such as measuring dissolved organic carbon concentration, concentration of residual chemicals, etc.

(v) *Quality criteria*—(A) *Reproducibility.* Generally good, especially so for chemicals of water solubility greater than 0.1 g/l.

(B) *Sensitivity.* (1) Oxygen consumption: Detection limit=1 mg (oxygen consumption by micro-organisms)

(2) Chemical analysis: Depends on the sensitivity of analytical methods.

(C) *Specificity.* Applicable to every kind of chemical, for which $C_{water}/C_{air}>1$. For volatile chemicals a "modified BOD meter," composed of capillary tubing and normal BOD meter, should be used under paragraph (b)(3) of this section.

(D) *Possibility of automation.* By using a BOD meter under paragraph (b)(3) of this section oxygen consumption by micro-organisms (in a closed system) is recorded automatically.

(2) *Description of the test procedure*—(i) *Preparations*—(A) *Apparatus.* BOD meter equipped with 6 bottles (300 ml each):

(1) Bottles 1 and 2: deionized water (Distilled water must never contain more than 10 percent of organic carbon introduced by the test substance), 300 ml+test chemical, 30 mg.

(2) Bottles 3 and 4: basal culture medium, 300 ml+activated sludge, 9 mg (dry base)+test chemical, 30 mg.

(3) Bottle 5: basal culture medium, 300 ml+activated sludge, 9 mg (dry base)+aniline, 30 mg.

(4) Bottle 6: basal culture medium, 300 ml+activated sludge, 9 mg (dry base).

(B) *Pretreatment of test chemical.* (1) Where the test compound is not soluble in water up to the test concentration, the test compound pulverized as finely as possible is employed.

(2) Where the test compound is volatile, test chemicals should be well cooled to prevent evaporation.

(*3*) If necessary, identification of the test sample should be undertaken.

(C) *Basal culture medium.* To each 3 ml of solution A, solution B, solution C and solution D, water is added to make up to 1000 ml. (Deionized water is used throughout.)

(*1*) *Solution A.* 21.75 g of dipotassium hydrogen phosphate, 8.5 g of potassium acid phosphate, 44.6 g of dibasic sodium phosphate dodecahydrate and 1.7 g of ammonium chloride are dissolved in water and the volume is made up to 1000 ml. (The pH of the solution is 7.2.)

(*2*) *Solution B.* 22.5 g of magnesium sulfate heptahydrate is dissolved in water and the volume is made up to 1000 ml.

(*3*) *Solution C.* 27.5 g of calcium chloride is dissolved in water and the volume is made up to 1000 ml.

(*4*) *Solution D.* 0.25 g of ferric chloride hexahydrate is dissolved in water and the volume is made up to 1000 ml.

(D) *Activated sludge*—(*1*) *Sludge sampling sites.* Sludge sampling is made, in principle, at not less than 10 places throughout the country, chiefly in those areas where a variety of chemical substances may be considered to be consumed and discarded. For example, standard activated sludge of the Japanese Chemical Biotesting Center is taken up from the following places and mixed:

(*i*) *City sewage plant.* 3 plants located in the northern, central and southern part of Japan.

(*ii*) *Industry sewage plant.* One plant used for the waste water treatment of chemical industries.

(*iii*) *River.* 3 rivers located in the northern, central and southern parts of Japan.

(*iv*) *Lake.* One lake located in the middle of Japan.

(*v*) *Sea.* 2 inland seas of Japan.

(*2*) *Frequency of sludge sampling.* Sludge sampling should be made, in principle, four times a year, in March, June, September and December.

(*3*) *Sludge sampling methods.* (*i*) City sewage: 1 liter of return sludge at a sewage disposal plant.

(*ii*) Rivers, lakes and marshes or sea: 1 liter of surface water and 1 liter of surface soil on the beach which is in contact with atmosphere.

(*4*) *Preparation.* The sludge samples collected from the sampling sites are mixed by stirring in a single container, and the mixture is allowed to stand. The floating foreign matters are removed, and the supernatant is filtered with No. 2 filter paper. The filtrate is adjusted to pH 7.0±1.0 with sodium hydroxide or phosphoric acid, transferred into a culture tank and aerated.

(*5*) *Culture.* Thirty minutes after stopping the aeration of the solution obtained above, approximately ⅓ of the whole volume of the supernatant is removed. An equal volume of 0.1 percent synthetic sewage (0.1 percent synthetic sewage: 1 g of glucose, 1 g of peptones and 1 g of monopotassium phosphate are dissolved in 1 liter of water and the solution is adjusted to pH 7.0±1.0 with sodium hydroxide) is added to the remaining portion of the supernatant and the mixture is aerated again. This procedure is repeated once every day. The culturing is carried out at 25±2 °C.

(*6*) *Control.* For the control of the culturing step, the following items are checked and necessary adjustments are made.

(*i*) Appearance of supernatant: The supernatant of active sludge should be clear.

(*ii*) Precipitability of active sludge: The active sludge, being in large flocks, must have high precipitability.

(*iii*) State of formation of active sludge: In the case where growth of flocks is not observed, either the volume of 0.1 percent synthetic sewage to be added or the frequency of addition of synthetic sewage is increased.

(*iv*) The pH of the supernatant is 7.0±1.0.

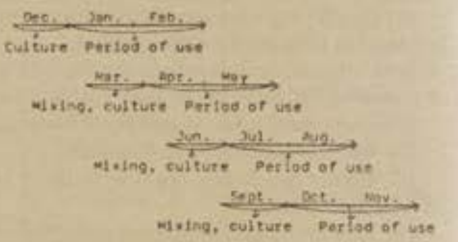
(*v*) Temperature: The temperature for cultivation of active sludge is 25±2 °C.

(*vi*) Amount of aeration: In replacing the supernatant with the synthetic sewage, the solution in the culturing tank must be sufficiently aerated to maintain the dissolved oxygen concentration of the solution above 5 ppm.

(*vii*) Microflora of activated sludge: When the active sludge is microscopically observed (at 100~400× magnification), a number of protozoa of different species together with cloudy flocks must be seen.

(*viii*) Mixing of fresh and old activated sludge: In order to maintain fresh and old activated sludges at the same activity, the filtrate of the supernatant of an activated sludge in use in the test is mixed with an equal volume of the filtrate of the supernatant of an activated sludge freshly collected and the mixture is cultured.

(*ix*) Checking of activity of activated sludge: Activity of activated sludge should be checked periodically, at least once every three months, with standard substances by applying the test method provided below. Where fresh and old activated sludge samples are mixed, especially, careful checking must be done in relation to the old activated sludge. Examples of preparation of activated sludge samples and period of use:



(The same pattern of preparation and use follows.)

(E) *Addition of test compound and preparation for test.* The following test vessels are provided and adjusted to the test temperature.

(*1*) A test vessel containing the basal culture medium, to which is added 100 ppm (W/V) of test compound: the pH of this solution is adjusted to 7 before the inoculation of active sludge, if necessary.

(*2*) A test vessel for the control blank test, containing only the basal culture medium.

(*3*) A test vessel containing water to which is added 100 pm (W/V) of the test compound.

(*4*) A test vessel containing basal culture medium to which is added 100 ppm (W/V) of aniline or any other of the control substances.

(F) *Inoculation of active sludge.* Inoculum is added to the test vessels 1, 2 and 4 above so that the suspended matter defined in the Japanese Industrial Standards described under paragraph (b)(4) of this section is contained at a concentration of 30 ppm (V/V).

(ii) *Test conditions.* (A) Concentration of test chemicals: 100 ppm (W/V).

(B) Concentration of activated sludge: 30 ppm (W/V).

(C) Test temperature: 25 °C ±2 °C.

(D) Period: 14 days.

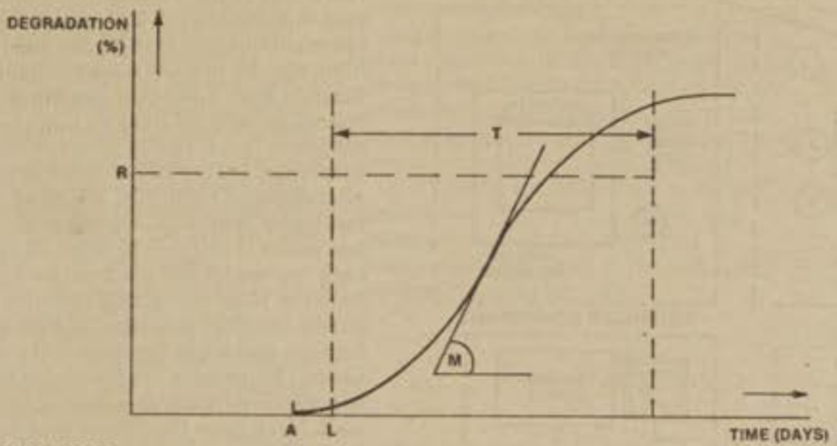
(E) Perform in darkness. Every day, the temperature, the change in color of the culturing vessel should be checked.

(F) Stir vigorously with mechanical stirrer.

(iii) *Performance of the test.* (A) The BOD curve is obtained continuously and automatically for 14 days.

(B) After the 14-day testing period, pH, residual chemicals, and intermediates in the testing vessels are analysed.

Figure 1—Degradation curve of a readily degradable compound.



A = ADAPTION PERIOD
L = PERIOD OF LOGARITHMIC GROWTH
M = MAXIMUM DEGRADATION RATE
R = REQUIRED LEVEL OF DEGRADATION
T = TIME WINDOW

The test chemicals in the testing vessel without activated sludge are also analyzed in order to confirm whether there is any change in the test chemical during the testing period or any loss of the original test chemical by evaporation or adsorption by the walls of test vessels, etc.

(iv) *Analytical means.* (A) If the test compound is soluble in water, the residual amount of total organic carbon is also determined.

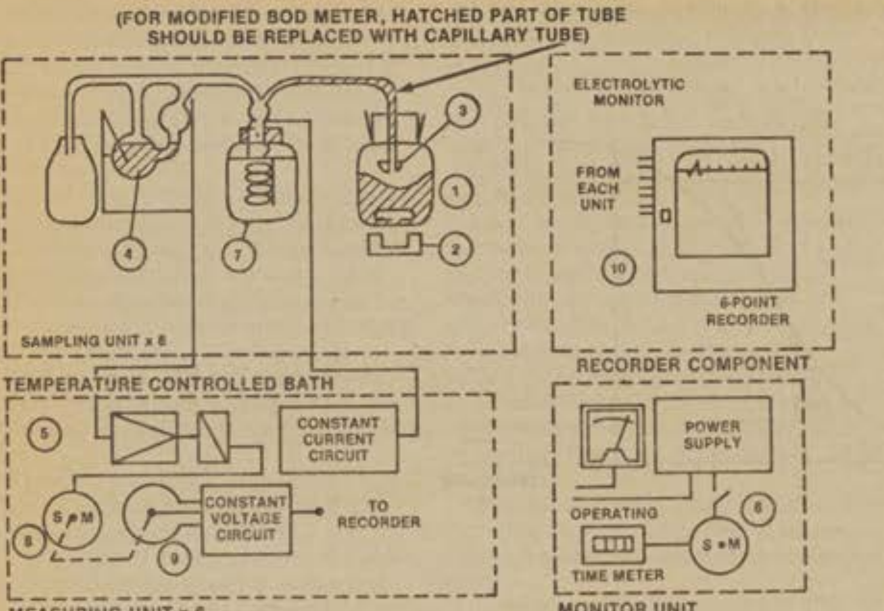
(*1*) Where a total organic carbon analyzer is used: 10 ml of the tested solution is sampled from the test vessel and centrifuged at 3000 g for five minutes. The residual amount of the total organic carbon in the supernatant is determined on a total organic carbon analyzer.

(*2*) Where other analyzers are used: The total content of a test vessel is extracted with a suitable solvent for the test compound, and, after proper pretreatment such as concentration, the residual amount of the test compound is determined on an analyzing instrument (gas chromatography, absorptiometry, mass spectrometry, atomic absorption spectrophotometry, etc.).

(B) For volatile chemicals, the temperature control bath of the BOD meter should be cooled to 10 °C and this temperature held for at least 30 min, in order to prevent evaporation. The analytical procedures (a) and (b) should then be started.

(3) *Principle of closed system oxygen consumption measuring apparatus.* (i) The coulometer is an instrument for measurement of the oxygen consumption of micro-organisms using electrochemical analysis process (coulometry).

(ii) The following is a block diagram. The BOD meter is produced by Ohkura Electric Co., Ltd., 1-11-16, Shibuya, Shibuya-ku, Tokyo, Japan. (For volatile chemicals, a capillary tube should be installed between each testing vessel and electrolytic bottle.) (For modified BOD meter, hatched part of tube should be replaced with capillary tube.)



THE SPECIMEN CONTAINED IN CULTIVATING BOTTLE (1) IS STIRRED BY MEANS OF A MAGNETIC STIRRER (2). WHEN THE REACTION PROGRESSES, THE DISSOLVED OXYGEN IN LIQUID WILL BE CONSUMED. OXYGEN (O_2) IN THE SPACE IN THE CULTIVATING BOTTLE IS DISSOLVED IN LIQUID, RESULTING IN GENERATION OF CO_2 IN ITS PLACE.

(A) The specimen contained in cultivating bottle (1) is stirred by means of a magnetic stirrer (2). When the reaction progresses, the dissolved oxygen in liquid will be consumed. Oxygen (O_2) in the space in the cultivating bottle is dissolved in liquid, resulting in generation of CO_2 in its place.

(B) As this CO_2 is absorbed by soda lime (3), the partial pressure of oxygen in the space and the total pressure decrease.

(C) The drop in pressure is detected and converted into an electric signal by means of an electrode type manometer (4) and is amplified by an amplifier (5) for operating a relay circuit (6), resulting in operation of a synchronous motor (8). Simultaneously, by constant current, electrolytic oxygen is generated from sulfuric acid copper solution contained in an electrolytic bottle (7).

(D) This oxygen is supplied to the cultivating bottle and restoration of pressure is detected by means of the manometer, resulting in switching off of the relay circuit and stopping the electrolytic and synchronous motor.

(E) The upper space in the cultivating bottle is always kept under a constant pressure of oxygen and the quantity of oxygen consumed in the cultivating bottle is proportional to the quantity of electrolytic oxygen. As this quantity of electrolytic oxygen is proportional to electrolytic time, there is a constant electrolysis current. Accordingly, the revolution angle of a synchronous motor (9) is converted to an mV signal by means of the interlocking potentiometer, resulting in an indicator quantity of consumed oxygen at the recorder (10).

(4) *Suspended matter.* (i) (From Japanese Industrial Standards K0102–10.2.)

(A) Suspended matter is that material which can be separated by filtration or by means of a centrifugal separator. It can be determined by any of the methods described below. When the test water is difficult to filter the centrifugal separation method should be applied; when the test water contains an extremely large quantity of suspended matter, the Büchner funnel method should be used.

(B) Test water is taken from the waste water passed through a 2 mm mesh sieve. At least 5 mg of the filtrate are necessary for the determination.

(ii) *Filtration through filter paper*—(A) *Sintered glass filter method*—(*1*) *Apparatus*—(*i*) *Sintered glass filter.* A crucible-type sintered glass filter IG2 or a Büchner funnel-type sintered glass filter 3G2.

(*ii*) [Reserved]

(*2*) *Procedure.* Prepare two sintered glass filters of the same type and of approximately the same weight; lay six sheets of filter paper in them and pour water through several times so that they adhere by suction. Then transfer the filters to an air oven and dry them for two hours at 105–110 °C. Allow them to cool in a desiccator, and weigh. (When a chemical balance is used, the lighter filter may be used as a supplementary weight.) Pour a suitable amount of the test water into the heavier filter (take sufficient test water to give a weight of suspended matter of not more than 5 mg after drying. Ordinarily, 200 ml of the test water is enough. However, if the test water is difficult to filter, 10 ml from each test water sample must be added from the 10 ml measuring cylinder during the filter process.), filter it by suction, and wash the wall of the filter several times with the filtrate, in order to remove substances adhering to the wall. Next, pour the filtrate into the lighter filter several times and filter it by suction. Dry the two filters in the air oven for two hours at 105–110 °C, and allow them to cool in a desiccator. Weigh each filter (when a chemical balance is used, the lighter filter may be used as a supplementary weight), obtain the difference in weight before and after the filtration, and calculate the quantity of the suspended matter in ppm according to the following formula:

$$S = (a - b) \times \frac{1000}{V}$$

where

S: Suspended matter (ppm)
a: Difference in weight before and after the filtration of test water (mg)
b: Difference in weight before and after the filtration of the filtrate (mg) (when a chemical balance is used, $b = 0$)
V: Amount of test water (ml)

(*3*) *Remarks.* (*i*) To determine the ignition loss of volatile suspended matter, a test should be carried out in accordance with the Glass Fiber Paper Method under paragraph (b)(4)(ii)(A)(*3*)(*iii*) of this section, or, after washing the suspended matter together with filter paper into a crucible or an evaporating dish, dry and ignite in muffle furnace.

(*ii*) When the soluble evaporated residue is less than 5000 ppm, correction (for difference in weight of the filtrate before and after filtration) may be omitted. However, when a chemical balance is used, the lighter filter should be used as a supplemental weight so the filtration of the filtrate can be carried out at the same time. Even when a direct reading balance is used the weight varies with the hygroscopic properties of substances contained in the test water, and with other conditions, so it is desirable that a correction be performed by obtaining the blank test value of the filter through which the filtrate is passed. In the case of the test water containing fats and oils, grease, wax, etc., a portion of these substances should be determined as the suspended matter. When the determination of the suspended matter exclusive of oils and fats is required, pour 10 ml volumes of n-hexane several times through the filter which has been dried and weighed after filtration and wash out the fats and oils. Then dry the filter and weigh.

(*iii*) Glass Fiber Paper Method (GFP Method): Fix an appropriate GFP (Whatman GF/B or equivalent) of known weight, which has been dried at 105 to 110 °C for 2 hours after washing, on a suitable supporting plate. Add the amount of test water to give a weight of the suspended matter after drying of over 5 mg. After filtration by suction, return a portion of the filtrate to the container holding the original test water. Wash down the suspended matter adhering to the walls of container and filter again on GFP by suction. Repeat this operation several times. Detach GFP from the filter and transfer it onto a water glass. Then operate as described for the Büchner funnel method under paragraph (b)(4)(ii)(B) of this section, and determine ppm of the suspended matter. After determination of the suspended matter determine the ignition residue of the suspended matter, if necessary, according to the operation described in the section on Filtration through asbestos layer, under paragraph (b)(4)(iii) of this section.

(B) *Büchner funnel method.* This method is applicable to samples containing a large quantity of suspended matter such as sludge.

(*1*) *Apparatus*—(*i*) *Perforated plate.* Stainless steel (SUS 27 or 28), approximately 0.5 mm in thickness, 50 mm or 90 mm in diameter. It is shaped like a watchglass with a slightly bent edge. Small holes about 0.5 mm in diameter are bored at suitable intervals all over its flat surface.

(*ii*) *Rubber packing.* A rubber ring 2 to 3 mm in thickness, 10 mm to 90 mm in diameter and about 10 mm in width, can be put in a Büchner funnel and can be used for filtration by suction, with the perforated plate on it.

(*iii*) *Büchner funnel.* 50 mm or 90 mm.

(*2*) *Testing procedure.* (*i*) Prepare two perforated plates. Put rubber packing in Büchner funnel and place the perforated plate on it. Position the filter paper (grade 6), pour water on the filter paper several times and suck. Remove the filter paper with the perforated plates and dry at a temperature of 105 to 110 °C for 2 to 3 hours. Allow to cool in a desiccator and weigh to constant weight. (When chemical balance is used, the lighter perforated plate is used as supplemental weight.)

(*ii*) Next put the heavier perforated plate together with the filter paper in the funnel and filter 200 to 400 ml of the test water by suction. Pour the filtrate into the lighter plate with filter paper several times and continue as for first plate.

(*iii*) Obtain the difference in weight before and after this operation, and calculate ppm of the suspended substances contained in the test water by the following formula:

$$S=(a-b)\times\frac{1000}{V}$$

where:

S: Suspended substances (ppm)

a: Difference in weight before and after filtration of the test water (mg)

b: Difference in the weight before and after filtration of the filtrate (mg) (when chemical balance is used b=0)

V: Test water (ml)

See also Remarks under paragraph (b)(4)(ii)(A)(*3*) of this section.

(iii) *Filtration through asbestos layer*—(A) *Apparatus.* Gooch crucible, 25 to 35 ml.

(B) *Reagents.* Suspension of asbestos: add water to 15 g asbestos and, after removing fine portion by decantation several times, add water to make 1 liter.

(C) *Procedure.* (*1*) Prepare two Gooch crucibles (same shape and approximately same weight). After drying, pour about 20 ml of the well-stirred asbestos suspension to obtain a layer of asbestos about 3 mm thick (about 0.3 g) (when half the amount of asbestos solution is poured out put in the perforated plate and pour the other half of the solution) and suck gently.

(*2*) Next put the Gooch crucibles into the air oven. After drying for two hours at a temperature of 105 to 110 °C, allow to cool to constant weight in the desiccator and measure the weight of each crucible (when chemical balance is used, the lighter crucible is used as the supplemental weight). Attach the heavier crucible to the suction bottle and pour in enough test water to give a weight of suspended matter of more than 5 mg after drying and gently filter by suction. At this time, repeat the filtration of the initial portion of the filtrate.

(*3*) Next pour a small amount of filtrate into the lighter crucible several times using suction, then dry in the air oven for two hours at 105 to 110 °C, and allow to cool in a desiccator. Weigh the crucible and obtain the difference in weight using the crucible as a supplemental weight and calculate ppm of the suspended matter by the following formula:

$$S=(a-b)\times\frac{1{,}000}{V}$$

(See paragraph (b)(4)(ii)(B)(*iii*) of this section for clarification of symbols.)

(D) *Remarks.* The test water should be sampled as specified in glass filter method. When the soluble volatile residue is less than 5000 ppm, refer to remark 2 in the same section.

(iv) *Centrifugation method* (A) This method is applicable to samples which are very difficult to filter due to their content of suspended matter.

(*1*) *Apparatus.* Centrifugal separator about 2000 rpm. Precipitation tube 50 to 100 ml.

(*2*) *Procedure.* (*i*) Pour into the precipitation tube enough test water to give more than 5 mg suspended matter.

(*ii*) After weighing each tube, centrifuge at about 2000 rpm for 20 minutes and precipitate the suspended matter in the test water. Remove the supernatant liquid by decantation. (When the determination of soluble evaporated residue is to be performed, keep the supernatant liquid).

(*iii*) Add 10 ml of the water to the precipitate, centrifuge again and remove the supernatant liquid by decantation.

(*iv*) Transfer the precipitate into an evaporating dish which has been previously heated to constant weight at 105 to 110 °C, and evaporate to dryness on the steam bath. After drying in the dryer at 105 to 110 °C, for 2 hours, allow to cool in a desiccator and weigh. (When a chemical balance is used, an evaporating dish of the same shape should be used as a supplemental weight after the blank test for it has been performed.) Obtain the difference in weight before and after this operation. Calculate ppm of the suspended matter by the following formula:

$$S=a\times\frac{1000}{V}$$

(See paragraph (b)(4)(ii)(B)(*2*) of this section for clarification of symbols.)

(*3*) *Remarks.* There should be a certain degree of difference in density between the dispersed phase and the dispersion medium to make centrifugal separation possible. When a particle of 1 mg is centrifuged at an angular velocity of w rad/sec at a position of r cm from the center of rotation, the centrifugal force which a particle receives is as follows:

(*i*) Supposing that the mass of the dispersion medium expelled by a particle is 1 mg

then $F = (m - m')\,w^2 r$

(*ii*) Supposing that the specific centrifugal force is RCF and rotational frequency per minute is N (rpm)

$$\text{then} \qquad RCF = \frac{F}{(m - m')g} = \frac{w^2 r}{g} = 0.00001118\,rN^2$$

(*iii*) From the equation under paragraph (b)(4)(iv)(A)(*3*) of this section, it is clear that the centrifugal force near the surface differs from that at the bottom portion of the liquid. For instance, when N=2000 rpm and the distance between the surface of the liquid in the precipitation tube and the center of rotation is 5 cm (r=5 cm), RCF is 223 g; when the distance between the bottom of the precipitation tube and the central axis of rotation is 13 cm, RCF becomes 581 g. Therefore, the RCF value near the surface and that at the bottom should both be reported.

$$\text{Depth of the liquid layer} = \frac{(\text{RCF at the bottom}') - (\text{RCF at the surface})}{(\text{RCF at the bottom})} \times (\text{distance from the bottom})$$

In this test, a centrifugal separator whose bottom is 13 cm from the central rotation axis at a rotational frequency of 2000 rpm is regarded as standard.

(B) Calculation of suspended matter from the difference in weight of evaporated residue: Calculate the suspended matter from the difference between the total evaporated residue and the soluble evaporated residue.

$A = B - C$

where:

A = suspended matter (ppm)
B = total evaporated residue (ppm)
C = soluble evaporated residue (ppm)

(5) *Suspended matter formed at pH 7.* (From Japanese Industrial Standards K0102 − 10.3)

For suspended matter formed when the test water is neutralized to pH 7±0.5.

(i) *Reagents.* (A) NaOH (sodium hydroxide) solution (4 to 24 w/v %)

(B) Acetic acid, diluted 1:2 to 1:16, acid:water.

(ii) *Procedure.* Place enough test water to give more than 5 mg suspended matter in a beaker and neutralize it with sodium hydroxide solution or with diluted acetic acid, according to the acidity/alkalinity of the test water, taking care to minimize the increase in the volume of the solution during neutralization. Then proceed according to procedures in the methods above to obtain amount of suspended matter at pH 7 and calculate ppm of the suspended matter formed at pH 7 by the following formula.

$A = B - C$ (See paragraph (b)(4)(iv)(B) of this section for clarification of symbols.)

(iii) *Remarks.* (A) Depending on the kind of waste water, the weight of suspended matter may decrease when it is neutralized. In such cases, the weight of suspended matter should be reported as suspended matter formed at pH 7.

(B) Suspended matter formed at pH 7 may be determined with the supernatant liquid or filtrate after removing the suspended matter. This method is applicable to test water which contains a relatively small amount of suspended matter but which forms a large amount of precipitate after neutralization (without changing the first suspended matter) or to waste water which forms a relatively small amount of precipitate. The method should not be applied to waste water which is apt to cause the formation of a complex precipitate or a dissolution reaction by neutralization.

(c) *Data and reporting*—(1) *Treatment of results.* (i) Method for calculating the percentage degradation from the oxygen consumption.

$$\text{Percentage degradation} = \frac{\text{BOD-B}}{\text{TOD}} \times 100(\%)$$

(See Definitions and units, under paragraph (b)(1)(ii) of this section for clarification of symbols.)

(ii) Method for calculating the percentage degradation from the result of direct analysis.

$$\text{Percentage degradation} = \frac{Sb - Sa}{Sb} \times 100(\%)$$

See Definitions and units, under paragraph (b)(1)(ii) of this section for clarification of symbols.

(2) *Evaluation of results.* The following calculations are to be made:

(i) Calculation of theoretical oxygen demand.

Element	Oxidised form
C	CO_2
H	H_2O
N	NO_2
S	SO_2
X(halogen)	X

(ii) Recovery rate of analytical procedure.

(3) *Test report.* The test report should include the following points:

(i) *Information on the test chemicals.* Name, structural formula, molecular weight, purity, kind of impurities, physical chemical properties of test chemical, identification data of test chemical.

(ii) *Test conditions.* (A) Activated sludge: sludge sampling site and concentration.

(B) Test chemical: concentration.

(C) Test period.

(D) Test temperature.

(iii) *Analytical procedure.*

(A) Pretreatment.

(B) Analytical conditions of instrument.

(C) Recovery rate of analysis.

(D) Identification of intermediate.

(iv) *Results.* (A) BOD curves and instrument name.

(*1*) BOD (mg).

(*2*) B (mg).

(*3*) Sa (mg).

(*4*) Sb (mg).

(*5*) TOD (mg).

(B) Percentage of degradation by BOD.

(C) Percentage of degradation by chemical analysis.

(D) Chromatograms or spectra of test chemicals obtained and used for the purpose of analysis.

(v) *Remarks.*

(4) *Interpretation of results.* (i) For the purpose of comparison with reference substances, the biodegradability of the test compound is categorised based on the relative degree of degradability compared to that of aniline.

(ii) If the percentage of degradation of aniline calculated from the oxygen consumption does not exceed 40 percent after 7 days or 65 percent after 14 days, the test is regarded as invalid.

(iii) If the recovery rate of Sb is found to be in the order of 10 percent or less, the test is also regarded as invalid.

See also Section 1.

(d) *Literature references.* For additional background information on this test guideline the following references should be consulted:

(1) *Biodegradability and Bioaccumulation Test of Chemical Substances* (C–5/98/JAP, 1978).

(2) The Chemical Substances Control Law in Japan, Chemical Products Safety Division, Basic Industries Bureau, MITI, (C–2/78/JAP, 1978).

(3) *The Biodegradability and Bioaccumulation of New and Existing Chemical Substances,* 5:8 (C–3/78/JAP, 1978).

§ 796.3240 Ready biodegradability: Modified OECD screening test.

(a) *Introductory information*—(1) *Prerequisites.* (i) Water solubility.

(ii) The organic carbon content of the test material must be established.

(2) *Guidance information.* (i) Information on the relative proportions of the major components of the test material will be useful in interpreting the results obtained, particularly in those cases where the result lies close to the "pass level."

(ii) Information on the toxicity of the chemical may be useful to the interpretation of low results and in the selection of appropriate test concentrations.

(3) *Interpretation of results.* Because of the stringency of this test a result of less than 70 per cent loss of DOC—dissolved organic carbon—(within 28 days) does not necessarily mean that the test compound is not biodegradable under environmental conditions, but indicates that more work will be necessary to establish biodegradability.

(4) *Qualifying statements.* (i) The method is only applicable to those organic test materials which, at the concentration used in the test,

(A) Are soluble in water (at least 5 to 40 mg dissolved organic carbon/liter).

(B) Have negligible vapour pressure.

(C) Are not inhibitory to bacteria.

(D) Do not significantly adsorb on glass surfaces.

(ii) This test has been found suitable by the OECD Expert Group Degradation/Accumulation for determining the ready biodegradability of organic chemicals under aerobic conditions. It has been tested in the OECD Laboratory Intercomparison Test Program (1978–1980).

(5) *Recommendations.* (i) Test chemicals giving a result of greater than 70 percent loss of DOC (within 28 days) should be regarded as readily biodegradable. This level is reached within 10 days of biodegradation exceeding 10 percent.

(ii) If the limits of sensitivity of organic carbon analysers are improved, the use of lower test concentrations may be an advantage, particularly for toxic compounds.

(6) *Standard documents.* This Test Guideline constitutes a modification of the OECD Screening Test (OECD) Environment Directorate, Proposed Method for the Determination of the Biodegradability of Surfactants Used in Synthetic Detergents, Paris 1976, and council directive of Nov. 22, 1973, on the approximation of the laws of the member states relating to methods of testing the biodegradability of anionic surfactants (73/405/EEC), Official Journal of the European Communities No. 4 347/53 of Dec. 17, 1973) for the application of the dissolved organic carbon (DOC) analysis.

(b) *Method*—(1) *Introduction, purpose, scope, relevance, application and limits of test* (i) The purpose of the method is the measurement of the ultimate biodegradability of water soluble, nonvolatile organic compounds in an aerobic, aqueous medium at a starting test concentration corresponding to 5–40 mg DOC/l (In order to avoid inhibitory effects, it is in the investigator's own interest to choose as low a starting concentration as his analytical capability permits.)

(ii) *Definitions and units.* (A) Definition of biodegradability:

$$D_t = \left[1 - \frac{C^t - C_{blt}}{C^o - C_{blo}}\right] \times 100$$

Where

D_t=degradation in percent DOC-removal at time t

C_o=starting DOC concentration of the culture medium (mg DOC/l)

C_t=DOC concentration of the culture medium at time t (mg DOC/l)

C_{blo}=starting DOC concentration of the blank (mg DOC/l)

C_{blt}=DOC concentration of the blank at time t (mg DOC/l)

(B) Degradation is stated as the percentage DOC-removal within 28 days with respect to the test material (% DOC-removal).

(iii) *Reference substances.* In some cases when investigating a new substance reference substances may be useful; however, specific reference substances cannot yet be recommended. In order to check the activity of the inoculum the use of control substances is desirable. Aniline, sodium acetate or sodium benzoate may be used for this purpose. They must exhibit DOC removal >70 percent within 28 days, otherwise the test is regarded as invalid and should be repeated using an inoculum from a different source.

(iv) *Principle of the test method.* (A) A predetermined amount of compound is dissolved in an inorganic medium (mineral nutrient solution, fortified with a trace element and essential vitamin solution), providing a concentration corresponding to 5–40 mg DOC/l. The solution is inoculated with a small number of micro-organisms from a mixed population and aerated at 20–25 °C in the dark or at least in diffuse light only. The degradation is followed by DOC analysis over a 28-day period. The procedure is checked by means of a standard.

(B) A control with inoculation, but without either test material or standard, is run parallelly for the determination of DOC blanks.

(v) *Quality criteria*—(A) *Reproducibility.* The reproducibility of the method is appropriate for a screening test which has solely an acceptance but no rejective function.

(B) *Sensitivity.* The sensitivity of the method is largely determined by the sensitivity limit of the organic carbon analysis, which is 0.5 mg C/liter at present.

(C) *Specificity.* This method is applicable for the biodegradability evaluation of water soluble, non-volatile organic compounds.

(D) *Possibility of standardization.* The test version with specific analyses for anionic and nonionic surfactants is standardized as the "OECD Screening Test."

(E) *Possibility of automation.* (*1*) Parts of the test, e.g., the analysis, can be automated, although hardly the total procedure.

(*2*) The procedure is, however, well suited for being operated with whole series of test materials.

(2) *Description of the test procedure*—(i) *Preparations*—(A) *Reagents*

(*1*) *Deionised water.*—(*i*) Deionized or distilled water free of toxic substances (copper in particular), for general use as a solvent. Water which has been deionized by distillation or ion exchange is suitable. Distilled water will never contain more than 10 percent of organic carbon introduced by the test material.

(*ii*) The high purity of this test water is necessary in view of the DOC analyses in the concentration range of 0–40 mg/l. Contamination may result from inherent impurities but also from the ion exchange resins and microbial developments (bacteria, algae under the influence of light, etc.). Only one water charge must be used for each test series, which is to be controlled beforehand by DOC analysis. If necessary, suitable water may be obtained by UV irradiation or other means.

(*2*) *Nutrient solution.* Mix 1 ml each of the following solutions (*i*) to (*vi*) and make up to a volume of 1 liter with water, under paragraph (b)(2)(i)(A)(*1*)(*i*) of this section. (A.R. means analytical reagent).

(*i*)

KH_2PO_4	A.R. 8.5 g
K_2HPO_4	A.R. 21.75g
$Na_2HPO_4 \cdot 2H_2O$	A.R. 33.4 g
NH_4Cl	A.R. 20.0 g

In 1000 ml of water under paragraph (b)(2)(i)(A)(*1*)(*i*) of this section the pH value should be 7.2.

(*ii*) 22.5 g of $MgSO_4 \cdot 7H_2O$ A.R. dissolved in 1000 ml of water.

(*iii*) 27.5 g of $CaCl_2$ A.R. dissolved in 1000 ml of water.

(*iv*) 0.25 g of $FeCl_3 \cdot 6H_2O$ A.R. dissolved in 1000 ml of water.

This solution is prepared freshly immediately before use.

(*v*) Trace element solution:

$MnSO_4 \cdot 4\,H_2O$	39.9 mg (30.23 mg $MnSO_4 \cdot H_2O$)
H_3BO_3	57.2 mg
$ZnSO_4 \cdot 7\,H_2O$	42.8 mg
$(NH_4)_6Mo_7O_{24}$	34.7 mg (36.85 mg $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$)

Fe—chelate ($FeCl_3$, EDTA), 100 mg; water under paragraph (b)(2) (i)(A)(*1*)(*i*) of this section, 1000 ml.

Sterilization of the trace element stock solution at 120 °C (393 K), 2 atm., 20 min.

(*vi*) Vitamin solution:

Biotin	0.2 mg.
Nicotinic acid	2.0 mg.
Thiamine	1.0 mg.
p-Aminobenzoic acid	1.0 mg.
Pantothenic acid	1.0 mg.
Pyridoxamine	5.0 mg.
Cyanocobalamine	2.0 mg.
Folic acid	5.0 mg.
Water (above)	100 ml.

The solution is filtered sterile (0.2 μm). Instead of solution (*vi*) under paragraph (b)(2)(i)(A)(*2*) of this section, 15 mg of yeast extract may be used per 100 ml of water under paragraph (b)(2)(i)(A)(*1*)(*i*) of this section.

(*3*) *Control substances.* See Reference substances. Aniline must be freshly distilled.

(B) *Materials.* (*1*) Shaking machine accommodating 2 liters. Erlenmeyer flasks either with automatic temperature control or used in a constant temperature room at 20–25 °C (293–298 K).

(*2*) Narrow neck, 2 liter Erlenmeyer flasks (creased fluted flasks are recommended). The flasks must be carefully cleaned with, e.g., alcoholic hydrochloric acid, before use, rinsed and dried in order to avoid contamination with residues from previous tests. The flasks must also be cleaned before the first use since they may be contaminated.

(*3*) Membrane filtration apparatus.

(*4*) Membrane filters, 0.2 μm.

(*5*) Carbon analyzer.

(C) *Inoculation.* Either of the following three alternatives under paragraph (b)(2)(i)(C) (*1*), (*2*), and (*3*) of this section may be used as inoculum or a composite sample thereof under paragraph (b)(2)(i)(C)(*4*) of this section.

(*1*) *Inoculum from secondary effluent.* The inoculum is gained preferably from a secondary effluent of good quality collected from a treatment plant dealing with a predominantly domestic sewage. The effluent must be kept under aerobic conditions in the period between sampling and use. To prepare the inoculum the sample is filtered through a coarse filter, the first 200 ml being discarded. The filtrate is kept aerobic until used. The inoculum must be used on the day of collection.

(*2*) *Inoculum from soil.* (*i*) 100 g of soil (fertile, not sterile) are suspended in 1000 ml of chlorine-free drinking water (soils with an extremely large content of clay, sand or organic carbon are unsuitable). After stirring the suspension is allowed to settle for 30 minutes.

(*ii*) The supernatant is filtered through coarse filter paper, the first 200 ml being discarded. The filtrate is aerated immediately and continuously until use. The inoculum must be used on the day of collection.

(*3*) *Inoculum from a surface water.* (*i*) An inoculum is drawn from a suitable surface water.

(*ii*) The sample is filtered through a coarse paper, the first 200 ml being discarded. The filtrate is kept aerobic until used. The inoculum must be used on the day of collection.

(*4*) *Composite inoculum.* Equal volumes of the 3 inoculum samples are combined, mixed well, and the final inoculum drawn from this mixture. The suitability of the inoculum is checked by means of a control substance.

(ii) *Procedure.* (A) The test materials are evaluated simultaneously in duplicate together with the biodegradability standard and a control test with inoculation but without either test or standard material for the determination of DOC blanks.

(B) The control material must attain >70 percent DOC removal within 28 days at a starting concentration corresponding to 20 mg DOC/l. If <70 percent DOC removal is not achieved the whole series must be discarded. (This limit is based on present experience with the 19-day version of the test. A revision of this limit or even of the standard might have to be considered after the accumulation of experience with the new 28-day version of the test.)

(C) A stock solution of the test material in water under paragraph (b)(2)(i)(A)(*1*)(*i*) of this section is prepared. Enough stock solution is added to the nutrient solution (above) to achieve a carbon concentration of 5–40 mg DOC/l. The starting concentration of the control substance is, however, 20 mg DOC/l.

(D) Two reaction vessels are each filled with 900 ml of the nutrient solution and inoculated with 0.5 ml/l of the inoculum. The opening of the vessel is covered (e.g., aluminum foil) in such a way that the exchange of air between the flask and the surrounding atmosphere is not unduly impeded. (Cotton wool is unsuited because of the DOC analysis.) The vessels are then inserted in the shaking machine. The temperature of 20–25 °C (293–298 K) must be maintained unchanged during the test, and the vessels should be shielded from light. The air should be free of pollutants and toxic materials (chlorinated solvents, etc.)

(E) In the course of the biodegradation test the DOC concentrations are determined in duplicate at the beginning (day 0), and on the 27th and 28th day. Three additional analyses must be performed at regular time intervals (7th, 14th, and 21st day)

(F) The analyses are registered on the form sheet (below) and evaluated.

(G) Only the necessary volumes of culture medium should be drawn for each determination; however, they must be large enough for the membrane filtration or centrifugation preceding the carbon determination. The latter requires differing volumes for the different instruments. Evaporation losses of the culture medium are to be made up by adding water under paragraph (b)(2)(i)(A)(*1*)(*i*) of this section in the required amounts. The culture medium is to be mixed well before withdrawing a sample. Material adhering to the wall of the vessel must be dissolved or suspended before sampling. The membrane filtration or centrifugation must be done immediately. The filtered or centrifuged samples must be analyzed on the same day, otherwise they must be preserved with 0.05 ml of the $HgCl_2$ solution for each 10 ml of nutrient medium or by storing at 2–4 °C. The biodegradability test is valid provided the standard exhibits a degradation rate within the specified range. The test can be finished before the 28th day if complete mineralization has been accomplished. Where degradation has obviously started on day 28 but did not reach a plateau on day 28, it is considered good practice to extend the experiments for one or two weeks longer.

(H) All steps require great care and cleanliness of the vessels, pipettes, etc. but not sterility.

(iii) *Analytical means*—(A) *Membrane filter*. (*1*) 0.2 µm, 25 mm diameter. Preparation of the filters: membrane filters are impregnated with surfactants for hydrophilization. Thus each filter contains up to several mg of soluble carbon which would interfere in the biodegradability determinations. Therefore, the filters are purified from surfactants and other soluble organic interferences by boiling them in deionized water for three periods each of one hour. These filters may be stored in water (above) for at least one week.

(*2*) Other membrane filters are suitable if it is assured that they neither release carbon nor adsorb the compound in the filtration step.

(*3*) If the samples are centrifuged, this must be done at 40,000 msec^{-2} (4000 g) for 15 minutes, preferably in a refrigerated centrifuge, in any case <40 °C.

(*4*) (Remark: the differentiation TOC: DOC by centrifugation at very low concentrations does not seem to work well since either not all bacteria are removed or carbon as part of the bacterial plasma is redissolved. At higher test concentrations (>10 mg C/l) and the same small inoculation the centrifugation error seems to be comparatively small.)

(B) *The DOC measurement*. (*1*) The sample withdrawn from the culture medium (about 30 ml) is centrifuged or membrane filtered immediately in the filtration apparatus using the membrane filters prepared as stated above. The first 20 ml of the filtrate are discarded.

(*2*) The DOC concentration is determined twice in the remaining filtrate (about 10 ml) by means of the TOC/DOC instrument. If the filtrate cannot be analyzed on the same day it must be preserved as stated above. The DOC measurements (mgC/l) obtained are registered on the attached data sheet and the DOC concentrations of the culture medium and of the blanks calculated for each sampling time.

(c) *Data and reporting*—(1) *Treatment of results*. (i) The degradation at time t is calculated from the determinations of the DOC concentrations at the beginning (C_o) and at time t (C_t) according to—

$$D_t = \left[1 - \frac{C_t - C_{blt}}{C_o - C_{blo}}\right] \times 100$$

where:

D_t = degradation in percent at time t
C_o = measured starting DOC concentration of the inoculated culture medium (mg DOC/l)
C_t = DOC concentration of the culture medium at time t (mg DOC/l)
C_{blo} = starting DOC blank of the mineral nutrient solution with inoculation, but without test material (mg DOC/l)
C_{blt} = DOC blank of the mineral nutrient solution with inoculation, but without test material at time t (mg DOC/l)

(ii) The degradation rates are calculated to the nearest 0.1 percent. The means of the D_t values are calculated and reported to the nearest full percent. Results ending in 0.5 are rounded up to the nearest whole number. The course of the degradation test is presented graphically in a diagram.

(2) *Test report*. The results may be reported on the following data sheet:

BILLING CODE 6560-50-M

SAMPLE FORM SHEET FOR THE MODIFIED OECD SCREENING TEST

Exp. no.: ____________________

Date of start of test: ____________________

Test / Standard material: ____________________

Theoretical test conc.: ____________________ mg DOC/l

Inoculum: ____________________

Carbon analyser: ____________________

PART I: CONTROLS:

	TOC[1]	DOC[2]
	mg/l	
Stock solution of the test material (1000 mg/l, dilution .../1000 ml of nutrient solution)		

PART II: CARBON DETERMINATIONS:

Culture medium	Flask No.	Analyses	Theor. conc. mg/l	DOC - concentrations after x days mg/l					
				0 (C_0)	~7	~14	~21	~27	28
Mineral nutrient solution <u>with</u> test material and <u>with</u> inoculum	1	a_1							
		a_2							
		$m_1 = \frac{a_1 + a_2}{2}$ (C_t)							
	2	b_1							
		b_2							
		$m_2 = \frac{b_1 + b_2}{2}$ (C_t)							
Mineral nutrient solution <u>without</u> test material but <u>with</u> inoculum	Blank	c_1							
		c_2							
		$m_3 = \frac{c_1 + c_2}{2}$ (C_{bl})							

PART III: EVALUATION OF RAW DATA:

	Flask No.		% DOC removal after x days				
			7	14	21	27	28
DOC - concentrations minus blanks	1	$D_1 = \left[1 - \frac{m_{1_t} - m_{3_t}}{m_1 - m_3}\right] \cdot 100$					
	2	$D_2 = \left[1 - \frac{m_{2_t} - m_{3_t}}{m_2 - m_3}\right] \cdot 100$					
	mean	$D = \frac{D_1 + D_2}{2}$ for day x					

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[1] Disagreement between DOC and TOC values points towards insufficient solubility of the test material.

[2] All DOC values determined after membrane filtration.

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(3) *Interpretation of results.* (i) The results of the degradation test are valid if the condition is met that in the same test series the control yields 70 percent DOC-removal.

(ii) Because of the stringency of this test a result inferior to the recommended pass level (70 percent loss of DOC) does not necessarily mean that the test compound is not biodegradable under environmental conditions, but indicates that more work will be necessary to establish this.

§ 796.3260 Ready biodegradability: Modified Sturm test.

(a) *Introductory information*—(1) *Prerequisites.* The total organic carbon content of the test material should be calculated or, if this is not possible, analyzed to enable the theoretical yield of CO_2 to be calculated.

(2) *Guidance information.* (i) Information on the relative proportions of the major components of the test material will be useful in interpreting the results obtained, particularly in those cases where the result lies close to the "pass level."

(ii) Information on the toxicity of the chemical may be useful in the interpretation of low results and in the selection of appropriate test concentrations.

(3) *Interpretation of results.* Because of the stringency of this test, a result of less than 60 percent yield of CO_2 (within 28 days) does not necessarily mean that the test compound is not biodegradable under environmental conditions, but indicates that more work will be necessary to establish biodegradability.

(4) *Qualifying statements.*

(i) The method is only applicable to those organic test materials which, at the concentration used in the test:

(A) Have negligible vapour pressure.

(B) Are not inhibitory to bacteria.

(C) Do not significantly adsorb to glass surfaces.

(ii) This test has been found suitable by the OECD Expert Group Degradation/Accumulation for determining the ready biodegradability of organic chemicals under aerobic conditions. It has been tested in the OECD Laboratory Intercomparison Test Programme (1978–1980).

(iii) Evolution of a significant amount of CO_2 from the blank flask during the test would indicate contamination of the medium, glassware or air supply. A total CO_2 evolution in the blank at the end of the test exceeding 50 mg CO_2 per 3 liters medium should be considered as invalidating the test.

(5) *Recommendations.* (i) Test chemicals giving a result of greater than 60 percent yield of CO_2 (within 28 days) should be regarded as readily biodegradable. This level must be reached within 10 days of biodegradation exceeding 10 percent.

(ii) If the test material is not soluble at the test concentration, special measures, such as the use of ultrasound dispersion, may have to be employed to achieve a good dispersion of the test material.

(6) *Standard documents.* This test guideline is based on biodegradability test methods described in paragraph (d) (1) and (2) of this section. Various improvements have been made in the test protocol, the latest ones being the object of the paper cited in paragraph (d)(3) of this section.

(b) *Method*—(1) *Introduction, purpose, scope, relevance, application and limits of test.* (i) (A) This test guideline has been developed for screening readily biodegradable chemicals. When a broader understanding of the biodegradability of a compound is required (particularly for new chemicals never discharged into the environment) environmentally relevant results may be obtained by operating the same test guideline but with inoculum preadapted to the test compound under paragraph (d) (1), (2), and (3) of this section.

(B) A high biodegradation result in this test provides the evidence that the test compound is highly biodegradable in aerobic systems.

(C) On the contrary, a low biodegradation result may have other causes than poor biodegradability of the test compound. Inhibition effects of the inoculum by the toxicity of the compound at the test concentration is often a cause for low biodegradation. In such cases the result is meaningless and further work is needed to assess the biodegradability of the test compound in systems and at concentrations where inhibition effects are overcome.

(D) An estimate of the expected environmental concentration will help to put toxicity effects into perspective and determine test concentrations.

(E) The proportion of carbon incorporated into cellular material to carbon released as CO_2 will vary depending on the organic substrate, on the particular micro-organism(s) carrying out the conversion and on the environmental conditions under which the conversion takes place. In principle, this uncertainty is a drawback in the interpretation of the results from this test.

(F) However, it has been observed that under the conditions of this test with a test compound concentration (sole source of carbon) ranging from 5 to 20 mg/l, a majority of substrate carbon is used to drive catabolic processes and a minimum is used to generate new cells under paragraph (d)(3) of this section. Under these conditions, the measure of CO_2 evolution becomes an accurate measure of the rate and extent of catabolism of a given material.

(ii) *Definitions and units.* The amount of CO_2 produced by the test compound during the test is measured and expressed as percent of the theoretical CO_2 it should have produced (TCO_2) calculated from the carbon content of the test compound. Biodegradability is therefore expressed as percentage TCO_2.

(iii) *Reference substances.* In some cases when investigating a new substance reference substances may be useful; however, specific reference substances cannot yet be recommended. In order to check the activity of the inoculum, the use of control substances is desirable. Aniline, sodium acetate or sodium benzoate may be used for this purpose. They must give a yield >60 percent CO_2 within 28 days, otherwise the test is regarded as invalid and should be repeated using an inoculum from a different source.

(iv) *Principle of the test method.* (A) A chemically defined liquid medium, essentially free of other organic carbon sources, is spiked with the test material and inoculated with sewage micro-organisms. The CO_2 released is trapped as $BaCO_3$.

(B) After reference to suitable blank controls, the total amount of CO_2 produced by the test compound is determined for the test period and calculated as the percentage of total CO_2 that the test material could have theoretically produced based on carbon composition.

(v) *Quality criteria*—(A) *Reproducibility.* In the absence of toxicity effects of the test compound on the inoculum, the reproducibility is around ±5 percent, relative. When toxicity effects start occurring, the reproducibility becomes poor.

(B) *Sensitivity.* The endogenous CO_2 production of the inoculum as measured in the blank flask (no test material) is the main reason why the test cannot use test compound concentrations lower than 5 mg/l. (When the test is adapted to handle ^{14}C labelled test compounds, test compound concentrations can be very much lower.)

(C) *Specificity.* (*1*) This test is adequate for soluble and insoluble organic materials. The test material must not be volatile. The purity of the test material should be high since organic impurities will complicate the interpretation of the CO_2 production data.

(*2*) Test results are meaningful only if, at the test concentration, the material has no toxic effect on the inoculum.

(D) *Possibility of standardization.* This possibility exists. The major difficulty is to standardise the inoculum in such a way that interlaboratory reproducibility is ensured. The selection of test organisms and their handling in the laboratory as described below (Test organisms) is one way to ensure "healthy" inoculum with a mutiplicity of micro-organism species. Procedures using preadaptation of the inoculum to the test compound usually reach higher reproducibility.

(E) *Possibility of automation.* None at present.

(2) *Description of the test procedure—*(*i*) *Preparations—*(A) *Apparatus equipment—(1) CO_2 scrubbing apparatus:* For a series of 12 carboys (3 test materials):

(*i*) Four one-liter plastic bottles, filled with 700 ml 10 N NaOH.

(*ii*) One one-liter Erlenmeyer flask containing 700 ml 0.025 N $Ba(OH)_2$ solution.

(*iii*) One empty one-liter Erlenmeyer to prevent liquid carry-over.

These bottles are connected in series, using Tygon tubing, to a pressurized air source, and air is sparged through the scrubbing solutions at a constant rate. For each additional set of carboys, add 1 additional one-litre plastic bottle filled with 700 ml 10 N NaOH.

(*2*) *CO_2 production apparatus.* (*i*) Four 5-litre disposable amber carboys for each test material.

(*ii*) Stoppers, flexible tubing, plastic tubing.

(*iii*) "French squares" (i.e. 100 ml barium hydroxide absorber bottles) or similar containers.

(*3*) *Analytical equipment.* (*i*) (Carbon analyser: optional if sample is well characterized.)

(*ii*) Analytical balance.

(*iii*) 100 ml-buret.

(B) *Chemicals/reagents/materials—*(*1*) *Stock solutions for test medium—*(*i*) *Ferric chloride solution:* dissolve 0.25 g $FeCl_3 \cdot 6H_2O$ in 1 liter distilled water.

(*ii*) *Magnesium sulfate solution:* dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in 1 liter distilled water.

(*iii*) *Calcium chloride solution:* dissolve 27.5 g anhydrous $CaCl_2$ in 1 liter distilled water.

(*iv*) *Phosphate buffer solution:* dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4 \cdot 7H_2O$ and 1.7 g NH_4Cl in 1 liter distilled water.

(*v*) *Ammonium sulfate solution:* dissolve 40 g $(NH_4)_2SO_4$ in 1 liter distilled water.

(*2*) *Test medium.* The test medium will contain per liter of high quality water (High quality water, free of toxic substances (copper, in particular), with low carbon content (<2.0 mg/liter TOC) and with a resistivity >18 megohms.cm. (Distilled water must never contain more than 10 percent of organic carbon introduced by the test material.)) the following reagents:

(*i*) 1 ml of magnesium sulfate solution under paragraph (b)(2)(i)(B)(*1*)(*ii*) of this section.

(*ii*) 1 ml of calcium chloride solution under paragraph (b)(2)(i)(B)(*1*)(*iii*) of this section.

(*iii*) 2 ml of phosphate buffer solution under paragraph (b)(2)(i)(B)(*1*)(*iv*) of this section.

(*iv*) 4 ml of ferric chloride solution under paragraph (b)(2)(i)(B)(*1*)(*i*) of this section.

(*v*) 1 ml of ammonium sulfate solution under paragraph (b)(2)(i)(B)(*1*)(*v*) of this section.

(*3*) *Barium hydroxide, 0.025 N.* Dissolve 4.0 g Ba $(OH)_2 \cdot 8H_2O$ per liter high quality water. Filter through filter paper and seal the clear solution to prevent absorption of CO_2 from the air. It is wise to prepare more than 5 liters at a time when running series.

(*4*) *Test materials.* (*i*) Basic physical-chemical data regarding water solubility, impurities solvent and percentage active must be specified by the sample submitter.

(*ii*) An initial stock solution is prepared from the test material by weighing out a homogeneous aliquot of the sample. The sample is dissolved in high quality water to give a solution of test material of 1000 mg/l. Stock solutions are made up on the basis of percentage of active compound in the test material. If the percentage of active compound is unknown, stock solutions are made up to concentration of 1000 mg/l on a weight basis. To obtain a homogeneous sample, it may be necessary to mix well, at the same time avoiding foaming which will tend to concentrate the active ingredient disproportionally. For solid samples, it may be necessary to melt and mix the entire contents of the sample bottle before taking the aliquot. This portion of the procedure is extremely important since the calculations of percentage of biodegradation depend directly on having added the correct amount of carbon to the test system.

(*iii*) The pH of the stock solution need not be adjusted unless it falls outside the range of 3–10, since the phosphate buffer in the test medium will control it. If the pH lies outside this range, adjust an aliquot of the stock solution to pH 7.0 (±1.0) with 1 N HCl or NaOH, making sure that the solution is being vigorously mixed during the addition of acid or base.

(*iv*) To confirm the nominal concentration of organic carbon of the test compound, the stock solution (or the neutralized aliquot) may be analyzed for total organic carbon (TOC). A TOC analysis is also required for the control stock solution.

(*v*) If a test material is insoluble in water, add the appropriate amount of test material directly to the carboy on a weight or volume basis. Total organic carbon analysis cannot be performed on insoluble test materials.

(*5*) *Test organisms.* (*i*) The source of test organisms is activated sludge freshly sampled from a well-operated municipal sewage treatment plant. This sewage treatment should receive no or minimal effluents from industry.

(*ii*) Upon arrival at the laboratory, the activated sludge is aerated for 4 hours. 500 ml of the mixed liquor is sampled and homogenized for 2 minutes at medium speed in a Waring blender. It is then settled for ½ hour.

(*iii*) If the supernatant still contains high levels of sludge solids at the end of 30 minutes, it may be settled for an additional 30–60 minutes or adapted to laboratory conditions to obtain better settleability.

(*iv*) The supernatant is decanted to provide sufficient volume for a 1 percent inoculum for each CO_2 test flask. Avoid carryover of sludge solids which would interfere with the measurement of CO_2 production.

(*v*) Although optional, it is useful to perform viable counts on the supernatant fraction to determine microbial numbers. This inoculum should normally contain 10^6 to 20×10^6 colony forming units per ml. It should be used on the day it is prepared. The CO_2 production test then proceeds as follows.

(ii) *Test conditions.* (A) (*1*) Since a 1 percent inoculum is used in the CO_2 test, it is necessary to make dilutions in the CO_2 test medium.

(*2*) This is most easily achieved as follows:

(*i*) To each of the 5-liter test carboys, add 2470 ml of high quality water.

(*ii*) To each of the 5-liter test carboys, add 3 ml each of the ammonium sulfate, magnesium sulfate, and calcium chloride stock solutions; add 6 ml of the phosphate buffer stock solution and 12 ml of the ferric chloride solution.

(*iii*) To each of the 5-liter test carboys, add 30 ml of the activated sludge inoculum.

(*iv*) These additions now total 2527 ml in each carboy.

(B) This mixture is aerated with CO_2-free air for 24 hours, to purge the system of carbon dioxide (see CO_2 scrubbing apparatus, above).

(C) After the aeration period, three CO_2 absorber bottles are filled with 100 ml 0.025 N $Ba(OH)_2$ and connected in series to the exit air line of each test carboy.

(D) Test material is added to two of the four carboys to begin the testing period. Each material is tested at two concentrations: 10 and 20 mg/l. The amount of test material stock solution required in the carboy is calculated as follows:

$$\text{ml of stock solution per carboy is: } \frac{B \times C}{A}$$

where:

B is test compound concentration in test carboy (mg/l).

A is test compound concentration in stock solution (mg/l).

C is final volume of test medium in test carboy (ml).

(E) Sufficient stock solution to reach the desired test concentration, as calculated above, plus enough distilled water to make 473 ml (stock solution + high quality water) are added to the appropriate carboys. To the third carboy, used as blank control and containing no test material, 473 ml of high quality water are added. The final volume of each carboy is now 3000 ml.

(F) A control substance at a concentration of 20 mg/l is added to the last of the four carboys (see paragraph (d) of this section on reference substances.

(iii) *Performance of test.* (A)(*1*) The test is started by bubbling CO_2-free air through the solution at a rate of 50–100 ml/min per carboy (approximately 1–2 bubbles/second). The CO_2 produced in each carboy reacts with the barium hydroxide and is precipitated out as barium carbonate; the amount of CO_2 produced is determined by titrating the remaining $Ba(OH)_2$ with 0.05 N standardized HCl (see below). Periodically (every 2 or 3 days), the CO_2 absorber nearest the carboy is removed for titration. The remaining two absorbers are each moved one place closer to the carboy, and a new absorber filled with 100 ml of fresh 0.025 N $Ba(OH)_2$ is placed at the far end of the series. Titrations are made as needed (before any $BaCO_3$ precipitate is evident in the second trap), approximately every other day for the first 10 days, and the every fifth day until the 28th day.

(*2*) For water-insoluble test materials, incorporated dry into the CO_2 test carboy, agitation can be done with a magnetic stirrer. For foaming chemicals, CO_2-free air bubbling can be replaced by overhead aeration and magnetic stirring.

(B) On the 26th day, the pH of the carboy contents is measured again, and then 1 ml of concentrated HCl is added to each of the test carboys to drive off inorganic carbonate. The carboys are aerated overnight, and samples are removed from each carboy for dissolved organic carbon (DOC) analysis. The final titration is made on day 28.

(C) Titrations of the 100-ml $Ba(OH)_2$ solution are made after removing the bottles closest to the carboys. The $Ba(OH)_2$ is titrated with 0.05 N HCl, using phenophthalein as an indicator.

(D) The test is run at room temperature and temperature is recorded during the test period.

(c) *Data and reporting*—(1) *Treatment of results.* (i) *TCO_2.* (A) The theoretical amount of CO_2 that can be generated by a test material, or TCO_2, is calculated as follows:

$$TCO_2 = \text{mg } CO_2/\text{mg test material} = \frac{\text{N° of carbons in test material} \times \text{mol. wt. of } CO_2}{\text{mol. wt. of active test material}}$$

(B) For instance, dextrose ($C_6H_{12}O_6$) contains 6 carbon, therefore TCO_2 of dextrose =

$$\frac{6 \times 44}{180} = 1.467 \text{ mg } CO_2/\text{mg dextrose}$$

(C) For mixtures, the TCO_2 of the total active material is a weighted average of the TCO_2's of the individual components.

(ii) *Amount of CO^2 produced.* (A) The first step in calculating the amount of CO_2 produced is to correct the test material carboys for endogenous CO_2 production. The control carboy serves as a "seed blank" to correct for CO_2 which may be produced through endogenous respiration of the bacteria. The amount of CO_2 produced by a test material is determined by the difference (in ml of titrant) between the experimental and blank $Ba(OH)_2$ traps.

For example:

Blank	48.0 ml HCl titrated
Experimental	45.0 ml HCl titrated
Test material	3.0 ml HCl titrated

(B) The next step is to convert ml HCl titrated into mg of CO_2 produced. When CO_2 enters the absorber bottle, it reacts in the following manner:

$$Ba(OH)_2 + CO_2 \rightarrow BaCO_3 \downarrow + H_2O$$

(C) The $BaCO_3$ formed is insoluble and precipitates. The amount of $Ba(OH)_2$ remaining in solution is determined by titration of the 100 ml with HCl according to the following equation:

$$Ba(OH)_2 + 2HCl \rightarrow BaCl_2 + 2H_2O$$

(D) From the above two equations, it can be seen that 1 mmol of CO_2 is produced for every 2 mmol of HCl titrated. This means that the number of mmol of CO_2 produced:

$$\text{mmol } CO_2 = \frac{\text{mmol HCl}}{2}$$

(E) The normality of HCl used is 0.05 N. Substituting for mmol gives:

$$\text{mmol } CO_2 = \frac{(0.05 \text{ N}) \times (\text{ml of HCl}}{2}$$

(F) To convert to mg CO_2, the value must be multiplied by the molecular weight of CO_2 which is 44:

$$\text{mg } CO_2 = \frac{((0.05) \times \text{ml titrated}}{2} \times 44 = 1.1 \times \text{ml of HCl titrated})$$

(G) Thus, to convert ml of HCl to mg CO^2, the former is multiplied by 1.1.

(iii) *Percentage of theoretical CO_2*. (A) The percentage of theoretical CO_2 produced is calculated from the following formula:

$$\%TCO_2 = \frac{\text{mg } CO_2 \text{ produced}}{(\text{mg test material added in test})\ (\text{mg } CO_2/\text{mg test material})} \times 100$$

$$= \frac{\text{mg } CO_2 \text{ produced}}{(\text{mg test material added in test})\ (TCO_2)} \times 100$$

Example

Blank	48.0 ml 0.05 N HCl titrated
Dextrose	45.0 ml 0.05 N HCl titrated
Difference	3.0 ml 0.05 N HCl titrated

(B) When multiplied by 1.1, 3.0 is equivalent to 3.3 mg CO_2 produced.

(C) Since dextrose has a TCO_2 of 1.47 mg CO_2/mg dextrose, and since 60 mg of dextrose were placed in the test (i.e. 3 litres × 20 mg/l), the percentage of theoretical CO_2 that was degraded on the particular day was:

$$\%TCO_2 = \frac{3.3 \text{ mg } CO_2 \text{ produced}}{(60 \text{ mg dextrose})\ (1.47 \text{ mg } CO_2/\text{mg dextrose})} \times 100 = 3.74$$

(2) *Test report*. (i) The test material should be well identified (source, physical-chemical data, organic carbon content, purity, etc.).

(ii) The test concentration at start should be reported with end results.

(iii) Indicate date and location where test organisms were sampled.

(iv) COD and TOC analyses on the stock solution of the test compound should be reported.

(v) Temperature range recorded during the test period must be noted.

(vi) If measured as suggested above (Test organisms), report number of micro-organisms per ml (colony forming units—CFU/ml).

(vii) For each titration, calculate percentage TCO_2 evolved. Plot cumulative percentage TCO_2 versus time until the end of the test. Not only the end result is important, but also the length of the lag phase and the slope (rate).

(viii) If the curve reaches a plateau before 28 days, the test can be ended and the plateau value considered as final.

(ix) If the curve shows that biodegradation started before day 28 but the plateau is not reached at day 28, then the test should be prolonged until the plateau is reached.

(x) If a more rigorous mathematical treatment of the data is desired, the cumulative CO_2 versus time data can be fit into a non-linear regression model to generate rate constants for mineralization and a final extent of degradation at infinite time (asymptote) (3).

(3) *Interpretation of results*. See paragraph (a) of this section.

(d) *Literature* references. For additional background information on this test guideline the following references should be consulted:

(1) Thompson, J.E., and Duthie, J.R., "The biodegradability of NTA," *Journal of Water Pollution Control Federation*. 40: 306–319 (1968).

(2) Sturm, R.N., "Biodegradability of Nonionic Surfactants: Screening Test for Predicting Rate and Ultimate Biodegradation," *Journal of American Oil Chemistry Society*. *50*: 159–167 (1973).

(3) Larson, R.J., "Estimation of Biodegradation Potential of Xenobiotic Organic Chemicals," *Applied Environmental Microbiology*. *38*: 1153–1161 (1979).

(4) American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*. *14*th Ed. (American Public Health Association, Inc.: New York, 1975) pp. 544–545.

(5) AFNOR T-90-302 Test. *Methode d'evaluation en milieu aqueux de biodegradabilite des produits organiques*. (1977).

(6) ISO TC 147 SC5 WG4. OECD Screening Test modified for the use of dissolved organic carbon analysis (1978).

§ 796.3300 Simulation test—aerobic sewage treatment: coupled units test.

(a) *Introductory information*—(1) *Prerequisites*. (i) Water solubility.

(ii) The organic carbon content of the test material must be established.

(2) *Guidance information*. (i) Information on the relative proportions of the major components of the test material will be useful in interpreting the results obtained, particularly in those cases where the result lies close to the "pass level".

(ii) Information on the toxicity of the chemical may be useful to the interpretation of low results and in the selection of appropriate test concentrations.

(3) *Qualifying statements*. (i) The method is only applicable to those organic test materials which, at the concentration used in the test:

(A) Are soluble in water to the extent necessary for the preparation of the test solutions.

(B) Have negligible vapor pressure.

(C) Are not inhibitory to bacteria.

(D) Do not significantly adsorb on glass surfaces.

(ii) This test is recommended by the OECD Expert Group on Degradation/Accumulation as a test for the determination of the ultimate biodegradability of test materials under conditions which stimulate treatment in an activated sludge plant.

(c) *Standard documents*. This test guideline constitutes a modification of the OECD Confirmatory Test (OECD Environmental Directorate, Proposed Method for the Determination of the Biodegradability of Surfactants Used in Synthetic Detergents, Paris 1976, and Council Directive of Nov. 22, 1973, on the Approximation of the Laws of the Member States Relating to Methods of Testing the Biodegradability of Anionic Surfactants [73/405/EEC], Official Journal of the European Communities No. 4 347/53 of Dec. 17, 1973). The modifications are described in the references cited under paragraph (d) (4) and (5) of this section.

(b) *Method*—(1) *Introduction, purpose, scope, relevance, application and limits of test* (i) The purpose of the method is the measurement of the ultimate biodegradability of the water-soluble, nonvolatile organic compounds in an activated sludge plant model at a concentration corresponding to ≥ 12 mg DOC/l (Dissolved organic carbon/litre) (or approximately 40 mg COD/l (Chemical oxygen demand/liter)), twenty mg DOC/l seem to be optimal.

(ii) *Definitions and units.* (A) Definition of *biodegradability:*

$$DR = \frac{T-(E-E_o)}{T} \times 100$$

Where

DR = degradation rate in percent DOC (or COD) removal within the given mean retention time with respect to the test material
T = concentration of the test material in mg DOC/litre (or mg COD/litre)
E = DOC (or COD) concentration in the effluent of the test unit in mg DOC/litre (or mg COD/litre)
E_o = DOC (or COD) concentration in the effluent of the blank unit in mg DOC/litre (or mg COD/litre)

(B) *Units.* The degradation is stated as the percentage DOC (or COD) removal within the given retention time with respect to the test material (percentage DOC [or percentage COD] removal).

(iii) *Reference substances.* In some cases when investigating a new substance reference substances may be useful; however specific reference substances cannot yet be recommended.

(iv) *Principle of the test method.* Two OECD Confirmatory Test units, i.e., model activated sludge plants, are operated in parallel whereby the parallelism is enhanced and assured by a transinoculation procedure. The test material is added to the influent (synthetic sewage) of one unit while the other is fed only with the synthetic sewage. The DOC (or COD) concentrations are measured in both effluents. The DOC (or COD) difference of these effluent values is due to non- or only partially degraded test material. The raw data obtained require a statistical procedure, i.e. the tolerance limits of the mean must be calculated in addition to the mean.

(v) *Quality criteria*—(A) *Reproducibility.* The reproducibility of the results obtained by this method is satisfactory.

(B) *Sensitivity.* A starting concentration of the test material corresponding to 12 mg DOC/litre (or approximately 40 mg COD/litre) constitutes the sensitivity limit at a mean retention time of 3 hrs; at a 6 hr mean retention time 9–10 DOC/litre are sufficient.

(C) *Specificity.* The method is applicable to the biodegradability evaluation of water-soluble, non-volatile organic compounds.

(D) *Possibility of standardization.* The test version with specific analyses for anionic and nonionic surfactants is standardized as the "OECD Confirmatory Test".

(E) *Possibility of automation.* Parts of the test, e.g., the analysis, can be automated, although not the total procedure.

(2) *Description of the test procedure*—(i) *Preparations*—(A) *Apparatus* (*1*)(*i*) OECD Confirmatory Test Units: The method employs two small activated sludge plants shown in the following Figure 1, and in greater detail in Figure 2, for each biodegradability determination.

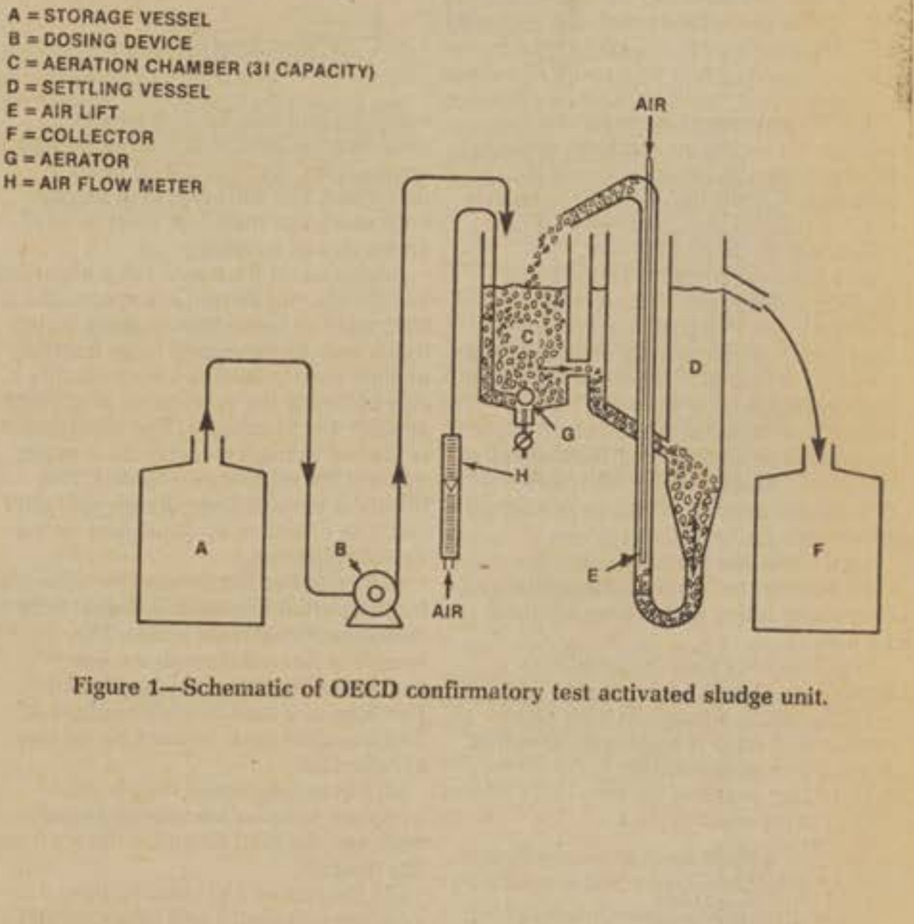
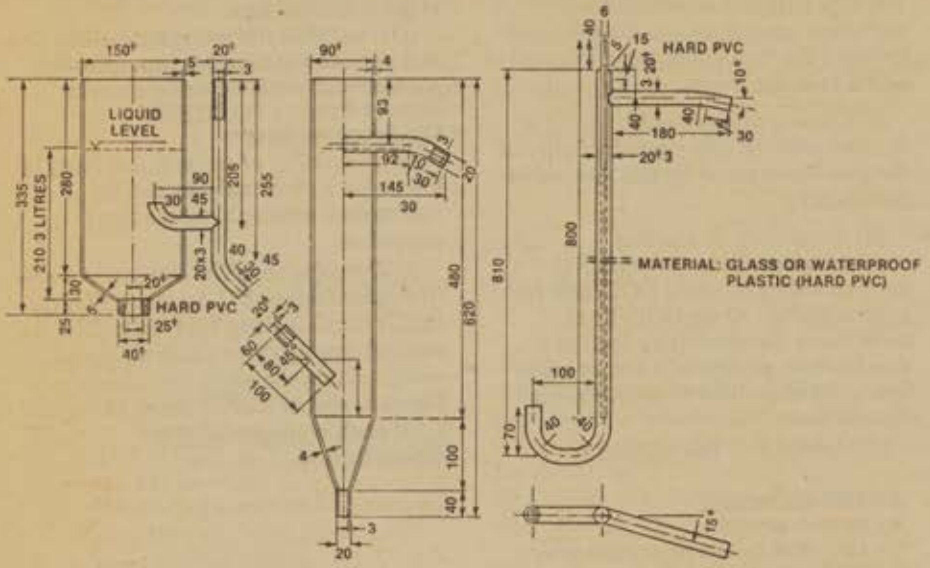


Figure 1—Schematic of OECD confirmatory test activated sludge unit.

Figure 2—Dimensions of OECD confirmatory test activated sludge unit.



(*ii*) The equipment consists of a storage vessel (A) for synthetic sewage, dosing pump (B), aeration vessel (C), separator (D), air-lift pump (E) to recycle the activated sludge, and vessel (F) for collecting the treated effluent.

(*iii*) Vessels (A) and (F) must be of glass or suitable plastic and hold at least 24 litres. Pump (B) must provide a constant flow of synthetic sewage to the aeration vessel; during normal operation this vessel contains 3 litres of mixed liquor. A sintered aeration cube (G) is suspended in vessel (C) at the apex of the cone. The quantity of air blown through the aerator should be monitored by means of a flowmeter.

(*2*) Carbon analyzer.

(*3*) Membrane filtration apparatus and membrane filters with a pore width of 1.2 μm.

(*4*) Glassware as it is commonly needed for sample preparation.

(*5*) Synthetic sewage: For the test a synthetic sewage is employed. Dissolve in each litre of tap water:

(*i*) 160 mg peptone.

(*ii*) 110 mg meat extract.

(*iii*) 30 mg urea.

(*iv*) 7 mg NaCl.

(*v*) 4 mg $CaCl_2 \cdot 2H_2O$.

(*vi*) 2 mg $MgSO_4 \cdot 7H_2O$.

(B) *Inoculation.* (*1*) A composite inoculum is used.

(*i*) *Inoculum from secondary effluent.* The inoculum should be obtained from a secondary effluent of good quality collected from a treatment plant dealing with predominantly domestic sewage. The effluent must be kept under aerobic conditions in the period between sampling and use. To prepare the inoculum the sample is filtered through a coarse filter, the first 200 ml being discarded. The filtrate is kept aerobic until used. The inoculum must be used on the day of collection.

(*ii*) *Inoculum from soil.* 100 g of garden soil (fertile, not sterile) are suspended in 1000 ml of chlorine-free drinking water. (Soils with an extremely large fraction of clay, sand or humus are unsuitable.) After stirring, the suspension is allowed to settle for 30 minutes. The supernatant is filtered through a coarse filter paper, the first 200 ml being discarded. The filtrate is aerated immediately and until use. The inoculum must be used on the day of collection.

(*iii*) *Inoculum from a surface water.* A further partial inoculum is drawn from a mesosaprobic surface water. The sample is filtered through a coarse paper, the first 200 ml being discarded. The filtrate is kept aerobic until used. The inoculum must be used on the day of collection.

(*2*) Equal volumes of the 3 partial inoculum samples are united, mixed well, and the final inoculum drawn from this mixture.

(ii) *Procedure.* (A) Initially, fill aeration vessels (C) and separator (D) of both units needed for one test with synthetic sewage. (It is strongly recommended that the synthetic sewage be prepared first in one batch. Then the storage vessels (A) of both units needed for one test are filled with the same synthetic sewage.) The height of separator (D) is so fixed that the volume contained in aeration vessel (C) is 3 litres. Inoculation is made by introducting 3 ml of the composite inoculum, above. The aerator, air lift (E) and dosing device (B) are then set in operation. The synthetic sewage must pass through aeration vessel (C) at the rate of one litre per hour; this gives a mean retention time of 3 hours; a rate of one half litre per hour results in a mean retention time of 6 hrs.

(B) The rate of aeration should be regulated so that the contents of vessel (C) are kept constantly in suspension while the dissolved oxygen content is at least 2 mg/l. Foaming must be prevented by appropriate means. Antifoaming agents which inhibit the activated sludge must not be used. Airlift pump (E) is set so that the activated sludge from the separator is continually and regularly recycled to aeration vessel (C). Sludge which has accumulated around the top of the aeration vessel (C), in the base of the settling vessel (D), or in the circulation circuit must be returned to the circulation at least once each day by brushing or some other appropriate means. When sludge fails to settle, its density may be increased by addition of 2-ml portions of a 5 percent solution of ferric chloride, repeated as necessary.

(C) The effluent from separator (D) is accumulated in vessel (F) for 24 hours, following which a sample is taken after thorough mixing. Vessel (F) must be carefully cleaned.

(D) In order to control the efficiency of the process the chemical oxygen demand (COD) or the dissolved organic carbon (DOC) of the filtrate of the effluent (especially of the blank unit) accumulated in vessel (F), is measured at least twice weekly, as well as that of the filtered synthetic sewage in vessel (A).

(E) The reduction in COD or DOC should level off when a roughly regular daily degradation is obtained, i.e. at the end of the running-in period.

(F) The dry matter content of the activated sludge in the aeration tank should be determined twice a week (in g/l). If it is more than 2.5 g/l, the excess activated sludge must be discarded.

(G) The test is performed at room temperature; this should be steady and should be kept between 18 °C and 25 °C.

(H) Two OECD Confirmatory Test units are run parallel in the fashion described above. Sufficient stock solution (approximately 1 percent) of the test material is added to the influent of the test unit that the desired concentration of test material (=

approximately 10–20 mg DOC/l or 40 mg COD/l) in the synthetic sewage is obtained. The blank unit is fed only with synthetic sewage.

(I) The coupling of the units is achieved by interchanging 1.5 litres—including sludge—from the activated sludge vessels between the two units once a day. In case of strongly adsorbing test materials 1.5 litres are drawn from the settling vessels and interchanged.

(J) The DOC (or COD) concentrations of the effluents of the test (E) and blank (E_o) units are determined daily after membrane filtration (pore size 1.2 μm).

(K) The working-in time should not exceed 6 weeks and the evaluation period should not be shorter than 3 weeks, i.e., about 20 determinations should be available for calculation of the final results.

(iii) *Analytical means.* (A) 250 ml effluent samples are drawn and immediately membrane filtered (1.2 μm). The first half of the filtrate is discarded. The DOC (or COD) concentration is determined in the remainder. If the analysis cannot be performed on the same day, the sample is stored in a refrigerator, but only after membrane filtration.

(B) The DOC concentrations are determined in duplicate with the carbon analyzer and the COD values according to APHA under paragraph (d)(1) of this section. The data are recorded on the following Figure 3 form sheet.

Figure 3.—Form sheet for the OECD confirmatory test modified for the application of unspecific analyses (coupled units test.)

Exp. No ________
Date of start of test ________
Test material ________
Theoretical test concentration:
  mg active substance/litre (or mg/l of product). ________
  mg DOC/litre ________
  mg COD/litre ________
Inoculum ________
Analysis or analytical instrument. ________
Mean retention time: (hrs) ________
Volume being interchanged (v_i): (l/d). ________
Daily throughput (v_d) ________
Transinoculation out of activated:
  Sludge vessels ________
  Settling vessel ________

REMARKS

A. Controls	mg/l	
	TOC [1]	DOC [2]
Stock solution of the test material (10,000 mg active substance/litre, dilution * * * / 1,000 ml of distilled water)		

[1] Disagreements between TOC and DOC values point towards insufficient solubility of the test material.

[2] All DOC values determined after membrane filtration (1.2 μm).

(c) *Data and reporting*—(1) *Treatment of results.* (i) The daily degradation rates DR are calculated according to

Equation 1

$$DR = \frac{T-(E-E_o)}{T} \times 100$$

(ii) For explanation of symbols, see Definitions and units, above.

(iii) These daily degradation rates are corrected for the material transfer due to the transinoculation procedure with equation 2 for a 3 hr, or equation 3 for a 6 hr, mean retention time.

Equation 2

$$DR_c = \frac{8}{7} DR - \frac{100}{7}$$

Equation 3

$$DR_c = \frac{4}{3} DR - \frac{100}{3}$$

(iv) The mean of the series of DR_c values is calculated and in addition the standard deviation according to equation 4

Equation 4

$${}^{s}DR_c = \sqrt{\frac{\sum_{i=1}^{n} (\overline{DR_c} - DR_{ci})^2}{n-1}}$$

Where

${}^{s}DR_c$ = Standard deviation of the series of DR_c values
DR_c = mean of DR_c value
n = number of determinations

(v) Outliers of the DR_c series are eliminated according to Nalimov under paragraph (d)(2) of this section at the 95 per cent probability level and the mean and the standard deviation of the outlier-free DR_c data set recalculated. The final result is then calculated with equation 5 as

Equation 5

$$DR_c = \overline{DR_c} \pm \frac{t_{n-1;\alpha}}{\sqrt{n}} \, {}^{s}DR_c$$

Where:

$t_{n-1;\alpha}$ = table[3] value of t for n value pairs of E and E_o and statistical confidence P ($P = 1-\alpha$) whereby P is set at 95 per cent.

(A) *Calculation of the results by computer.* A computer program BILAH available from Henkel A.G. (Düsseldorf, FRG) performs the above calculations automatically and is recommended for use. The program is written in Fortran IV and has been tested on a Honeywell-Bull series 6000 computer (Mark III Service). This program is designed for any continuous flow biodegradability test run with a transinoculation procedure.

(B) *Calculation of the results by pocket calculator.* The calculation described above can also be performed automatically by the programme BILAW for a pocket calculator (Texas Instruments 59 with printer). The programme instructions, the programme itself, and an example for a complete input and output are available from Henkle A.G. (Düsseldorf, FRG)

(2) *Test report.* (i) The report must contain a statement referring to the analytical accuracy with respect to the particular test material.

(ii) The result is stated as the mean with tolerance limits at the 95 percent probability level, the respective standard deviation and the number of data of the outlier-free DR_c data set, e.g.

$DR_c = 98.6 \pm 2.3)$ % DOC removal.
s = 4.65 % DOC removal
n = 18

(iii) The running-in time also constitutes pertinent information.

(iv) Some indication of the general purification such as DOC or COD removal with respect to the total organic feed, especially of the blank unit, and the effluent concentrations of the latter must be provided.

(v) All this information is summarized in the following Figure 4 report sheet:

Figure 4—Sample Reporting Sheet

Date of start of test:
Test material:
Test concentration:
  mg AS/l (or mg/l product)
  mg DOC/l
  mg COD/l
Result with respect to test material
Mean and tolerance limits at the 95 per cent probability level; standard deviation and number of determinations (outlier-free):
DR_e= ( ) % DOC removal
s= ( ) % DOC removal
n=
DR_e= ( ) % COD removal
s= ( ) % COD removal
n=
Working-in time: d
Evaluation period: d
Analysis:
Analysis of instrument response:
Analytical correctness with respect to test material:
  Percentage recovery with respect to theory at conc . . . mg DOC (or COD)/l
General parameters:
Mean retention time: hrs
Volume being interchanged (v_i): l/d
Daily throughput (v_d): l/d
Transinoculation out of:
  activated sludge vessel:
  settling vessel:
Origin and preparation of inoculum:
Mean organic dry matter:
  Test unit: g/l
  Blank unit: g/l
Mean DOC (COD) removal with respect to total organic load:
  Test unit: % DOC (or COD) removal
  Blank unit: % DOC (or COD) removal
Effluent of blank unit
  Concentration: mg DOC/l
  Standard deviation: mg DOC/l n:
  Concentration: mg COD/l
  Standard deviation: mg COD/l n:

Remarks:

(vi) Special observations, e.g. "excessive formation of bulking sludge", are to be reported under "remarks".

(vii) Finally, a degradation diagram must be provided whereby the outliers in the evaluation period are not excluded in the graph.

(d) *Literature references.* For additional background information on this test guideline the following references should be consulted:

(1) "Oxygen Demand (chemical)," *Standard Methods for the Examination of Water and Waste Water,* 14 Ed. American Public Health Association, American Water Works Association Water Pollution Control Federation, (1975) p. 550

(2) Kaiser, R., and Gottschalk G., *Elementare Tests zur Beurteilung von Messdaten,* (Bibliographisches Institut: Mannheim, Wien, Zürich, 1971) p. 18.

(3) Sachs, L., *Statistische Auswertungsmethoden,* Springer-Verlag (1971) p. 111.

(4) Fischer, W.K., Gerike, P., Holtmann, W., "Biodegradability Determinations Via Unspecific Analyses (chemical oxgen demand, dissolved organic carbon) in coupled units of the OECD Confirmatory Test. 1. The Test". *Water Research,* 9:1131–1135 (1975).

(5) Gerike, P., Fischer, W.K., and Holtmann, W. "Biodegradability Determination in Trickling Filter Units Compared with the OECD Confirmatory Test," *Water Research,* 14:753 (1980).

§ 796.3340 Inherent biodegradability: Modified SCAS test.

(a) *Introductory information*—(1) *Prerequisites.* (i) Water solubility.

(ii) The organic carbon content of the test material must be established.

(2) *Guidance information.* (i) Information on the relative proportions of the major components of the test material will be useful in interpreting the results obtained, particularly in those cases where the result lies close to the "pass level".

(ii) Information on the toxicity of the chemical may be useful to the interpretation of low results and in the selection of appropriate test concentrations.

(3) *Qualifying statements.* (i) The method is only applicable to those organic test materials which, at the concentration used in the test,

(A) Are soluble in water (at least 20 mg dissolved organic carbon/l).

(B) Have negligible vapour pressure.

(C) Are not inhibitory to bacteria.

(D) Do not significantly adsorb on glass surfaces.

(E) Are not lost by foaming from the test solution.

(ii) This test has been found suitable by the OECD Expert Group Degradation/Accumulation for determining the inherent biodegradability of organic chemicals under aerobic conditions.

(4) *Recommendations.* Test chemicals giving a result of greater than 20 per cent loss of DOC in this test may be regarded as inherently biodegradable, whereas a result of greater than 70 per cent loss of DOC is evidence of ultimate biodegradability. The use of a compound specific analytical technique on ^{14}C-labelled test substance may allow greater sensitivity. In these last cases a lower level may be regarded as evidence of inherent biodegradability.

(5) *Standard documents.* This Test Guideline has been based on the paper cited under paragraph (d) (1) of this section.

(b) *Method*—(1) *Introduction, purpose, scope relevance, application and limits of test.* (i)(A) The method is an adaptation of the Soap and Detergent Association semi-continuous activated sludge (SCAS) procedure for assessing the primary biodegradation of alkyl benzene sulfonate. The method involves exposure of the chemical to relatively high concentrations of micro-organisms over a long time period (possibly several months). The viability of the micro-organisms is maintained over this period by daily addition of a settled sewage feed.

(B) Because of the long detention period (36 hours) and the intermittent addition of nutrients the test does not simulate those conditions experienced in a sewage treatment plant. The results obtained with the test substance indicate that it has a high biodegradation potential, and for this reason it is most useful as a test of inherent biodegradability.

(c) Since the conditions provided by the test are highly favorable to the selection and/or adaptation of mircro-organisms capable of degrading the test compound, the procedure may also be used to produce acclimatized inocula for use in other tests. The test is applicable to water soluble, non-volatile, organic chemicals that are not inhibitory to bacteria at the test concentration.

(ii) *Reference substances.* In some cases when investigating a new substance reference substances may be useful; however, specific reference substances cannot yet be recommended. Data on several compounds used in ring tests are provided (see Annex) primarily so that calibration of the method may be performed from time to time and to permit comparison of results when another method is employed.

(iii) *Principle of the test method.* (A) Activated sludge from a sewage treatment plant is placed in an aeration (SCAS) unit. The test compound and settled domestic sewage are added, and the mixture is aerated for 23 hours. The aeration is then stopped, the sludge allowed to settle and the supernatant liquor is removed. The sludge remaining in the aeration chamber is then mixed with a further aliquot of test compound and sewage and the cycle is repeated.

(B) Biodegradation is established by determination of the dissolved organic carbon content of the supernatant liquor. This value is compared with that found for the liquor obtained from a control tube dosed with settled sewage only.

(iv) *Quality criteria*—(A) *Reproducibility.* The reproducibility of this modification of the method based on removal of dissolved organic carbon has not yet been established. When primary biodegradation is considered, very precise data is obtained for materials that are extensively degraded. The results reported in reference (1) suggest 95 per cent confidence limits of less than ±3 per cent, and this includes interlaboratory tests. As would be expected, wider confidence limits are obtained for less biodegradable materials.

(B) *Sensitivity.* The sensitivity of the method largely depends on the precision of the determination of dissolved organic carbon and the level of test compound in the liquor at the start of each cycle. At the end of the aeration period about 10 mg/liter of dissolved organic carbon remain in the supernatant liquor of the control experiment. Assuming that the dissolved organic carbon determination is within ±5 per cent and a level of 20 mg/litre of carbon as test material is added at the start of the aearation period, then the assessment of the extent of biodegradation should be within ±6 per cent for the range 80–100 per cent biodegradation.

(C) *Specificity.* The method is applicable to any non-volatile, water soluble, organic compound.

(D) *Possibility of standardization.* Since the method uses a feed of real settled sewage, absolute standardization is not possible unless this feed were replaced by an artificial one. However, since the method is designed to give an indication of the biodegradability potential of a chemical and is not a simulation test, such standardization is unnecessary.

(E) *Possibility of automation.* Automation of this method would be possible but would be expensive. As the method is not labor intensive, the exercise would offer few advantages.

(2) *Description of the test procedure*—(i) *Preparations.* (A) The aeration units are cleaned and fixed in a suitable support. The air inlet tubes are connected to the supply manifold. A small laboratory scale air compressor is used to aerate the units, and the air is presaturated with water to reduce evaporation losses from the units.

(B) A sample of mixed liquor from an activated sludge plant treating predominantly domestic sewage is obtained. Approximately 150 ml of the mixed liquor are required for each aeration unit.

(C) The organic carbon analyzer is calibrated using potassium hydrogen phthalate.

(D) Stock solutions of the test compounds are prepared: the concentration normally required is 400 mg/litre as organic carbon which gives a test compound concentration of 20 mg/litre carbon at the start of each aeration cycle if no biogradegradation is occurring.

(E) The organic carbon content of the stock solutions is measured.

(ii) *Test conditions.* A high concentration of aerobic micro-organisms is used, and the effective detention period is 36 hours. The carbonaceous material in the sewage feed is oxidized extensively within 8 hours of the start of each aeration cycle. Thereafter, the sludge respires endogeneously for the remainder of the aeration period, during which time the only available substrate is the test compound unless this is also readily metabolized. These features, combined with daily reinoculation of the test when domestic sewage is used as the medium, provide highly favorable conditions for both acclimatization and biodegradation.

(iii) *Performance of the test.* (A) A sample of mixed liquor from a suitable activated sludge plant is obtained and aerated during transportation to the laboratory. Each aeration unit is filled with 150 ml of mixed liquor and the aeration is started. After 23 hours, aeration is stopped, and the sludge is allowed to settle for 45 minutes. The tap is opened and 100 ml of the supernatant liquor withdrawn. A sample of settled domestic sewage is obtained immediately before use, and 100 ml are added to the sludge remaining in each aeration unit. Aeration is started anew. At this stage no test materials are added, and the units are fed daily with domestic sewage only until a clear supernatant liquor is obtained on settling. This usually takes up to two weeks, by which time the dissolved organic carbon in the supernatant liquor at the end of each aeration cycle should be less than 12 mg/litre.

(B) At the end of this period the individual settled sludges are mixed, and 50 ml of the resulting composite sludge are added to each unit.

(C) 100 ml of settled sewage are added to the control units and 95 ml plus 5 ml of the appropriate test compound stock solution (400 mg/l) to the test units. Aeration is started again and continued for 23 hours. The sludge is then allowed to settle for 45 minutes and the supernatant drawn off and analzed for dissolved organic carbon content.

(D) The fill and draw procedure under paragraph (b)(2)(iii)(A) of this section is repeated daily throughout the test.

(E) Before settling it may be necessary to clean the walls of the units to prevent the accumulation of solids above the level of the liquid. A separate scraper or brush is used for each unit to prevent cross contamination.

(F) Ideally, the dissolved organic carbon in the supernatant liquors is determined daily, although less frequent analysis is permissible. Before analysis the liquors are filtered through washed 0.45 μm membrane filters and certifuged. Temperature of the sample must not exceed 40° C while it is in the centrifuge.

(G) The length of the test for compounds, showing little or no biodegradation is indeterminate, but experience suggests that this should be at least 12 weeks.

(c) *Data and reporting*—(1) *Treatment of the results.* (i) The dissolved organic carbon results in the supernatant liquors of the test units and the control units are plotted against time. As biodegradation is achieved the level found in the test will approach that found in the control. Once the difference between the two levels is found to be constant over three consecutive measurements, 3 further measurements are made and the percentage biodegradation of the test compound is calculated by the following equation:

$$\%\ \text{biodegradation} = \frac{100\,[O_T - (O_t - O_c)]}{O_T}$$

Where

O_T = concentration of test compound as organic carbon added to the settled sewage at the start of the aeration period.

O_t = concentration of dissolved organic carbon found in the supernatant liquor of the test at the end of the aeration period.

O_c = concentration of dissolved organic carbon found in the supernatant liquor of the control

(ii) The level of biodegradation is therefore the percentage elimination of organic carbon, under the following Figure 1:

FIGURE 1.—EXAMPLES OF RESULTS OF SCAS TEST ON VARIOUS COMPOUNDS USED IN THE OECD/EEC RING TEST

Test compound	O_T (mg/l)	O_i-O_t (mg/l)	Percentage biodegradation/bioelimination
4-acetyl aminobenzene sulphonate	17.2	2.0	85
Tetra propylene benzene sulphonate	17.3	8.4	51.4
4–nitrophenal	16.9	0.8	95.3
Diethylene glycol	16.5	0.2	98.8
Aniline	16.9	1.7	95.9

Duration of test 40 days.

RESULTS FOUND FOR CYCLOPENTANE TETRA CARBOXYLATE

O_T (mg/l)	(O_i-O_t) (mg/l)	Percentage biodegradation/bioelimination
17.9	3.2	81.1

Duration of test 120 days.

(iii) If from the outset there is no difference between the control and the test, or the difference between the two remains constant at a level less than would be expected if no degradation had taken place, further tests are necessary to distinguish between biodegradation and adsorption. This may be done by using the supernatant liquors as a source of inoculum for tests such as the Sturm or the Closed Bottle Tests under §§ 796.3200 and 796.3260.

(d) *Literature references.* For additional background information on this test guideline the following references should be consulted:

(1) "A Procedure and Standards for the Determination of the Biodegradability of Alkyl Benzene Sulphonate and Linear Alkylate Sulphonate", *Journal of the American Chemical Society,* Vol. 42 (1965), p. 986.

(2) [Reserved]

§ 796.3360 Inherent biodegradability: Modified Zahn-Wellens test.

(a) *Introductory information*—(1) *Prerequisite.* Water solubility.

(2) *Guidance information.* (i) Information on the relative proportions of the major components of the test material will be useful in interpreting the results obtained, particularly in those cases where the result lies close to the "pass level".

(ii) Information on the toxicity of the chemical may be useful to the interpretation of low results and in the selection of appropriate test concentrations.

(3) *Qualifying statements.* (i) The method is only applicable to those organic test materials which, at the concentration used in the test:

(A) Are soluble in water to the extent necessary for the preparation of the test solutions.

(B) Have negligible vapour pressure.

(C) Are not inhibitory to bacteria.

(D) Do not significantly adsorb on glass surfaces.

(E) Are not lost by foaming from the test solution.

(ii) This test has been found suitable by the OECD Expert Group "Degradation/Accumulation" for determining the inherent biodegradability of organic chemicals under aerobic conditions. It has been tested in the OECD Laboratory Intercomparison Test Program (1978–1980).

(4) *Recommendations.* Test chemicals giving a result of greater than 20 percent loss of DOC (dissolved organic carbon) in this test may be regarded as inherently biodegradable, whereas a result of greater than 70 per cent loss of DOC is evidence of ultimate biodegradability. The use of a compound specific analytical technique on ^{14}C-labeled test substances may allow greater sensitivity. In these last cases a lower level may be regarded as evidence of inherent biodegradability.

(5) *Standard documents.* This Test Guideline is based on a modification of the method cited under paragraph (d) (1) and (2) of this section.

(b) *Method*—(1) *Introduction, purpose, scope, relevance, application and limits of test* (i)(A). The static test is a simple, reproducible method for evaluating the ultimate biodegradability of organic substances in water by micro-organisms in an aerobic milieu.

(B) The static method is limited to the examination of the biodegradability of water soluble, non-volatile organic compounds. The compounds to be studied are used in concentrations corresponding to DOC-values in the range of 50–400 mg/litre or COD-values in the range of 100–1000 mg/litre (DOC = dissolved organic carbon; COD = chemical oxygen demand).

(C) These relatively high concentrations have the advantage of analytical reliability. Compounds with toxic properties may delay or inhibit the degradation process.

(ii) *Definition and units.* (A) The amount of degradation attained at the end of the test is reported as the "Biodegradability in the Static Test":

$$D_T\ (\%) = 1 - \frac{(C_T - C_B)}{C_A} \cdot 100$$

D_T = biodegradation (%) at time T

C_A = initial value (DOC or COD values in the test mixture calculated from the DOC or COD values in the stock solution, [mg/l]

C_T = DOC or COD values at time of sampling, [mg/l]

C_B = DOC or COD value of the blank, [mg/l]

(B) The degradation rates are rounded to the nearest full percent.

(C) Percentage degradation is stated as the percentage DOC (or COD) removal of the tested substance.

(iii) *Reference substances.* In some cases when investigating a new substance reference substances may be useful, however specific reference substances cannot yet be recommended.

(iv) *Principle of the test method.* Activated sludge, mineral nutrients and the test material as the sole carbon source in an aqueous solution are placed together in a 1–4 litre glass vessel equipped with an agitator and an aerator. The mixture is agitated and aerated at 22 °C (±3°) under diffuse illumination or in a dark room for up to 28 days. The degradation process is monitored by determination of the DOC (or COD) values in the filtered solution at daily or other appropriate regular time intervals. The ratio of eliminated DOC (or COD) after each interval to the value at the start is expressed as percentage biodegradation and serves as the measure for the rate of degradation at this time. The result is plotted versus time to give the biodegradation curve.

(v) *Quality criteria*—(A) *Reproducibility.* Reproducibility has been proven in ring tests. Detection of <20 per cent; >20 to <70 per cent; >70 per cent DOC-removal as required for testing inherent biodegradability is possible.

(B) *Sensitivity.* The limits for sensitivity are given by the sensitivity of the carbon analysis (0.5–1 mg C/l) and the COD-analysis (5–10 mg O_2/l).

(C) *Specificity.* Applicable for tests with water soluble (>100 mg/l), non-volatile organic substances.

(D) *Possibility of standardization.* Standardization is possible.

(E) *Possibility of automation.* Automation of analysis is possible.

(2) *Description of the test procedure*—(i) *Preparations*—(A) *Reagents.* (*1*) Test water: drinking water with an organic-carbon content <5 mg/l. The concentration of calcium and magnesium ions together must not exceed 2.7 mole/l; otherwise adequate dilution with deionised or distilled water is required.

(*2*) Sulfuric acid, analytic reagent (A.R.) 50 g/l.

(*3*) Sodium hydroxide solution, A.R., 40 g/l.

(*4*) Mineral nutrient solution: dissolve in one litre deionized water:

38.5 g ammonium chloride, NH_4Cl, A.R.

33.4 g sodium dihydrogenphosphate, $NaH_2PO_4{\cdot}2H_2O$, A.R.

8.5 g potassium dihydrogenphosphate, KH_2PO_4, A.R.

21.75 g di-potassium monohydrogenphosphate K_2HPO_4, A.R.

The mixture serves both as a nutrient compound and as a buffering system.

(B) *Apparatus.* (*1*) Glass vessels with a volume of 1—4 litre (e.g., cylindrical vessels).

(*2*) Agitator with a glass or metal stirrer on a suitable shaft. (The stirrer should rotate about 5 to 10 cm above the bottom of the vessel.) A magnetic stirrer with a 7–10 cm long rod can be used instead.

(*3*) Glass tube of 2–4 mm inner diameter to introduce air. The opening of the tube should be about 1 cm above the bottom of the vessel.

(*4*) Centrifuge (at least 2000 rpm).

(*5*) pH-meter.

(*6*) O_2-measuring instrument.

(*7*) Paper filters.

(*8*) Membrane filtration apparatus.

(*9*) Membrane filters, pore size 0.2 μm.

(*10*) Analytical equipment for determining organic carbon content and chemical oxygen demand.

(C) *Preparation of the inoculum.* (*1*) Activated sludge from a biological treatment plant is washed by (repeatedly) centrifuging or settling with testwater under paragraph (b)(2)(i)(B)(*4*) of this section.

The activated sludge must be in an appropriate condition. Such sludge is available from a properly working sewage treatment plant. To get as many possible different species or strains of bacteria in special cases it may be preferred to make a mixture from different sources (e.g. different treatment plants, soil extracts, river waters, etc.). The mixture is to be treated as described under paragraph (b)(2)(i)(A) of this section.

(*2*) For checking the activity of the activated sludge see Functional control, under paragraph (b)(2)(ii)(E)(*1*) of this section.

(D) *Preparation of the test solutions.* (*1*) To the test vessel add 500 ml of test water 2.5 ml/litre mineral nutrient solution and activated sludge in an amount corresponding to 0.2–1.0 g/l (normally 0.2 or 1.0 g/l (The lower concentrations: 0.2 g dry matter of activated sludge/litre and a DOC-concentration of 50 mg/l are introduced to make the test instructions compatible with the instructions of the EMPA–Test (Switzerland) under paragraph(d)(4) of this section.)) dry matter in the final mixture. Add sufficient stock solution of the test material or of the waste water to be tested that a DOC concentration of 50–400 mg/l (normally 50 or 400 mg/l (The lower concentrations: 0.2 g dry matter of activated sludge/litre and a DOC-concentration of 50 mg/l are introduced to make the test instructions compatible with the instructions of the EMPA–Test (Switzerland) under paragraph(d)(4) of this section.)) results in the final mixture. The corresponding COD-values are 100–1000 mg/l. Make up with test water to a total volume of 1–4 litres. The total volume to be chosen is dependent on the number of samples to be taken for DOC or COD determination and the volumes necessary for the analytical procedure.

(*2*) Normally a volume of 2 litres can be regarded as satisfactory.

(*3*) At least one control vessel (blank) is set up to run in parallel with each test series: it contains only activated sludge and mineral nutrient solution made up with test water to the same total volume as in the test vessels.

(*4*) Note: Before starting the test it is advisable to make certain with appropriate methods that no inhibition occurs at the chosen concentration of test material. Run the test with a smaller concentration if an inhibitory effect is found.

(ii) *Performance of the test.* (A) The test vessels are agitated with magnetic stirrers or screw propellers under diffuse illumination or in a dark room at 22 °C (±3°). Aeration is accomplished by compressed air cleaned by a cotton wool strainer and a wash bottle if necessary. It must be ensured that the sludge does not settle and the oxygen concentration does not fall below 2 mg/l.

(B) The pH-value must be checked at regular intervals (for example daily) and adjusted to pH 7–8 with NaOH or H_2SO_4, if necessary.

(C) Losses from evaporation are made up just before each sampling with deionized or distilled water in the required amounts. The best procedure is to mark the liquid level on the vessel before starting the test and after each sampling (without aeration and stirring). The first samples are always taken 3 hours after the start of the test in order to detect adsorption of test material by the activated sludge.

(D) The elimination of the test material is followed by DOC- or COD-determinations made daily or at some other regular interval. The samples from the test vessel and the blank are filtered through a carefully washed paper filter. The first 5 ml of test solution-filtrate are returned to the test vessel. Sludges difficult to filter may be removed previously by centrifugation. DOC and COD determinations are made at least in duplicate. The test is run for up to 28 days.

(E) Note: Turbid remaining samples are filtered through membrane filters. The membrane filters must not release or adsorb any organic material. Otherwise they are to be purified by boiling in deionised water previously. For procedure in connection with adaptation processes, see paragraph (b)(2)(ii)(E)(2) of this section.

(*1*) *Functional control.* A vessel with a known substance should be run parallel with each test series in order to check the functional capacity of the activated sludge. For this purpose compounds, such as diethyleneglycol, sodium benzoate, and aniline, are recommended.

(*2*) *Adaptation.* (*i*) If analyses are carried out at relatively short intervals (e.g. daily), adaptation can be clearly recognized from the degradation curve (see Figure 2).

(*ii*) If the adaptation occurs in the final days of the test time, the test time can be prolonged until the degradation is finished.

(*3*) Note: If a broader knowledge of the behaviour of the adapted sludge is needed, the same activated sludge is exposed once again to the same test material in accordance with the following procedure:

(*i*) Switch off the agitator and the aerator and allow the activated sludge to settle. Draw off the supernatant liquid, fill up to 2 liters with test water, stir for 15 minutes and allow to settle again. After the supernatant liquid is drawn off again use the remaining sludge to repeat the test with the same test material in accordance with Preparation of test solutions and Performance of the test, above. The activated sludge can also be isolated by centrifuging instead of settling.

(*ii*) The adapted sludge may be mixed with fresh sludge to a total amount of 0.2–1 g dry weight/litre.

(iii) *Analytical means.* (A) Normally samples are filtered through a carefully washed paper filter (for washing use deionised water).

(B) Samples which remain are filtered through membrane filters (0.2 μm, diameter 25 mm). Membrane filters are suitable if it is assured that they neither release nor adsorb organic compounds. Otherwise the membrane filters must be purified from soluble organic material by boiling them 3 times in deionized water. The purified filters may be stored in water.

(C) The DOC concentration is determined twice in the sample filtrates (the first 5 ml are discarded) by means of the TOC instrument. If the filtrate cannot be analyzed on the same day, it must be stored in the refrigerator until the next day. Longer storage cannot be recommended.

(D) The COD concentration is determined in the sample filtrates with a COD analytical set up by the procedure described under paragraph (d)(3) of this section.

(c) *Data and reporting*—(1) *Treatment of results.* (i) DOC and COD concentrations are determined at least in duplicate in the samples according to Performance of the Test and Analytical means, above. The degradation at the time T is calculated according to the formula (with definitions) given under Definitions and units, under paragraph (b)(1)(ii) of this section.

(ii) The degradation rates are rounded to the nearest full percent. The amount of degradation attained at the end of the test is reported as the "Biodegradability in the Static Test".

(iii) Note: If complete degradation is attained before the test time is over and this result is confirmed by a second analysis on the next day, the test can be concluded.

(2) *Test report.* (i) The test report comprises information about:

(A) The test substance (name, structure, impurities, solubility, concentration, etc.).

(B) The inoculum (sampling of the inoculum, concentration, status of adaptation).

(C) The kind of analysis.

(D) The toxicity evaluations.

(E) The functional control (calibration compound).

(ii) The test results at different sampling times, are seen in the following example:

Evaluation Example

Organic compound: 4-Ethoxybenzoic acid
Theoretical test concentration: 600 mg/l
Theoretical DOC: 390 mg/l
Inoculum: Treatment plant of HOECHST AG in Frankfurt/M.-Höchst
Concentration: 1 g dry material/litre
Adaptation status: not adapted
Analysis: DOC-determination
Amount of sample: 3ml
Functional control: Diethyleneglycol
Toxicity of compound: No toxic effects below 1000 ppm (Gärröhrchentest)

Functional control				*Test compound*			
Sampling blank time	DOC [1] mg/l	DOC [1] mg/l	DOC net mg/l	Degrad. %	DOC [1] mg/l	DOC [1] net mg/l	Degrad. %
0			300			390	
3 h	4.0	298	294	2	371.6	367.6	6
1 d	6.1	288.3	282.2	6	373.3	367.2	6
2 d	5.0	281.2	276.2	8	360.0	355.0	9
5 d	6.3	270.5	264.2	12	193.8	187.5	52
6 d	7.4	253.3	245.9	18	143.9	136.5	65
7 d	11.3	212.5	201.2	33	104.5	93.2	76
8 d	7.8	142.5	134.7	55	58.9	51.1	87
9 d	7.0	35.0	28.0	91	18.1	11.1	97
10 d	18.0	37.0	19.0	94	20.0	2.0	99

[1] Mean values of triplicate determinations

(iii) A second part of the test report is made up by the biodegradation curve in the following Figures 1 and 2:

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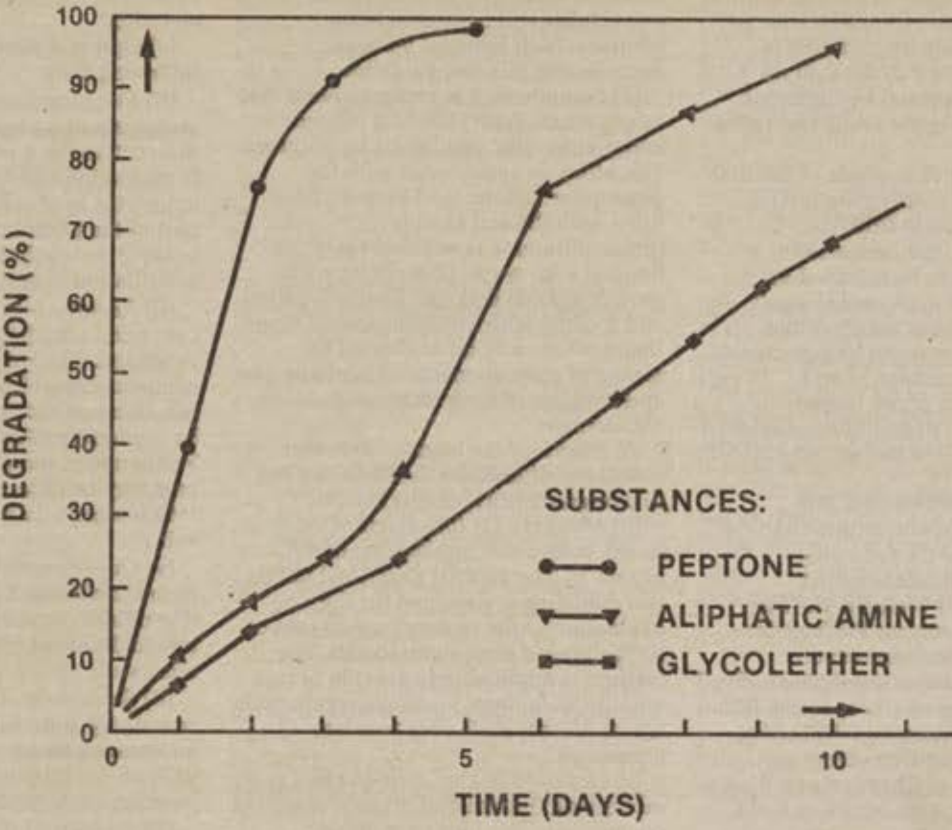


FIGURE 1 — STATIC TEST — EXAMPLES OF BIODEGRADATION CURVES

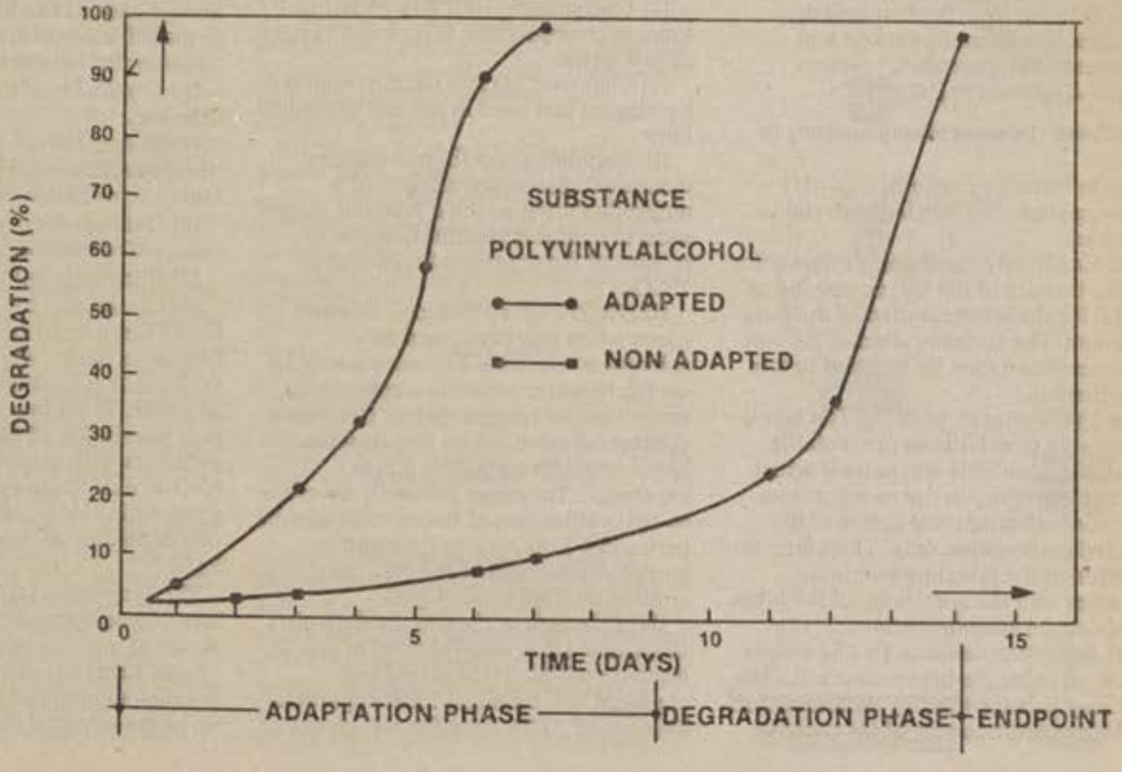


FIGURE 2 — STATIC TEST — EXAMPLES OF SLUDGE ADAPTATION

(3) *Interpretation/evaluation of results.* (i) The degree of biodegradation attained at the end of the test after 28 days or, if complete degradation is attained in less than 28 days, at an earlier time, is reported as "inherent biodegradability in the static test (after x days)".

(ii) If the result of analysis of the first sample (3 h after starting the test) is significantly different from the theoretical value, the amount of deficient DOC is to be reported as "adsorbed by the activated sludge".

(iii) The significant points of the degradation curve are to be reported as

(A) Adaptation-phase (days).

(B) Degradation-phase (days).

(C) Endpoint of degradation reached after * * * days (see paragraph (c)(2)(iii) of this section.

(d) *Literature references.* For additional background information on this test guideline the following references should be consulted:

(1) Zahn, R. und Wellens, H. "Ein einfaches Verfahren zur Prüfung der biologischen Abbaubarkeit von Produkten und Abwasserinhaltsstoffen." *Chemiker-Zeitung* 98 (1974) p. 228–232

(2) Umweltbundesamt: OECD-Ring-Test Programme on Detecting Biodegradability of Chemicals in Water, Berlin July 31, Test prescription No. 4, 1978.

(3) *Standard Methods for the Examination of Water and Waste Water*, 13th ed., (No. 220), (American Public Health Association, 1971) pp. 495–499.

(4) Schefer, W., "Bestimmung den biologischen Eliminierbackeit von Abwasser-Inhaltsstoffen," *Forum-Stadte-Hygiene* 110 (1978).

§ 796.3400 Inherent biodegradability in soil.

(a) *Introductory information*—(1) *Prerequisites.* ^{14}C-labeled material is required.

(2) *Guidance information.* Information on the toxicity of the test compound is useful for the interpretation of the data obtained. The concentration of the test compound can then be adapted to this information.

(3) *Qualifying statements.* The test is applicable to volatile or non-volatile, soluble or insoluble compounds which are not inhibitory to micro-organisms. The mineralization rate refers to the labeled carbonation only. Therefore, the location of the labelling within the structure and the specificity of the label need careful consideration.

(4) *Recommendations.* (i) The results obtained using the basic mineralization test may be supported by determination of the evaporation rate of the parent compound and some of possible volatile metabolites and by determination of soil extractable and nonextractable residues. Both optional tests are described in this test guideline.

(ii) Sometimes it is recommended that information about chemical degradation under anaerobic conditions be obtained. Therefore, in accordance with the description below, the biometer flask filled with the soil sample (preconditioning is not necessary), is flooded with water (2–3 cm layer) to protect against leakage, then evacuated and flushed with nitrogen several times. Degradation may be evaluated by means of measurements of methane gas and analysis of both water and soil for ^{14}C-content.

(5) *Standard documents.* This test guideline is based on the method cited in paragraph (d)(1) of this section.

(b) *Method*—(1) *Introduction, purpose, scope, relevance, application and limits of test.* (i) The method described in this test guideline is designed for the evaluation of the mineralization rate of a ^{14}C-labeled compound in soil. The method is applicable to volatile or non-volatile, soluble or insoluble compounds which are not inhibitory to micro-organisms.

(ii) *Definitions and units* (A) Soil is a mixture of mineral and organic chemical constituents, the latter containing compounds of high carbon and nitrogen content and of high molecular weights, animated by small (mostly micro-) organisms. Soil may be handled in two states:

(*1*) Undisturbed, as it has grown with time, in characteristic layers of a variety of soil types,

(*2*) Disturbed, as it is usually sampled by digging and used in the test described here.

(B) Mineralisation (in this context) means extensive degradation of a molecule during which a labelled carbon atom is oxidised quantitatively with release of the appropriate amount of $^{14}CO_2$.

(iii) *Reference substances.* In some cases when investigating a new substance reference substances may be useful; however, reference substances cannot yet be recommended. Reference substances need not be employed in all cases when investigating a new substance. They may primarily be used so that calibration of the method may be performed from time to time and to permit comparison of results when another method is employed.

(iv) *Principle of the test method*—(A) *Basic test.* (*1*) A small sample of soil is treated with the ^{14}C-labeled test chemical in a biometer flask apparatus. Release of $^{14}CO_2$ from the test chemical is measured by means of alkali absorption and liquid scintillation counting.

(*2*) Optional experiments include the following tests.

(B) *Evaporation test.* When testing chemicals of a vapour pressure higher than 0.0133 Pa, a polyurethane foam plug is placed into the biometer flask apparatus to absorb the labelled volatile part of the parent compound and volatile metabolites for liquid scintillation counting.

(C) *Residue test.* At the point of 50 per cent mineralization the test soil may be extracted. The extractable portion of the compound, and its metabolites remaining in the soil, may be determined by liquid scintillation counting. Furthermore, data on the bound residue part may be obtained by measuring the $^{14}CO_2$ released after combustion of the soil.

(v) *Quality criteria*—(A) *Reproducibility.* Reproducibility is good if standard conditions, especially preconditioning of the soil, are strictly observed.

(B) *Sensitivity.* The evaluation of sensitivity is not relevant because a moderate amount as 37–185 kBq (1–5 µCi) of ^{14}C-labelled test chemicals is used for each experiment.

(C) *Specificity.* The method is only applicable if ^{14}C-labeled test chemicals are available. The specificity is very good.

(D) *Possibility of standardization.* This procedure is standardized to a limited extent. The limitation is related to the difficulty of standardization of soil samples between laboratories.

(E) *Possibility of automation.* Not foreseen.

(2) *Description of the test procedure*—(i) *Preparations*—(A) *Equipment.* (*1*) Liquid scintillation counter.

(*2*) Oxidizer for combustion of radioactive material.

(*3*) Ultrasonic bath, 500 ml.

(*4*) Glassware: 250 ml Erlenmeyer flasks fused to 50 ml round bottom tubes (biometer flasks, see Figure 1); 25 ml syringes (e.g. Luer-lock); syringe needle 15 gauge, 15 cm in length; 100 µl syringes (e.g. Hamilton); 25 ml graduated cylinders with stopper; 1 ml pipettes; soxhlet extraction apparatus; scintillation vials; polyurethane plugs, 30 mm diameter, 30 mm length, density 16 kg/m^3.

(B) *Reagents*—(*1*) *Test substance:* ^{14}C-labelled compounds are dissolved in water or acetone to give radioactivity of 37–185 KBq (≃1 – 5 µCi)/100 µl. Using unlabelled material this solution is made up to the required concentration (e.g. 0.5

mg/100 μl≅10 mg/kg soil, or depending on the toxicity of the substance).

(*2*) *Chemicals.*

(*i*) KOH, analytical grade, 0.1 N solution.

(*ii*) Acetone, analytical grade.

(*iii*) Methanol, analytical grade (for optional tests).

(*iv*) n-Hexane, analytical grade (for optional test).

(*v*) Ascarite (A.H. Thomas Co. Philadelphia or equivalent).

(*vi*) Scintillation cocktail.

(*3*) *Soil.*

(*i*) Alfisol: pH between 5.5 and 6.5 organic C content between 1 and 1.5 percent clay content (i.e. particles <0.002 mm in diameter) between 10 and 20 percent cation exchange capacity between 10 and 15 mval.

(*ii*) Spodosol: pH between 4.0 and 5.0 organic C content between 1.5 and 3.5 per cent clay content <per cent cation exchange capacity <10 mval.

(*iii*) Entisol: pH between 6.6 and 8.0 organic C content between 1 and 4 per cent clay content between 11 and 25 per cent cation exchange capacity >10 mval.

In special cases it is recommended that two additional soils be used: one with high silt-fraction (Diameter between 0.002 and 0.063 mm) content, the other with a high clay content (30 per cent). Air dried test soil stored at +4 °C is remoisturized to 40 per cent maximum water capacity. After incubation for 2 weeks at 22 °C+2 °C in the dark it is ready for the experiments.

(ii) *Test conditions*—(A) *Test temperature.* During the whole test period the flasks are incubated in the dark at 22 °C±2 °C

(B) *Soil characterization data.* (*1*) for determination of the pH value of the soil for selecting the soil type, 10 g air-dried soil are suspended in 25 ml 0.01 M $CaCl_2$.

(*2*) After standing overnight the sample is disturbed once more and measured in a potentiometric apparatus with a 0.1 M KCl electrode. Immediately before the measurement the instrument must be calibrated with two standard solutions within the measuring range of the sample values expected.

(*3*) For determination of the organic carbon content of the soil for selecting the soil type, 1.0 g air-dried soil is heated with 15 ml 2M $K_2Cr_2O_7$ and 20 ml H_2SO_4 (analytical reagent, $\rho c=1.84$ g/cm³) at 145–155 °C for 15 minutes. After cooling to room temperature sample volume is made up to 150 ml with distilled water. A 20 ml aliquot is measured spectrophotometrically, after centrifuging, in a 1 cm cuvette at 590 nm compared to distilled water. The self-destroying property of the $K_2Cr_2O_7$ reagent must be determined by two blank samples. Calculation is conducted using the following equation:

$$C = \frac{\cdot 1000 \cdot v \cdot E_2(E_x - \alpha_2 \cdot c)}{e \cdot E_1 \cdot (\alpha_1 - \alpha_2 \cdot F)}$$

Where

C=carbon content (%)
V=gross volume (ml)
E_1=equivalent weight of Cr_2O_3 (25.332)
E_2=equivalent weight of carbon (3.0028)
E_x=extinction at 590 nm and 1 cm layer thickness
F=factor calculating $K_2Cr_2O_7$ from Cr_2O_3
c=concentration of Cr (g) per 100 ml (=1.9356)
e=sample weight (mg)
α_1=extinction coefficient of Cr (III) α_1 is an average value from five single determinations for the calibration curve, each obtained by division of E_x by the amount of Cr_2O_3 (in g)
α_2=extinction coefficient of Cr (VI) α_2 is an average value from two single estimations, each obtained by division of E_x by the respective amounts of $K_2Cr_2O_7$.

(*4*) For determination of particle size of the soil for selecting the soil type, 10.0 g air-dried soil are reacted with 100 ml H_2O_2 (15 per cent w/v for 15 hours, then heated until CO_2 evolution is complete. Afterwards the suspension is left to stand overnight with 25 ml 0.4 N $Na_4P_2O_7$, then water is added to make it up to 250 ml and the solution is sieved through a mesh of 0.2 mm width. The portion >0.2 mm is fractionated further by sieving. The smaller particles (silty fractions) are fractionated by homogenous partitioning of the particles in the aqueous medium, which is made up to 1000 ml with water in an elutriating cylinder.

(*5*) 10 ml portions are removed by pipette from various heights of the cylinder after different sedimentation times; measurement of the dry weights of the suspended material in these portions yields the particle composition according to the following scheme:

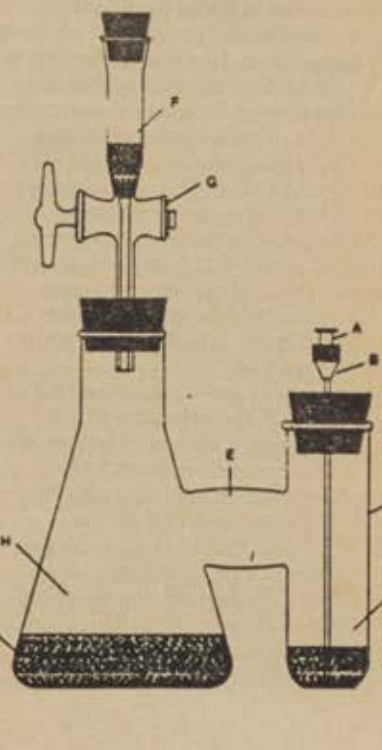
Particle fraction diameter (mm)	Dipping depth (cm)			
	20	15	10	5
<0.002			7h 45m	3h 52m
<0.0063	1h 33m 49s	1h 10m 52s	46m 55s	23m 27s
<0.02	9m 19s	6m 59s	4m 39s	2m 20s
<0.063	59s	42s		

(*6*) For determination of the cation exchange capacity of the soil, in order to select the soil type, a glass column 15 cm in length and 30 mm inner diameter is reduced in diameter at one end like a funnel. This side is stuffed with filter wool. About 1 cm quartz sand is strewn on the wool, followed by 10.0 g air-dried test soil, which is in turn covered by about 1 cm quartz sand. Above these layers comes 40 ml of a mixed solution [consisting of 100 g triethanolamine in 2 l water (adjusted to ph 8.1 with HCl) plus 100 g $BaCl_2 \cdot 2H_2O$ in 2 litres). After 1 h the solution is collected in an Erlenmyer flask of 250 ml. The procedure is then repeated. In addition, 40 ml of a solution of 25 g $BaCl_2 \cdot 2H_2O$ in 1 l are poured into the column.

(*7*) After standing overnight, this solution is also collected and the column is washed with 100 ml water. The combined eluates are titrated against HCl (bromocresol green plus methyl red as indicators) to measure H^+, Ca^{2+}, K^+, Na^+. For the determination of Ba^{2+} the column is leached in a similar mannner with 200 mil of 20 g $MgCl_2 \cdot 6H_2O$ in 1 litre water. This cation is determined by flame absorption spectrophotometry. The cation exchange capacity is expressed as the sum of all the cation equivalents sorbed by 100 g soil.

(iii) *Performance of test*—(A) *Basic test.* (*1*) Fifty grams of soil (dry weight basis) are placed into each Erlenmeyer part (H) of the biometer flask (see the following Figure 1). 100 μl of the radioactive test solution are added in 50 drops over the whole soil surface (I) of each flask. Then, the soil is carefully mixed with a Pasteur pipette (from which the lower part is cut off) and left in the flask.

Figure 1—Test flask



(*2*) In addition, an equivalent volume of test solution is placed in a 100 ml volumetric flask for direct determination of the added radioactivity.

(*3*) The biometer flask is closed with a teflon-coated silicon rubber stopper through which an Ascarite filter (F) is inserted. The filter (F) is provided with a stopper and stopcock (G). The side tube (C) is sealed with a teflon stopper pierced by a 15-gauge needle (B), 15 cm long. The needle (B) is capped by a silicone rubber stopper (A), and its tip at (D) is covered with a short length of silicone tubing that remains in contact with the base of the side tube (C).

(*4*) The unit is charged by injecting 10 ml of alkali solution into the side tube (C) in the following manner: the small stopper (A) is replaced by a calibrated Luer lock syringe containing 0.1 N KOH; then the filter stopper on (F) is removed and the stopcock (G) is opened; the alkali solution is introduced through the needle (B) into the side tube (C); then the stopcock is closed; the syringe is removed; the small stopper (A) and filter stopper on (F) are then returned to their initial positions. The ^{14}C-carbon dioxide produced is absorbed by the alkali.

(*5*) To recover the $^{14}CO_2$-loaded alkali for liquid scintillation analysis, the procedure for charging each parallel unit at increasing time intervals after start of the experiment is performed in reverse order. Thereafter the side tube (C) is rinsed with 5 ml alkali. Before recharging the side tube (C) fresh alkali, 3 volumes of 25 ml air are sucked through the system with the empty syringe to maintain the soil in an aerobic condition. A 1 ml aliquot of the alkali solution is taken for liquid scintillation counting.

(*6*) Experiment duration times of 1, 2, 4, 8, 16, 32 and—if necessary—64 days should be chosen for measurement. The test requires parallel determinations. The $^{14}CO_2$ radioactivity recovered is plotted versus time. This graph shows when to terminate the experiment. Incubation time is sufficient, when a total of 50 per cent CO_2 expressed as ^{14}C originally applied can be measured. Incubation should be stopped after reaching 64 days, whether or not this value is obtained.

(B) *Optional tests*—(*1*) *Estimation of evaporation.* If the volatility of a chemical is higher than 10^{-5} torr at 20 °C, it is recommended that a 3 cm diameter polyurethane foam plug be introduced into the arm E of the biometer flask. This plug absorbs the volatile parent compound as well as volatile organic degradation products but does not absorb $^{14}CO_2$. The plugs are extracted in a soxhlet extraction apparatus with an n-hexane/methanol mixture (1/4), and aliquots are taken for liquid scintillation counting.

(*2*) *Determination of soil-extractable and non-extractable residues.* (*i*) In cases of relatively persistent chemicals (50 per cent mineralisation in 10 days), further information concerning the soil-extractable radioactivity (parent compound plus degradation products) and soil bound residues is recommended.

(*ii*) For this purpose two further biometer flasks in addition to the four others must be prepared. At the point of 50 (or x-) percent mineralisation in the basic test, the soil in the two separate biometer flasks is extracted with 100 ml acetone (5 min ultrasonic treatment) followed by an extraction with methanol in the same manner. Aliquots of the combined extracts are taken for liquid scintillation counting. Other extract portions may be used—if necessary—for further identification studies.

(*iii*) Aliquots of the air dried soil are combusted to $^{14}CO_2$ and measured by liquid scintillation counting to determine the soil bound residues.

(c) *Data and reporting*—(1) *Treatment of results*—(i) *Basic test.* Radioactivity values for $^{14}CO_2$ (average of 4 parallel experiments) obtained from the alkali solution after 1, 2, 4, 8, 16, 32 and 64 days are expressed as the percentage of test chemical (radioactivity) initially applied and are plotted in a graph versus time. The time at which 50 per cent of the radioactivity is recovered as $^{14}CO_2$ is considered to be the "50% mineralisation" level. If this level has not been reached by the 64th day, the data at this time are taken and expressed as "x-percent-mineralisation".

(ii) *Evaporation test.* The radioactivity of vaporised (and trapped) original compound plus degradation products at the point of 50 (or x-) percent mineralisation is extracted, measured and interpreted as the percentage of volatilisation at the point of 50 (or x-) percent-mineralisation.

(iii) *Residue test.* Radioactivity values for extractable and non-extractable residues of the parent compound plus degradation products obtained after the extraction procedure of the soil at the point of 50 (or x-) percent mineralisation are given.

(2) *Test report.* The report of the degradability of a test chemical must include:

(i) Name of the test chemical, formula.

(ii) Amount applied, if not standard.

(iii) Exact characteristic data of the soil used.

(iv) Dates of the performance of the measurements.

(3) *Interpretation and evaluation of results.* The results are artificial because they are obtained with disturbed soil. However, since standardised soils are used, the test data are intercomparable and enable the experimentator to group relatively the chemicals tested within one scale for this property.

(d) *Literature references.* For additional background information on this test guideline the following references should be consulted:

(1) Bartha, R., and Pramer D., *Soil Science* 100: 68–70 (1965).

(2) Soil Survey Staff, U.S. Department of Agriculture, *Soil Taxonomy* Handbook No. 436, (Washington, DC, 1975).

(3) Butler, B.E., *Soil Classification for Soil Survey*. (Oxford, 1980) p. 129.

§ 796.3480 Complex formation ability in water.

(a) *Introductory information.* (Polarographic Method)

(1) *Prerequisites.* (i) Suitable analytical method.

(ii) Water solubility.

(iii) Dissociation constant.

(2) *Guidance information.* Structural fomula.

(3) *Qualifying statement.* (i) This method is applicable only if the water solubility is higher than 10^{-5} M.

(ii) This method should be applied to pure substances.

(iii) This method is not applicable to the determination of mercury complexes.

(4) *Standard documents.* This Test Guideline is based on the consensus method of polarography.

(b) *Method*—(1) *Introduction, purpose, scope, relevance, application and limits of test.* (i)(A) The ability of a new chemical to form soluble metal complexes may increase the availability to food chains of metals that might otherwise become inaccessible, e.g. by incorporation in sediments. This is of outstanding importance in the case of cadmium, copper, cobalt, chromium, lead and zinc. The ability of a substance to form complexes with the above metals can be assessed by means of polarographic techniques which allow the determination of stability constants for some complexes.

(B) The polarographic method can be applied to substances with a water solubility greater than 10^{-5} M. Direct measurement of the shift in half-wave potential $E_{1/2}$ can be made, provided that reactions at the dropping-mercury electrode occur reversibly. With suitable modifications, irreversible cases can occasionally be dealt with. For example, the Gelling's method under paragraph (d)(6) of this section can be applied to calculate $E_{1/2}$ values for a reversible process from irreversible reduction data. For quasi-reversible systems the method proposed by Grabaric et al. under paragraph (d)(6) of this section may be conveniently used.

(C) Two or more ions may be determined successively, if their half-wave potentials differ by at least 0.4 V for univalent ions and 0.2 V for bivalent ions, provided that their concentrations are approximately equal.

(D) When a substance forms a series of complexes with a metal ion, the determination of stability constants for intermediate species is also possible, but generally not necessary for environmental assessment. With strong chelating agents care must be taken not to overload the system (i.e. that not all metal is in the complex form).

(ii) *Definitions and units.* (A) The potential E of the dropping-mercury electrode is given by the equation

$$E = E^{\circ} - \frac{RT}{nF} \ln \frac{[M_a]}{[M_s]}$$

Where

E° is the standard electrode potential.
R is the gas constant,
T the absolute temperature,
n the number of electrons involved in the electrode process,
F the Faraday constant, and
$[M_a]$ and $[M_s]$ the concentrations of simple metals in the amalgam and at the electrode surface, respectively.

(B) The equation given above implies that the diffusion coefficients of the free metal ions and the complexed ones are equal.

(C) The stability constant for the complexation reaction

$$M^{n+} + pX^{b-} \rightleftharpoons MX_p(n-pb)+$$

is given by

$$K = \frac{[MX_p(n-pb)+]}{[M^{n+}][X^{b-}]^p}$$

Where

p is the co-ordination number.

(D) The complex formation constant depends on the temperature and diffusion coefficient.

(E) By combination of the above equations, the following relation is obtained, which is valid for the reversible reduction of a complex species at 25 °C:

$$E_{1/2} = E_m - \frac{0.0591}{n} \log K\,[X]\,P$$

Where

$E_{1/2}$ is the half-wave potential of the complex,
E_m that of the metal ion in the absence of complexing agents, and
[X] the concentration of the test compound.

(F) SI unit of the half-wave potential is volt [V].

(iii) *Reference substances.* The following reference substances need not be employed in all cases when investigating a new substance. They are primarily provided so that calibration of the method may be performed from time to time and to offer the chance to compare the results when another method is applied.

(A) ethylenediaminetetraacetic acid (EDTA).

(B) nitrilotriacetic acid (NTA).

(C) thioglycolic acid.

(D) o-nitrophenol.

(iv) *Principle of the test method.* This method is based on the fact that the reduction potentials of metal ions are shifted, usually to more negative values, as a result of complex formation. A positive shift can also occur, but only if another metal ion is present in the solution that is capable of binding to excess complex-forming agent. The potential at the point on the polarographic wave where the current is equal to one half of the diffusion current is termed the "half-wave potential" ($E_{1/2}$). This parameter is a characteristic constant for each metal ion. In the presence of a complex-forming substance the extent of the $E_{1/2}$ shift depends on its concentration as well as on the stability of the complex. Measurements of $E_{1/2}$ as a function of substance concentrations permit the calculation of both the formulae and the stability constants of some metal complexes. It is assumed that the $E_{1/2}$ shift is entirely due to complex formation, with no contribution from liquid junction potential.

(v) *Quality criteria*—(A) *Repeatability.* Under normal conditions (particularly when working with metal ions in the concentration range 10^{-3} to 10^{-4} M the repeatability can be as good as ±2 per cent. The temperature coefficient is about 1.5–2% per °C. Precise measurements therefore require temperature control to within ±0.2 °C.

(B) *Sensitivity.* This depends upon the particular procedure adopted. Generally, metal ion concentrations down to 10^{-5} M can be determined.

(C) *Specificity.* The method can be applied to substances which are capable of forming stable aqueous solutions at concentrations greater than 10^{-5} M.

(D) *Possibility of standardization.* Yes.

(E) *Possibility of automation.* Not known.

(2) *Description of the test procedure*—(i) *Preparations.* Suitable polarographic apparatus has been fully described in the literature under paragraph (d)(1) through (8) of this section.

(ii) *Test conditions.* (A) The mercury used in the dropping electrode should be at least redistilled commercial grade and should be filtered before use. Solutions to be tested need to be well-buffered under paragraph (d) (4) of this section. High-quality pure nitrogen should be used to de-oxygenate solutions for 10 to 15 minutes before determining the current-electrode potential curve.

(B) A minimum of four known concentrations of the chemical being tested should be investigated with a known concentration of metal ions. Solutions are most conveniently prepared directly within the polarographic cell by means of a precision burette. The chemical being tested should normally be present in at least a 25-fold excess over the metal ion concentrations so that its concentration at the electrode surface will be essentially the same as in the solution bulk. The current should be measured at applied potentials in the range −0.2 V to −1.0 V.

(C) In order to detect complexes which form slowly, it is necessary to allow the solutions to stand under a nitrogen atmosphere for a minimum period of 24 hours and, by re-examination of a sufficient number of samples, to ensure that complex formation was complete at the time of original measurements.

(D) The use of buffers and the use of surface-active substances to suppress polarographic maxima should be carefully considered, in order to avoid undesirable effects on the chemical reaction-controlled current as well as on the polarographic wave slope.

(iii) *Performance of the test.* The performance of the polarographic technique has been fully described in the literature paragraph (d) (1) through (8) of this section. The test should be run at 25 °C (±0.2 °C).

(e) *Data and reporting*—(1) *Treatment of results.* The stability constant K can be easily determined by plotting $E_{1/2}$ versus log [X]P and extrapolating to [X] = 1.0 M. If a straight line is obtained (whose slope is therefore 0.0591/n) the formation of a 1:1 complex is assumed. Smooth curves may be caused either by reaction irreversibility or by formation of two or more complex species in equilibrium with each other. In this last case it is necessary to apply the method by De Ford and Hume paragraph (d) (8) of this section to calculate stepwise formation constants.

(2) *Test report.* (i) The test report should list for each metal ion investigated the half-wave potential $E_{1/2}$, co-ordination number and overall stability constant.

(ii) In addition, the following should also be reported:

(A) Type of polarisable micro-electrode, the reference electrode and, in the case of the dropping mercury electrode, the flow rate in mg/sec and drop time.

(B) IR correction (if applied).

(C) Maxima suppressors (if used).

(D) Supporting electrolyte.

(E) Buffer.

(F) Temperature of measurement.

(G) Total ionic strength.

(H) Technique applied (pilot ion, standard addition, or other).

(I) Technical difficulties encountered.

(J) Estimate of accuracy.

(K) Polarographic technique employed (e.g., DC-, AC, polarography, single sweep polarography, radio frequency polarography or square-wave polarography).

(3) *Interpretation and evaluation of results.* (i) Stability constants determined for a new substance can be compared with literature values for standard substances (see Reference substances, above) and used therefore to evaluate the strength of its complexing ability.

(ii) The system is physically meaningful if (A) the value of the stability constant is positive and (B) the standard error is less than the constant (the t-test should be used as a criterion).

(iii) If data are not significant, it is necessary to use methods based on different physicochemical principles, such as spectrophotometry or nuclear magnetic resonance spectrometry.

(d) *Literature references.* For additional background information on this test guideline the following references should be consulted:

(1) Vogel, A.I., Basset, J. *Vogel's Textbook of Qualitative Inorganic Analysis.* Chapter XIX, 4th Ed. (Wiley: New York, 1978).

(2) Crow, D.R., Westwood, J.V., "The Study of Complexed Metal Ions by Polarographic Methods", *Quarterly Review 19:* 51 (1965).

(3) Irving, H., "The Stability of Metal Complexes and Their Measurement Polarographically," *Advances in Polarography Proceedings of the 2nd International Congress,* Ed. I.S. Langmuir (Pergamon Press, 1960).

(4) Perrin, D.D., Dempsey, B., *Buffer for pH and Metal Ion Controls.* (Chapman and Hall: London, 1974).

(5) "Stability Constants of Metal-ion Complexes," Part B, *Organic Ligands,* Compiled by D.D. Perrin, IUPAC Publication on Chemical Data Series, No. 22 (Pergamon Press, 1979)

(6) Grabaric, B., Tkalcec, M., Piljac, I., Filipovic, I., Simeon, V., "Numerical Evaluation of Complex Stability Constants from Polarographic Data for Quasi-Reversible Processes," *Analytica Chimica Acta 74*: 147, (1975).

(7) Piljac, I., Grabaric, B., Filipovic, I., "Improved Technique for Determination of Stability Constants by Polarographic Method," *Journal of Electroanalytical Chemistry Interfacial Electrochemistry, 42*:433 (1973).

(8) De Ford, D.D., Hume, D.N., "The Determination of Consecutive Formation Constants of Complex Ions from Polarographic Data," *American Chemical Society Journal, 73*:5321 (1951).

[FR Doc. 85-23077 Filed 9-26-85; 8:45 am]

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federal register

Friday
September 27, 1985

Part III

Department of Labor

**Employment Standards Administration,
Wage and Hour Division**

**Minimum Wages for Federal and
Federally Assisted Construction; General
Wage Determination Decisions; Notice**

DEPARTMENT OF LABOR

Employment Standards Administration, Wage and Hour Division

Minimum Wages for Federal and Federally Assisted Construction; General Wage Determination Decisions

General wage determination decisions of the Secretary of Labor specify, in accordance with applicable law and on the basis of information available to the Department of Labor from its study of local wage conditions and from other sources, the basic hourly wage rates and fringe benefit payments which are determined to be prevailing for the described classes of laborers and mechanics employed on construction projects of the character and in the localities specified therein.

The determinations in these decisions of such prevailing rates and fringe benefits have been made by authority of the Secretary of Labor pursuant to the provisions of the Davis-Bacon Act of March 3, 1931, as amended (46 Stat. 1494, as amended 40 U.S.C. 276a) and of other Federal statutes referred to in 29 CFR 5.1 (including the statutes listed at 36 FR 306 (1970) following Secretary of Labor's Order No. 24–70) containing provisions for the payment of wages which are dependent upon determination by the Secretary of Labor under the Davis-Bacon Act; and pursuant to the provisions of part 1 of subtitle A of title 29 of Code of Federal Regulations Procedure for Predetermination of Wage Rates, 48 FR 19533 (1983) and of Secretary of Labor's Orders 9–83, 48 FR 35736 (1983), and 6–84, 49 FR 32473 (1984). The prevailing rates and fringe benefits determined in these decisions shall, in accordance with the provisions of the foregoing statutes, constitute the minimum wages payable on Federal and federally assisted construction projects to laborers and mechanics of the specified classes engaged on contract work of the character and in the localities described therein.

Good cause is hereby found for not utilizing notice and public procedure thereon prior to the issuance of these determinations as prescribed in 5 U.S.C. 553 and not providing for delay in the effective date as prescribed in that section, because the necessity to issue construction industry wage determination frequently and in large volume causes procedures to be impractical and contrary to the public interest.

General wage determination decisions are effective from their date of publication in the **Federal Register** without limitation as to time and are to be used in accordance with the provisions of 29 CFR Parts 1 and 5. Accordingly, the applicable decision together with any modifications issued subsequent to its publication date shall be made a part of every contract for performance of the described work within the geographic area indicated as required by an applicable Federal prevailing wage law and 29 CFR Part 5. The wage rates contained therein shall be the minimum paid under such contract by contractors and subcontractors on the work.

Modifications and Supersedeas Decisions to General Wage Determination Decisions

Modifications and supersedeas decisions to general wage determination decisions are based upon information obtained concerning changes in prevailing hourly wage rates and fringe benefit payments since the decisions were issued.

The determinations of prevailing rates and fringe benefits made in the modifications and supersedeas decisions have been made by authority of the Secretary of Labor pursuant to the provisions of the Davis-Bacon Act of March 3, 1931, as amended (46 Stat. 1494, as amended, 40 U.S.C. 276a) and of other Federal statutes referred to in 29 CFR 5.1 (including the statutes listed at 36 FR 306 (1970) following Secretary of Labor's Order No. 24–70) containing provisions for the payment of wages which are dependent upon determination by the Secretary of Labor under the Davis-Bacon Act; and pursuant to the provisions of Part 1 of Subtitle A of Title 29 of Code of Federal Regulations Procedure for Predetermination of Wage Rates, 48 FR 19533 (1983) and of Secretary of Labor's Order 6–84, 49 FR 32473 (1984). The prevailing rates and fringe benefits determined in foregoing general wage determination decisions, as hereby modified, and/or superseded shall, in accordance with the provisions of the foregoing statutes, constitute the minimum wages payable on Federal and federally assisted construction projects to laborers and mechanics of the specified classes engaged in contract work of the character and in the localities described therein.

Modifications and supersedeas decisions are effective from their date of publication in the **Federal Register** without limitation as to time and are to be used in accordance with the provisions of 29 CFR Parts 1 and 5.

Any person, organization, or governmental agency having an interest in the wages determined as prevailing is encouraged to submit wage rate information for consideration by the Department. Further information and self-explanatory forms for the purpose of submitting this data may be obtained by writing to the U.S. Department of Labor, Employment Standards Administration, Wage and Hour Division, Office of Program Operations, Division of Wage Determinations, Washington, D.C. 20210. The cause for not utilizing the rulemaking procedures prescribed in 5 U.S.C. 553 has been set forth in the original General Determination Decision.

Modification to General Wage Determination Decisions

The numbers of the decisions being modified and their dates of publication in the **Federal Register** are listed with each State.

Arkansas: AR84–4090	Jan. 13, 1984.
California: CA85–5035	Sept. 6, 1985.
Iowa:	
IA84–4031	May 11, 1984.
IA84–4109	Dec. 21, 1984.
IA84–4042	June 15, 1984.
Kansas:	
KS84–4052	Aug. 24, 1984.
KS84–4053	Do.
KS84–4107	Dec. 14, 1984.
New Jersey:	
NJ85–3031	Aug. 2, 1985.
NJ85–3032	July 19, 1985.
New Mexico: NM85–4014	June 14, 1985.
Nevada: NV84–5014	June 8, 1984.
New York: NY85–3039	July 26, 1985.

Supersedeas Decision to General Wage Determination Decisions

The numbers of the decisions being modified and their dates of publication in the **Federal Register** are listed with each State. Supersedeas decision numbers are in parentheses following the numbers of the decisions being superseded.

Iowa: IA82–4044 (IA85–4040).	Aug. 27, 1982.
Maryland: MD85–3052 (MD85–3053).	Sept. 13, 1985.

Signed at Washington, D.C. this 20th day of September 1985.

James L. Valin,

Assistant Administrator.

BILLING CODE 4510-27-M

MODIFICATIONS P. 1

DECISION NO. AR84-4090 MOD. # 7 (49FR1844 - January 13, 1984)	Basic Hourly Rates	Fringe Benefits
Garland, Clark & Hot Springs Cos., Arkansas		
Change		
Bricklayers:		
Garland & Clark Cos.	$12.95	$1.09
Sprinkler fitters	14.80	3.40

DECISION NO. CA85-5035 - Mod. #1 (50 FR 36519 - September 6, 1985)	Basic Hourly Rates	Fringe Benefits
Imperial, Inyo, Kern, etc., Counties, CA		
Omit:		
Electricians:		
Area 3:		
Alarm Technician		

DECISION NO. KS84-4053 - MOD. # 7 - (49FR33784 - August 24, 1984	Basic Hourly Rates	Fringe Benefits
LEAVENWORTH CO., KANSAS		
CHANGE:		
PLUMBERS	19.61	2.90
PIPEFITTERS	18.99	3.52
SPRINKLER FITTERS	17.00	3.40

DECISION #IA84-4031-MOD.#1 (49 FR 20225-May 11, 1984)	Basic Hourly Rates	Fringe Benefits
POTTAWATTAMIE COUNTY, IOWA		
CHANGE:		
Boilermakers	$17.345	$3.50
Sheet Metal Workers	17.64	1.34
Sprinkler Fitters	17.00	3.40

DECISION #IA84-4109-MOD.#2 (49 FR 49811-Dec. 21, 1984)	Basic Hourly Rates	Fringe Benefits
SCOTT COUNTY, IOWA		
CHANGE:		
Boilermakers	$17.345	$3.50
Bricklayers	14.13	2.30
Sprinkler Fitters	17.00	3.40

DECISION #IA84-4042-MOD.#5 (49 FR 24855- June 15, 1984)	Basic Hourly Rates	Fringe Benefits
WOODBURY COUNTY, IOWA		
Cement Masons:		
On projects $750,000 over	$12.80	
On projects over $750,000	14.08	
Laborers:		
Group 1	8.30	$2.36
Group 2	8.45	2.36
Sheet Metal Workers	11.83	2.62

(2)

MODIFICATIONS P. 2

DECISION NO. KS84-4052- MOD. # 3 - (49FR33780 August 24, 1984	Basic Hourly Rates	Fringe Benefits
Sedgwick County, Kansas		
OMIT: LABORERS (SITE PREPARATION & GRADING: GROUPS 3 & 4 WAGE RATES AND DEFINITIONS		
CHANGE:		
ASBESTOS WORKERS	$15.88	$3.45
BOILERMAKERS	16.20	3.975
LABORERS (SITE PREPARATION AND GRADING):		
Group 1	6.35	2.30
Group 2	6.60	2.30
LINE CONSTRUCTION		
Lineman	16.97	1.25+ 3½%
Cable Splicers	17.17	1.25+ 3½%
Groundman	10.17	1.25+ 3½%
Powderman	13.97	1.25+ 3½%
Line Truck & Equipment Operators	13.97	1.25+ 3½%
PLASTERERS	14.25	
SPRINKLER FITTERS	17.00	3.40
TILE, MARBLE & TERRAZZO WORKERS	13.70	
TRUCK DRIVERS (SITE PREPARATION & GRADING):		
Group 1	9.40	1.75
Group 2	9.50	1.75
Group 3	9.65	1.75

Change: Laborers (Site Preparation & Grading) Definition for Groups 1 & 2 to Read:

Group 1: Board Mat Weavers & Cable Tiers, Georgia Buggy (Manually operated), Mixerman-No Skip Lift, Salamander Tenders, Track Men, Tractor Swamper, Truck Dumper, Wire Mesh Setter, Water Pump up to 4 inches, and all other general laborers including Flaggers.

Decision #KS84-4052(cont'd)	Basic Hourly Rates	Fringe Benefits
Laborers Definition Cont'd		
Group 2: Air Tool Operators, Cement Handler (Bulk), Chain Saw, Georgia Buggy (Mechanically Operated), Grademan, Hot Mastic Kettleman, Crusher Feeder, Joint Man, Jute Man, Mason Tender, Material Batch Hopper & Scale Man, Mixer Man, Pier Hole Man (Working 10 feet deep) Pipelayer - Drainage (Concrete and/or Corrugated Metal), Signal Man (Crane), Truck Dumper (Dry Batch), Vibrator Operator, Wagon & Churn Drill Operator, Asphalt Raker, Barco Tamper, Concrete Saw, Creosote Material (Handling and Applying), Nozzle Burner (Cutting Torch & Burning Bar), Conduit Pipe, Water and Gas Distribution Line, Tile and Duct Line Setter, Form Setter & Liner on Concrete Paving, Powderman, Sandblasting & Gunite Nozzleman, Sanitary Sewer Pipe Layer, Steel Plate Structure Erectors, Screed Man.		

(3)

	Basic Hourly Rates	Fringe Benefits
DECISION NO. NJ85-3031 MOD. NO. 6 (50 FR 31459 - August 2, 1985) Bergen, Essex, Hudson (excluding Ellis Island and Statue of Liberty Island), Hunterdon, Middlesex, Morris, Passaic, Somerset, Sussex, Union and Warren Counties, New Jersey		
OMIT:		
LABORERS:		
ZONE 18:		
(Heavy & Highway Const.):		
Group 2		
Power Tool Operators		
ADD:		
LABORERS:		
ZONE 18:		
Heavy & Highway Const:		
Group 1		
Flaggers		
Group 3		
Power Tool Operators		

	Basic Hourly Rates	Fringe Benefits
DECISION NO. NJ85-3032 MOD. NO. 5 (50 FR 29594 - July 19, 1985) Atlantic, Burlington, Camden, Cape May, Cumberland, Gloucester, Mercer, Monmouth, Ocean, and Salem Counties, New Jersey		
ADD:		
HEAVY & HIGHWAY CONST:		
LABORERS:		
ZONE 1		
Group 1		
Flaggers		

	Basic Hourly Rates	Fringe Benefits
DECISION NO. KS84-4107 - MOD. # 1 - (49FR48881 December 14, 1984) Shawnee Co., Kansas		
CHANGE:		
BRICKLAYERS	$14.89	$1.25
CARPENTERS	12.70	1.60
MILLWRIGHTS	13.70	1.80
SHEET METAL WORKERS	14.68	3.65+ 3%
SOFT FLOOR LAYERS	11.00	1.78+ 10%
SPRINKLER FITTERS	17.00	3.40
TILE SETTERS	15.50	

	Basic Hourly Rates	Fringe Benefits
DECISION NO. NV84-5014 - Mod. #14 (49 FR 23988 - June 8, 1984) Statewide (does not include the Test Range, or Building construction in Churchill, Lyon and Mineral Counties, or Highway construction in Douglas County), Nevada		
Change:		
Marble Setters:		
Area 1	$16.59	
Terrazzo Workers: Tile Setters:		
Area 1	16.59	

	Basic Hourly Rates	Fringe Benefits
DECISION NO. NY85-3039 MOD #1 BROOME & CHENANGO COUNTIES NEW YORK (50 FR 30591 July 26, 1985)		
CHANGE		
PLUMBERS & STEAMFITTERS	15.75	2.51

	Basic Hourly Rates	Fringe Benefits
DECISION #NM85-4014- MOD. #2 (49 FR 24997- June 14, 1985) Statewide (excluding Eddy and Lea Counties for Building Construction in New Mexico)		
CHANGE:		
Sprinkler Fitters	$16.66	$3.40

	Basic Hourly Rates	Fringe Benefits

SUPERSEDEAS DECISION

STATE: Iowa COUNTY: Webster
DECISION NO.: IA85-4040 DATE: Date of Publication
Supersedes Decision No. IA82-4044, dated August 27, 1982 in 47 FR 38032.
DESCRIPTION OF WORK: Building Projects (does not include single family homes and apartments up to and including 4 stories).

	Basic Hourly Rates	Fringe Benefits
BRICKLAYERS	$11.55	$ 1.77
CARPENTERS	8.84	
CEMENT MASONS	8.31	
ELECTRICIANS	10.10	1.30
IRONWORKERS	10.59	
LABORERS	5.79	
PAINTERS	6.00	
PLUMBERS & PIPEFITTERS	12.74	3.15
ROOFERS	$ 6.87	
SHEET METAL WORKERS	8.54	.86
SOFT FLOOR LAYERS	7.44	
SPRINKLER FITTERS	17.00	3.40

WELDERS: receive rate prescribed for craft performing operation to which the welding is incidental.

Unlisted classifications needed for work not included within the scope of the classifications listed may be added after award only as provided in the labor standards contract clauses (29 CFR, 5.5 (a) (1) (ii).

(6)

SUPERSEDEAS DECISION

STATE: MARYLAND COUNTIES: ALLEGANY AND GARRETT

DECISION NO.: MD85-3053 DATE: DATE OF PUBLICATION
Supersedes Decision No. MD85-3052 dated September 13, 1985 in 50 FR 37475

DESCRIPTION OF WORK: Building Construction (does not include single family homes and apartments up to and including 4 stories)

	Basic Hourly Rates	Fringe Benefits
ASBESTOS WORKERS	16.45	5.72
BOILERMAKERS	18.90	3.75
BRICKLAYERS, MARBLE MASONS STONE MASONS, TERRAZZO WORKERS, & TILE SETTERS	15.34	1.55
CARPENTERS	14.41	3.84
CEMENT MASONS & PLASTERERS	16.43	1.50
ELECTRICIANS:		
Allegany & Garrett (East of Rt. 219 at the Lake)	15.00	3.50+ 3.75%
Garrett County (West of Rt. 219 at the Lake)	15.40	3.50+ 3.75%
ELEVATOR CONSTRUCTORS:		
Elevator Constructors	16.46	3.29+ a&b
Elevator Constructors' Helpers	11.53	3.29+ a&b
Elevator Constructors' Probationary Helpers	8.235	
GLAZIERS	13.44	3.98
IRONWORKERS:		
Structural, Ornamental, & Reinforcing	15.60	3.55
LABORERS:		
Water Boy	10.33	3.70
Laborer, landscape worker, tool checker, dumpman, spotter, handyman	10.61	3.70
Power tool operator, mason & plaster tender, mortar mixer by hand, scaffold builder, mortar mixer by machine, hod carrier, concrete puddler rammer, grade checker, tamper, pipelayer, asbestos worker, burner, large wacker	10.78	3.70
Blaster/dynamite, air track driller, diamond bit driller	11.03	3.70
Tunnel workers: caisson, driller, mucker, laborer	11.63	3.70
Gunnite workers: nozzlemen, gun operator, laborer on scaffold, laborer on ground	10.78	3.70
LATHERS	9.25	.36
LINEMEN-Allegany and Garrett (East of Rt. 219)		
Linemen	14.20	2.20+ 3.75%
Equipment Operator	13.49	2.20+ 3.75%
Truck Driver & Groundmen	9.23	2.20+ 3.75%
LINEMEN-Garrett County (West of Rt. 219)		
Linemen	14.60	2.20+ 3.75%
Equipment Operator	13.89	2.20+ 3.75%
Truck Driver & Groundmen	9.63	2.20+ 3.75%
MILLWRIGHTS	14.92	3.84
PAINTERS:		
Group I		
Brush, wall covering hangers, installation of seamless type floors, use of flame burning and power tools	11.20	.50
Spray, roller, and sandblasting	12.20	.50
Toxic materials-brush and roller	11.70	.50
Toxic materials-spray	12.70	.50

(7)

DECISION NO.: MD85-3053

	Basic Hourly Rates	Fringe Benefits
PAINTERS (Continued):		
Group II		
Brush, roller, wall covering hangers, installation of seamless type floors	12.60	.50
Spray, sandblasting, use of flame burning and power tools	13.60	.50
Toxic materials-brush and roller		
Toxic materials-spray	14.10	.50
Group III		
Brush, wall covering hangers, installation of seamless type floors	13.60	.50
Spray, roller, sandblasting, use of flame burning and power tools	14.60	.50
Epoxy materials-brush	14.10	.50
Epoxy materials-roller and spray	15.10	.50
Group IV	13.10	.50
PILEDRIVERMEN	14.84	3.84
PLUMBERS & STEAMFITTERS	15.10	3.23

	Basic Hourly Rates	Fringe Benefits
POWER EQUIPMENT OPERATORS		
Group I	15.88	3.30+c
Hourly additional pay for long boom cranes (including jibs), pile driver machines with leads:		
130' to 169' plus $ .40		
170' to 209' plus .60		
210' to 249' plus .80		
250' to 299' plus 1.00		
300' & over plus 1.25		
Group II	15.31	3.30+c
Group III	14.72	3.30+c
Group IV	14.25	3.30+c
Group V	13.74	3.30+c
Group VI	13.26	3.30+c
ROOFERS:		
Composition	12.15	2.25
Mopmen-composition	12.40	2.25
Slaters	12.30	2.25
SHEET METAL WORKERS	16.28	2.42
SPRINKLER FITTERS	16.90	2.40
TRUCK DRIVERS:		
Group I	11.95	3.45
Group II	12.17	3.45
Group III	12.45	3.45
Group IV	12.70	3.45
Group V	13.04	3.45
Group VI	13.39	3.45
Group VII	13.75	3.45

FOOTNOTES:

a. Paid Holidays: New Year's Day, Memorial Day, Independence Day, Labor Day, Thanksgiving Day, and Christmas Day.

b. Employer contributes 6% of basic hourly rate for under 5 years of service and 8% of basic hourly rate for over 5 years of service as Vacation Pay Credit.

c. Paid Holidays: New Year's Day, Memorial Day, Independence Day, Labor Day, Veterans Day, Thanksgiving Day, and Christmas Day.

DECISION NO.: MD85-305

PAINTERS DEFINITIONS

Group I - Residential and Commercial Work - schools, college dormitories, churches, retail and wholesale outlet stores, apartment buildings, hospitals, banks, residential structures, and buildings of a like nature.

Group II - Light Industrial Work - new construction and remodeling in existing manufacturing plants, including repaint work in all areas not defined as hazardous, or over 50 feet in height.

Group III - Heavy Industrial and Hazardous Work - new industrial buildings under construction, power plants, hydro-electric plants, or any type of electric generating facility, boiler houses, and all other industrial work over 50 feet in height.

Group V - Dry Wall Work (finishing and taping)

DECISION NO.: MD85-3053

POWER EQUIPMENT OPERATORS CLASSIFICATION DEFINITIONS

Group I - Operators handling or setting steel, stone, pre-stressed concrete or machine. Tower Cranes.

Group II - Backfiller, backhoe, concrete mixing plants, batching plants, cable way, derrick, derrick boat, dragline, elevating grader, compressors (2 or more), space heaters, hoist (2 active drums or more), pile driving machine, power crane, power shovel, standard guage locomotive, trenching machine, tunnel mucking machine, shirley rig, certified welders, concrete paver, double concrete pump, front end loader (over 2 yds.), over welders top scale (more than 6, another man), Elimco type overhead loader, wellpoint systems, mighty midget with compressor, equipment foreman, twin engine scraper (25 yds. & over), mechanic, mechanic's welder, grader, all bulldozer, tire maintenance trucks.

Group III - Tractor with attachments (2 or more).

Group IV - Concrete mixer, concrete pump, one drum hoist, narrow guage locomotive, power roller, asphalt spreader, pumps (not exceeding 4), well drill, engine driven welders (not exceeding 4), single compressors (over 210 CFM), steam hammer, pile extractor, conveyor, stone crusher, hi-lift, excavating scoop, front end loader (up to & including 2 yds.), finishing machine, bull float, longitudinal float, screeding machine, concrete spreader, sub grader.

Group V - Fireman, truck crane oiler, wheel tractor, grease truck operator.

Group VI - Oiler, greaser, mechanic's helper, (singe compressor over 180 CFM and up to 210 CFM).

TRUCK DRIVERS CLASSIFICATION DEFINITIONS

Group I - Dumpmen.

Group II - Pick-ups, dump trucks (under 5 yds., capacity), straight trucks.

Group III - Helpers, panel trucks, straight trucks with multiple axle, dumpsters (under 5 yds. capacity), Transit mix, dumps (5 to 9 yds. capacity), flatbody material trucks (straight jobs), greasers, tiremen, mechanics' helpers, rubber-tired (towing & pushing flatbody vehicles), form trucks, dispatcher, yardmen.

Group IV - Dump trucks (10 to 15 yds. capacity).

Group V - Dump trucks (over 15 yds. capacity), button and end dump euclids, all other euclid type trucks, turnarockers, ross carriers, athey wagons, mechanics, semi-trailers or tractor-trailers, low boys, asphalt distributors, agitator mixer, dumpcretes or batch trucks, specialized earth moving equipment, off-highway tandem back-dump, twin engine equipment & double hitched equipment (where not self-loaded).

Group VI - All equipment listed in Group V over 49 tons to 59 tons.

Group VII - All equipment listed in Group V over 59 tons.

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federal register

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September 27, 1985

Part IV

Department of the Interior

Fish and Wildlife Service

50 CFR Part 17

Endangered and Threatened Wildlife and Plants; Review of Plant Taxa for Listing as Endangered or Threatened Species; Notice of Review

DEPARTMENT OF THE INTERIOR

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Endangered and Threatened Wildlife and Plants; Review of Plant Taxa for Listing as Endangered or Threatened Species

AGENCY: Fish and Wildlife Service, Interior.

ACTION: Notice of review.

SUMMARY: The Service issues an updated notice of vascular plant taxa native to the United States that are being reviewed for possible addition to the List of Endangered and Threatened Plants under the Endangered Species Act of 1973, as amended. Changes from previous notices primarily involve additions of taxa to and deletions of taxa from active consideration, changes in category for candidate taxa, and additions and deletions in State distributions. While it is prudent to take candidate taxa into account in environmental planning, none of the substantive or procedural provisions of the Act apply to a species that is designated as a candidate for listing.

DATE: Comments are requested until further notice.

ADDRESSES: Interested persons or organizations should submit comments to the appropriate Regional Director(s) below or to: Director (OES), 500 Broyhill Building, U.S. Fish and Wildlife Service, Washington, D.C. 20240. Comments and materials relating to this notice are available for public inspection by appointment during usual business hours at the Service's Office of Endangered Species, Suite 500, 1000 North Glebe Road, Arlington, Virginia.

Information relating to particular taxa may be obtained from the Endangered Species Coordinator(s) in the appropriate Service Regional Office(s) listed below:

Region 1.—California, Hawaii, Idaho, Nevada, Oregon, Washington, and Pacific territories
Regional Director (ARD/FA), U.S. Fish and Wildlife Service, Suite 1692, Lloyd 500 Building, 500 N.E. Multnomah Street, Portland, Oregon 97232, Telephone: 503/231-6131 (FTS: 8/429-6131);

Region 2.—Arizona, New Mexico, Oklahoma, and Texas
Regional Director (ARD/AFF), U.S. Fish and Wildlife Service, 500 Gold Avenue, S.W., Albuquerque, New Mexico 87103, Telephone: 505/766-3972 (FTS: 8/474-3972);

Region 3.—Illinois, Indiana, Iowa, Michigan, Minnesota, Missouri, Ohio, and Wisconsin
Regional Director (ARD/AFF), U.S. Fish and Wildlife Service, Federal Building, Fort Snelling, Twin Cities, Minnesota 55111, Telephone: 612/725-3276 (FTS: 8/725-3276);

Region 4.—Alabama, Arkansas, Florida, Georgia, Kentucky, Louisiana, Mississippi, North Carolina, South Carolina, Tennessee, Puerto Rico, and the Virgin Islands.
Regional Director (ARD/FA), U.S. Fish and Wildlife Service, The Richard B. Russell Federal Building, 75 Spring Street, S.W., Atlanta, Georgia 30303, Telephone: 404/221-3583 (FTS: 8/242-3583);

Region 5.—Connecticut, Delaware, District of Columbia, Maine, Maryland, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont, Virginia, and West Virginia
Regional Director (ARD/FA), U.S. Fish and Wildlife Service, Suite 700, One Gateway Center, Newton Corner, Massachusetts 02158, Telephone: 617/965-5100, ext. 316 (FTS: 8/829-9316, 7, 8);

Region 6.—Colorado, Kansas, Montana, Nebraska, North Dakota, South Dakota, Utah, and Wyoming
Regional Director (ARD/FA), U.S. Fish and Wildlife Service, P.O. Box 25486, Denver Federal Center, Denver, Colorado 80225, Telephone: 303/236-7398 (FTS: 8/776-7398);

Region 7.—Alaska
Regional Director (ARD/AFF), U.S. Fish and Wildlife Service, 1101 East Tudor Road, Anchorage, Alaska 99503, Telephone: 907/786-3435 (FTS: 8/907/786-3435).

FOR FURTHER INFORMATION CONTACT: Endangered Species Coordinator(s) in the appropriate Regional Office(s), or Mr. John L. Spinks, Jr., Chief, Office of Endangered Species, U.S. Fish and Wildlife Service, Washington, D.C. 20240, Telephone: 703/235-2771 (FTS: 8/235-2771).

SUPPLEMENTARY INFORMATION:

Background

The Endangered Species Act of 1973, as amended (Act), requires the Service to identify species of wildlife and plants that are endangered or threatened, based on the best available scientific and commercial data. Recognizing a special need to focus on the conservation of endangered and threatened plants, which had not previously been eligible for Federal protection, the 1973 Act directed the Secretary of the Smithsonian Institution to prepare a report on endangered and threatened plant species and to recommend necessary conservation measures. The Smithsonian report, published as House Document No. 94-51, included a list of more than 3,000 native taxa. The Service published a notice on July 1, 1975 (40 FR 27823), in which it announced that this report had been accepted as a petition under the terms of the Act, and that the taxa named in the report and notice were being reviewed for possible inclusion in the List of Endangered and Threatened Plants.

A revision of the Smithsonian's report was published in 1978 as a book: E.S. Ayensu and R.A. DeFilipps, *Endangered and Threatened Plants of the United States*, Smithsonian Institution and World Wildlife Fund, Washington, D.C.; this revision was also accepted as a petition for the taxa newly included therein (48 FR 6752). The July 1975 notice was superseded on December 15, 1980, by the Service's publication in the **Federal Register** (45 FR 82479) of a new comprehensive notice of review for native plants, which took into account the Smithsonian petitions and other accumulated information (*Endangered Species Technical Bulletin*, January, 1981). A petition on one additional species (*Serianthes nelsonii*) was accepted in the February 15, 1983, **Federal Register** (48 FR 6752). On November 28, 1983 (48 FR 53640), the Service published a supplement to its 1980 notice, noting changes in the status of various taxa.

Present Notice

This notice reflects the Service's current judgment of the possible vulnerability of all native candidate plant taxa. Taxa in the notice are assigned to several status categories, noted in the "Status" column of the table. Unlike previous plant notices, this one lists all taxa in one table, with coded entries to indicate current category. The codes are explained below:

LE—Taxa formally listed as endangered.

LE—Taxa formally listed as threatened.

PE—Taxa proposed to be formally listed as endangered.

PT—Taxa proposed to be formally listed as threatened.

S—Synonyms.

1—Taxa for which the Service currently has on file substantial information on biological vulnerability and threat(s) to support the appropriateness of proposing to list them as endangered or threatened species. Presently, data are being gathered concerning precise habitat needs and, for some of the taxa, concerning the precise boundaries for critical habitat designations. Development and publication of proposed rules on these taxa are anticipated, but, because of the large number of such taxa, could take some years.

Also included in category 1 are taxa whose status in the recent past is known, but that may already have become extinct. These plants may retain a high priority for addition to the List, subject to the confirmation of extant populations. Such possibly extinct taxa are indicated by an asterisk (*). Double asterisks (**) indicate taxa believed to be extinct in the wild, but known to be extant in cultivation.

2—Taxa for which information now in possession of the Service indicates that proposing to list them as endangered or threatened species is possibly appropriate, but for which substantial data on biological vulnerability and threat(s) are not currently known or on file to support the immediate preparation of rules. Such taxa that are are possibly extinct are again indicated by an asterisk (*). Taxonomically questionable taxa that are believed extinct in the wild, but are extant in cultivation, are marked by double asterisks (**). Further biological research and field study usually will be necessary to ascertain the status of the taxa in category 2, and some of the taxa are of uncertain taxonomic validity. It is likely that some of these will not warrant listing, while others will be found to be in greater danger of extinction than some taxa in category 1. It is hoped that this notice will encourage necessary research on vulnerability, taxonomy, and/or threats for these taxa. To organize and elaborate status information that may be submitted, contributors are encouraged to use the status report guidelines of Henifin *et al.*, pages 261-282 in L.E. Morse and M.S. Henifin, editors, *Rare Plant Conservation*, 1981, the New York Botanical Garden, Bronx, New York; copies of these guidelines are available from the Service.

3—Taxa that are no longer being considered for listing as threatened or endangered species. Such taxa are further coded to indicate three subcategories, depending on the reason(s) for removal from consideration.

3A—Taxa for which the Service has persuasive evidence of extinction. If rediscovered, however, such taxa might acquire high priority for listing. At this time, the best available information indicates that the taxa included in this subcategory, or the habitats from which they were known, are in fact extinct or destroyed, respectively.

3B—Names that, on the basis of current taxonomic understanding, usually as represented in published revisions and monographs, do not represent taxa meeting the Act's definition of "species." Such supposed taxa could be reevaluated in the future on the basis of subsequent research.

3C—Taxa that have proven to be more abundant or widespread than was previously believed and/or those that are not subject to any identifiable threat. Should further research or changes in land use indicate significant decline in any of these taxa, they may be reevaluated for possible inclusion in category 1 or 2.

The taxa in categories 1 and 2 of this notice are candidates for possible addition to the List of Endangered and Threatened Plants. The Service encourages their consideration in environmental planning, such as in environmental impact analysis under the National Environmental Policy Act of 1969 (implemented at 40 CFR Parts 1500-1508). Information regarding the range, status, and habitat needs of such species is available from the Service's Regional Offices (see "**ADDRESSES**" above) to assist in such planning.

The Service hereby requests that further information on the vulnerable taxa named in this notice be submitted as soon as possible and on a continuing basis. Especially sought are data for our files—

(1) Indicating that a taxon would more properly be assigned to a category other than the one in which it appears;

(2) Nominating a taxon not included;

(3) Recommending an area as critical habitat for a candidate taxon, or indicating that proposal of critical habitat would not be prudent for a taxon;

(4) Documenting threats to any of the included taxa;

(5) Informing the Service of the intensity and immediacy of threats to any of the taxa;

(6) Pointing out taxonomic or nomenclatural changes for any of the taxa;

(7) Suggesting appropriate common names; or

(8) Noting errors, such as any in the indicated historic distributions.

The Service intends to consider all information received in response to this notice, to amend the contents of categories 1, 2, and 3 to reflect current knowledge concerning affected plant taxa, and to indicate its intentions with regard to future listing actions (in accord with 50 CFR 424.15). Substantive changes will be announced by periodic supplemental or revised notices in the **Federal Register.**

Summary of Status Categories

For ease of reference, numerical totals for taxa included in the various status categories are provided below:

LE—88
LT—25
PE—25
PT—3
1—894
  1*—177
  1**—4
2—1623
  2*—27
  2**—1
3—1414
  3A—60
  3B—310
  3C—1044

Relationship To Petition Requirements

All candidate plant taxa (those in category 1 or 2 of this notice) are treated as under petition for listing, and this notice initiates the review of status required by section 4(b)(3)(A) of the Act, as amended in 1982.

Organization of Table

The following table is arranged alphabetically by names of genera and species. Synonyms have been provided when necessary to avoid confusion. In some cases, taxa have been included that have not yet been formally described in the scientific literature. Such taxa are identified by a generic name followed by "SP. (SSP., VAR.) NOV./INED." Following the scientific name of each species (subspecies, variety) are a family designation and any common name. Known historical ranges are given on the right for all included taxa, usually indicated by abbreviations for States. Some taxa may no longer occur in some of the areas shown. For each taxon, the assigned status category appears on the left.

This notice was prepared by Dr. John J. Fay in the Service's Office of Endangered Species in Washington (703/235-1975; FTS 8/235-1975), from evaluations by appropriate staff botanists or biologists in the Washington Office and in the Service's Endangered Species Program in Regional Offices and Field Stations. The Service gratefully acknowledges the computer assistance of Dr. Larry E. Morse, The Nature Conservancy, Arlington, Virginia, and Dr. John Nagy, Brookhaven National Laboratory, Upton, New York, in compiling the lists of taxa.

List of Subjects in 50 CFR Part 17

Endangered and threatened wildlife, Fish, Marine mammals, Plants (agriculture).

Dated: September 20, 1985.

P. Daniel Smith,

Acting Deputy Assistant Secretary for Fish and Wildlife and Parks.

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STATUS		SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
S		ABAMA AMERICANUM	*** SEE ***	NARTHECIUM AMERICANUM	
S		ABAMA MONTANA	*** SEE ***	NARTHECIUM AMERICANUM	
2		ABIES FRASERI	PINACEAE	FIR, FRASER	GA NC TN VA
1		ABRONIA ALPINA	NYCTAGINACEAE	SAND-VERBENA, ALPINE	CA
3C		ABRONIA BIGELOVII	NYCTAGINACEAE		NM
1		ABRONIA MACROCARPA	NYCTAGINACEAE		TX
3B		ABRONIA ORBICULATA	NYCTAGINACEAE		NV
2		ABRONIA UMBELLATA SSP. ACUTALATA	NYCTAGINACEAE	SAND-VERBENA, ROSE-PURPLE	WA
2		ABRONIA UMBELLATA SSP. BREVIFLORA	NYCTAGINACEAE		CA OR
3B		ABUTILON COMMUTATUM	MALVACEAE	TERCIOPELO	PR
1	*	ABUTILON EREMITOPETALUM	MALVACEAE	ABUTILON, HIDDEN-PETALED	HI
S		ABUTILON MARSHII	*** SEE ***	ALLOWISSADULA HOLOSERICEA	
PE		ABUTILON MENZIESII	MALVACEAE	KO'OLOA 'ULA	HI
1		ABUTILON SANDWICENSE	MALVACEAE		HI
2		ABUTILON VIRGINIANUM	MALVACEAE		PR VI, British V.I.
3B		ACACIA EMORYANA	FABACEAE	ACACIA, EMORY	TX
1		ACACIA KOAIA	FABACEAE	KOA OHA	HI
1		ACAENA EXIGUA	ROSACEAE	LILI-WAI	HI
2		ACANTHOMINTHA ILICIFOLIA	LAMIACEAE	THORN-MINT, SAN DIEGO	CA, Mexico
LE		ACANTHOMINTHA OBOVATA SSP. DUTTONII	LAMIACEAE	THORN-MINT, SAN MATEO	CA
3C		ACER GRANDIDENTATUM VAR. SINUOSUM	ACERACEAE		TX
1	*	ACHYRANTHES ATOLLENSIS	AMARANTHACEAE		HI
3A		ACHYRANTHES MUTICA	AMARANTHACEAE		HI
3B		ACHYRANTHES NELSONII	AMARANTHACEAE		HI
3B		ACHYRANTHES SPLENDENS VAR. REFLEXA	AMARANTHACEAE		HI
S		ACHYRANTHES SPLENDENS VAR. ROTUNDATA	*** SEE ***	ACHYRANTHES ROTUNDATA	HI
3C		ACHYRANTHES SPLENDENS VAR. SPLENDENS	AMARANTHACEAE		HI
2		ACLEISANTHES CRASSIFOLIA	NYCTAGINACEAE		TX, Mexico
LT		ACONITUM NOVEBORACENSE	RANUNCULACEAE	MONKSHOOD, WILD, NORTHERN	IA NY OH WI
S		ACTINEA HERBACEA	*** SEE ***	HYMENOXYS ACAULIS VAR. GLABRA	
S		ACTINELLA DEPRESSA	*** SEE ***	HYMENOXYS DEPRESSA	
S		ACTINOSTACHYS GERMANII	*** SEE ***	SCHIZAEA GERMANII	
1		ADENOPHORUS PERIENS	POLYPODIACEAE	FERN, PENDANT KIHI	HI
1	*	AERVA SERICEA	AMARANTHACEAE		HI
2		AESCHYNOMENE VIRGINICA	FABACEAE	JOINT-VETCH, SENSITIVE	DE MD NJ NC PA VA
1		AGALINIS ACUTA	SCROPHULARIACEAE	GERARDIA, SANDPLAIN	CT MA NY RI
2		AGALINIS AURICULATA	SCROPHULARIACEAE	FOXGLOVE, FALSE, AURICULATE	IL IN KS MI MN MO OH OK TN WI
1	*	AGALINIS CADDOENSIS	SCROPHULARIACEAE		LA
2		AGALINIS PSEUDAPHYLLA	SCROPHULARIACEAE		AL MS TN
2		AGALINIS PURPUREA VAR. CARTERI	SCROPHULARIACEAE		FL
2	*	AGALINIS STENOPHYLLA	SCROPHULARIACEAE	FALSE FOXGLOVE,	FL
3C		AGASTACHE CUSICKII	LAMIACEAE		ID NV OR
3C		AGASTACHE PARVIFOLIA	LAMIACEAE	AGASTACHE, SMALL-LEAVED	CA
LE		AGAVE ARIZONICA	LILIACEAE	AGAVE, ARIZONA	AZ
S		AGAVE CHISOSENSIS	*** SEE ***	AGAVE GLOMERULIFERA	
2		AGAVE EGGERSIANA	LILIACEAE		VI
2		AGAVE GLOMERULIFERA	LILIACEAE		TX
3C		AGAVE MCKELVEYANA	LILIACEAE		AZ
2		AGAVE PARVIFLORA	LILIACEAE		AZ
2		AGAVE SCHOTTII VAR. TRELEASEI	LILIACEAE		AZ
3C		AGAVE TOUMEYANA VAR. BELLA	LILIACEAE		AZ
3C		AGAVE UTAHENSIS VAR. EBORISPINA	LILIACEAE		CA NV
3C		AGAVE UTAHENSIS VAR. KAIBABENSIS	LILIACEAE		AZ
3C		AGAVE UTAHENSIS VAR. NEVADENSIS	LILIACEAE		CA NV
S		AGERATINA SHASTENSIS	*** SEE ***	EUPATORIUM SHASTENSE	
2		AGRIMONIA INCISA	ROSACEAE		FL MS SC
2		AGROSTIS ARISTIGLUMIS	POACEAE	BENT GRASS, AWNED	CA
2		AGROSTIS BLASDALEI VAR. BLASDALEI	POACEAE	BENT GRASS, BLASDALE'S	CA
2		AGROSTIS BLASDALEI VAR. MARINENSIS	POACEAE	BENT GRASS, MARIN	CA
2		AGROSTIS CLIVICOLA VAR. CLIVICOLA	POACEAE	BENT GRASS, COASTAL BLUFF	CA
2		AGROSTIS CLIVICOLA VAR. PUNTA-REYESENSIS	POACEAE	BENT GRASS, POINT REYES	CA
S		AGROSTIS HENDERSONII	*** SEE ***	AGROSTIS MICROPHYLLA VAR. HENDERSONII	
3C		AGROSTIS HOWELLII	POACEAE		OR
2		AGROSTIS MICROPHYLLA VAR. HENDERSONII	POACEAE	BENT GRASS, HENDERSON'S	CA OR
2		AGROSTIS ROSSIAE	POACEAE	BENT GRASS, ROSS	WY
S		AJANIA SENJAVINENSIS	*** SEE ***	ARTEMISIA SENJAVINENSIS	
1		ALECTRYON MACROCOCCUM	SAPINDACEAE	MAHOE,	HI
3C		ALECTRYON MAHOE	SAPINDACEAE	MAHOE,	HI
3C		ALETES FILIFOLIUS	APIACEAE		NM TX
2		ALETES HUMILIS	APIACEAE		CO
3B		ALLIONIA CRISTATA	NYCTAGINACEAE		AZ
1		ALLIUM AASEAE	LILIACEAE	ONION, AASE	ID
2		ALLIUM DICTUON	LILIACEAE	ONION, BLUE MOUNTAIN	WA
2		ALLIUM DOUGLASII VAR. CONSTRICTUM	LILIACEAE		WA
2		ALLIUM FIMBRIATUM VAR. MUNZII	LILIACEAE	ONION, MUNZ'S	CA
1		ALLIUM GOODDINGII	LILIACEAE		AZ NM
1		ALLIUM HICKMANII	LILIACEAE	ONION, HICKMAN'S	CA
2		ALLIUM HOFFMANII	LILIACEAE	ONION, BEEGUM	CA
3C		ALLIUM MADIDUM	LILIACEAE		ID OR
2		ALLIUM OXYPHILUM	LILIACEAE		VA WV
3C		ALLIUM PASSEYI	LILIACEAE	ONION, PASSEY'S	UT
3C		ALLIUM PERDULCE VAR. SPERRYI	LILIACEAE		TX

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
3B	ALLIUM PLEIANTHUM	LILIACEAE		OR
3C	ALLIUM ROBINSONII	LILIACEAE		OR WA
1	ALLIUM SANBORNII VAR. TUOLUMNENSE	LILIACEAE		CA
3C	ALLIUM SCILLOIDES	LILIACEAE		WA
2	ALLIUM SPECULAE	LILIACEAE		AL
3C	ALLIUM TOLMIEI VAR. PERSIMILE	LILIACEAE		ID
3C	ALLIUM YOSEMITENSE	LILIACEAE	ONION, YOSEMITE	CA
3C	ALLOWISSADULA HOLOSERICEA	MALVACEAE		TX
3C	ALNUS MARITIMA	BETULACEAE	ALDER, SEASIDE	DE MD OK
2	ALOPECURUS AEQUALIS VAR. SONOMENSIS	POACEAE	ALOPECURUS, SONOMA	CA
3C	ALPHITONIA PONDEROSA	RHAMNACEAE	KAUILA	HI
1	ALSINODENDRON OBOVATUM	CARYOPHYLLACEAE		HI
1	ALSINODENDRON TRINERVE	CARYOPHYLLACEAE		HI
1	* ALSINODENDRON VISCOSUM	CARYOPHYLLACEAE		HI
2	ALSOPHILA BROOKSII	CYATHEACEAE		PR, Cuba, Hispaniola
1	ALSOPHILA DRYOPTEROIDES	CYATHEACEAE		PR
1	AMARANTHUS BROWNII	AMARANTHACEAE		HI
2	AMARANTHUS PUMILUS	AMARANTHACEAE	PIGWEED, SEA-BEACH	DE MA MD NC NJ NY RI SC VA
1	AMBROSIA CHEIRANTHIFOLIA	ASTERACEAE	RAGWEED,	TX Mexico
2	AMBROSIA LINEARIS	ASTERACEAE		CO
2	AMBROSIA PUMILA	ASTERACEAE	AMBROSIA, SAN DIEGO	CA
3C	AMMOBROMA SONORAE	LENNOACEAE	SANDFOOD	AZ CA Mexico
3C	AMOREUXIA WRIGHTII	COCHLOSPERMACEAE		TX Mexico
3B	AMORPHA BRACHYCARPA	FABACEAE		MO
LE	AMORPHA CRENULATA	FABACEAE	LEAD-PLANT, CRENULATE	FL
1	AMORPHA OUACHITENSIS	FABACEAE	FALSE INDIGO,	AR OK
2	AMORPHA ROEMERANA	FABACEAE		TX
S	AMORPHA TEXANA	*** SEE ***	AMORPHA ROEMERANA	TX
2	AMPHIANTHUS PUSILLUS	SCROPHULARIACEAE	AMPHIANTHUS, LITTLE	AL GA SC
2	AMSINCKIA CARINATA	BORAGINACEAE		OR
2	AMSINCKIA FURCATA	BORAGINACEAE		CA
LE	AMSINCKIA GRANDIFLORA	BORAGINACEAE	FIDDLENECK, LARGE-FLOWERED	CA
2	AMSINCKIA VERNICOSA	BORAGINACEAE		CA
S	AMSINCKIA VERNICOSA VAR. FURCATA	*** SEE ***	AMSINCKIA FURCATA	
2	AMSONIA GLABERRIMA	APOCYNACEAE		TX
2	AMSONIA GRANDIFLORA	APOCYNACEAE		AZ, Mexico
1	AMSONIA KEARNEYANA	APOCYNACEAE		AZ
2	AMSONIA LUDOVICIANA	APOCYNACEAE	BLUE-STAR, LOUISIANA	LA MS
3C	AMSONIA PALMERI	APOCYNACEAE		AZ
2	AMSONIA PEEBLESII	APOCYNACEAE		AZ
3C	AMSONIA REPENS	APOCYNACEAE		TX
2	AMSONIA TABERNAEMONTANA VAR. GATTINGERI	APOCYNACEAE		TN
1	AMSONIA THARPII	APOCYNACEAE		TX
LE	ANCISTROCACTUS TOBUSCHII	CACTACEAE	FISHHOOK CACTUS, TOBUSCH	TX
3C	ANDRACHNE ARIDA	EUPHORBIACEAE		TX
3C	ANDROPOGON ARCTATUS	POACEAE	BEARD GRASS,	AL FL
3C	ANEMONE EDWARDSIANA VAR. EDWARDSIANA	RANUNCULACEAE		TX
2	ANEMONE EDWARDSIANA VAR. PETRAEA	RANUNCULACEAE		TX
3C	ANEMONE MINIMA	RANUNCULACEAE	ANEMONE,	VA WV
3C	ANEMONE OREGANA VAR. FELIX	RANUNCULACEAE		OR WA
3C	ANGELICA CALLII	APIACEAE	ANGELICA, CALL'S	CA
1	ANGELICA SCABRIDA	APIACEAE		NV
3C	ANGELICA WHEELERI	APIACEAE		UT
2	ANGURIA COOKIANA	CUCURBITACEAE	ALGODONCILLO	PR
3C	ANODA ABUTILOIDES	MALVACEAE	FALSE INDIAN-MALLOW	AZ, Mexico
S	ANODA PYGMAEA	*** SEE ***	FRYXELLIA PYGMAEA	
2	ANTENNARIA ARCUATA	ASTERACEAE	PUSSYTOES, MEADOW	ID NV WY
2	ANTENNARIA AROMATICA	ASTERACEAE		WY
1	ANTENNARIA SOLICEPS	ASTERACEAE		NV
3C	ANTENNARIA SUFFRUTESCENS	ASTERACEAE	EVERLASTING, EVERGREEN	CA OR
2	ANTHERICUM CHANDLERI	LILIACEAE		TX, Mexico
3B	ANTIDESMA CRENATUM	EUPHORBIACEAE		HI
2	ANTIRHEA PORTORICENSIS	RUBIACEAE	QUINA	PR
3C	ANTIRRHINUM SUBCORDATUM	SCROPHULARIACEAE	SNAPDRAGON, DIMORPHIC	CA
3C	APACHERIA CHIRICAHUENSIS	CROSSOSOMATACEAE		AZ NM
2	APHANISMA BLITOIDES	CHENOPODIACEAE		CA
1	APIOS PRICEANA	FABACEAE	POTATO-BEAN (GROUNDNUT), PRICE'S	AL IL KY MS TN
S	APLOPAPPUS SALICINUS	*** SEE ***	HAPLOPAPPUS SALICINUS	
2	APOCYNUM JONESII	APOCYNACEAE	DOGBANE, JONES'	AZ
S	AQUILEGIA AUSTRALIS	*** SEE ***	AQUILEGIA CANADENSIS VAR. AUSTRALIS	
3C	AQUILEGIA BARNEBYI	RANUNCULACEAE		CO UT
3B	AQUILEGIA CAERULEA VAR. DAILEYAE	RANUNCULACEAE		CO
2	AQUILEGIA CANADENSIS VAR. AUSTRALIS	RANUNCULACEAE	COLUMBINE, CANADIAN,	FL
3C	AQUILEGIA CHAPLINEI	RANUNCULACEAE	COLUMBINE,	NM TX
3C	AQUILEGIA CHRYSANTHA VAR. RYDBERGII	RANUNCULACEAE	COLUMBINE,	AZ CO NM
3C	AQUILEGIA DESERTORUM	RANUNCULACEAE		AZ
2	AQUILEGIA HINCKLEYANA	RANUNCULACEAE	COLUMBINE, HINCKLEY'S	TX
3C	AQUILEGIA JONESII	RANUNCULACEAE	COLUMBINE, JONES'	MT WY, Canada (Alta.)
3C	AQUILEGIA LARAMIENSIS	RANUNCULACEAE	COLUMBINE, LARAMIE	WY
3B	AQUILEGIA LONGISSIMA	RANUNCULACEAE	COLUMBINE, LONG SPUR	Mexico
2	* AQUILEGIA MICRANTHA VAR. MANCOSANA	RANUNCULACEAE	COLUMBINE,	CO
3C	AQUILEGIA SAXIMONTANA	RANUNCULACEAE		CO

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
3C	ARABIS ACULEOLATA	BRASSICACEAE		CA OR
3C	ARABIS BLEPHAROPHYLLA	BRASSICACEAE	ROCK CRESS, COAST	CA
2	ARABIS BREWERI VAR. AUSTINIAE	BRASSICACEAE		CA
2	ARABIS BREWERI VAR. PECUNIARIA	BRASSICACEAE	ROCK CRESS, SAN BERNARDINO	CA
2	ARABIS CONSTANCEI	BRASSICACEAE	ROCK CRESS, CONSTANCE'S	CA
3C	ARABIS CRANDALLII	BRASSICACEAE		CO WY
3C	ARABIS DEMISSA VAR. LANGUIDA	BRASSICACEAE	ROCK CRESS, DAGGETT,	UT WY
3C	ARABIS DEMISSA VAR. RUSSEOLA	BRASSICACEAE	ROCK CRESS, DAGGETT,	CO UT WY
3C	ARABIS DISPAR	BRASSICACEAE		CA NV
3B	ARABIS FRUCTICOSA	BRASSICACEAE	ROCK CRESS, FRUIT	WY
2	ARABIS GEORGIANA	BRASSICACEAE		AL GA
3C	ARABIS GRACILIPES	BRASSICACEAE		AZ
3C	ARABIS GUNNISONIANA	BRASSICACEAE	ROCK CRESS,	CO
1	* ARABIS HOFFMANNII	BRASSICACEAE	ROCK CRESS, HOFFMANN'S	CA
1	ARABIS JOHNSTONII	BRASSICACEAE	ROCK CRESS, JOHNSTON'S	CA
2	ARABIS KOEHLERI VAR. KOEHLERI	BRASSICACEAE	ROCK CRESS, KOEHLER'S	OR
2	ARABIS KOEHLERI VAR. STIPITATA	BRASSICACEAE		OR
LE	ARABIS MCDONALDIANA	BRASSICACEAE	ROCK CRESS, MCDONALD'S	CA
2	ARABIS MISSOURIENSIS VAR. DEAMII	BRASSICACEAE		IN MI MO WI
3C	ARABIS MODESTA	BRASSICACEAE	ROCK CRESS, MODEST	CA OR
3C	ARABIS OREGANA	BRASSICACEAE		CA OR
3C	ARABIS OXYLOBULA	BRASSICACEAE	ROCK CRESS, .	CO
2	ARABIS PARISHII	BRASSICACEAE	ROCK CRESS, PARISH'S	CA
1	* ARABIS PERSTELLATA VAR. AMPLA	BRASSICACEAE	ROCK CRESS, LARGE	TN
1	ARABIS PERSTELLATA VAR. PERSTELLATA	BRASSICACEAE	ROCK CRESS, SMALL	AL KY
3C	ARABIS PETIOLARIS	BRASSICACEAE		TX
2	ARABIS PINZLIAE	BRASSICACEAE	ROCKCRESS, PINZL'S	CA
2	ARABIS PUSILLA	BRASSICACEAE		WY
3C	ARABIS PYGMAEA	BRASSICACEAE		CA
2	ARABIS SEROTINA	BRASSICACEAE		VA WV
2	ARABIS SERPENTINICOLA	BRASSICACEAE		CA OR
3C	ARABIS SHOCKLEYI	BRASSICACEAE	ROCK CRESS, SHOCKLEY'S	CA NV UT
2*	ARABIS SP. NOV. /INED.	BRASSICACEAE	ROCK CRESS (GRAY KNOLLS, UINTAH CO.)	UT
2	ARABIS SP. NOV. /INED. (DEL NORTE, CURRY COS.)	BRASSICACEAE		CA OR
3C	ARABIS SP. NOV. /INED. (JONES HOLE, UINTA CO.)	BRASSICACEAE	ROCK CRESS (JONES HOLE, UINTAH CO.)	UT
2	ARABIS SUFFRUTESCENS VAR. HORIZONTALIS	BRASSICACEAE		OR
S	ARABIS SUFFRUTESCENS VAR. PERSTYLOSA	*** SEE ***	ARABIS CONSTANCEI	
2	ARABIS WILLIAMSII	BRASSICACEAE		WY
3C	ARCEUTHOBIUM APACHENSE	LORANTHACEAE		AZ
2	ARCTOMECON CALIFORNICA	PAPAVERACEAE	DESERT-POPPY,	AZ NV
LE	ARCTOMECON HUMILIS	PAPAVERACEAE	BEAR-POPPY, DWARF	UT
3C	ARCTOMECON MERRIAMII	PAPAVERACEAE	DESERT-POPPY,	CA NV
S	ARCTOSTAPHYLOS ANDERSONII VAR. PALLIDA	*** SEE ***	ARCTOSTAPHYLOS PALLIDA	
3C	ARCTOSTAPHYLOS AURICULATA	ERICACEAE	MANZANITA, MT. DIABLO	CA
S	ARCTOSTAPHYLOS BAKERI	*** SEE ***	ARCTOSTAPHYLOS PUNGENS SSP. BAKERI	
2	ARCTOSTAPHYLOS CATALINAE	ERICACEAE	MANZANITA, SANTA CATALINA ISLAND	CA
2	ARCTOSTAPHYLOS CONFERTIFLORA	ERICACEAE	MANZANITA, SANTA ROSA ISLAND	CA
3C	ARCTOSTAPHYLOS CRUZENSIS	ERICACEAE	MANZANITA, ARROYO DE LA CRUZ	CA
S	ARCTOSTAPHYLOS DENSIFLORA	*** SEE ***	ARCTOSTAPHYLOS UVA-URSI VAR. DENSIFLORA	
S	ARCTOSTAPHYLOS EDMUNDSII VAR. EDMUNDSII	*** SEE ***	ARCTOSTAPHYLOS UVA-URSI SSP. EDMUNDSII	
S	ARCTOSTAPHYLOS EDMUNDSII VAR. PARVIFOLIA	*** SEE ***	ARCTOSTAPHYLOS UVA-URSI VAR. PARVIFOLIA	
3B	ARCTOSTAPHYLOS GLANDULOSA SSP. CRASSIFOLIA	ERICACEAE	MANZANITA, EASTWOOD	CA
2	ARCTOSTAPHYLOS GLUTINOSA	ERICACEAE	MANZANITA, SCHREIBER'S	CA
3C	ARCTOSTAPHYLOS HISPIDULA	ERICACEAE		CA OR
S	ARCTOSTAPHYLOS HOOKERI SSP. FRANCISCANA	*** SEE ***	ARCTOSTAPHYLOS UVA-URSI VAR. FRANCISCANA	
S	ARCTOSTAPHYLOS HOOKERI SSP. HEARSTIORUM	*** SEE ***	ARCTOSTAPHYLOS UVA-URSI VAR. HEARSTIORUM	
S	ARCTOSTAPHYLOS HOOKERI SSP. MONTANA	*** SEE ***	ARCTOSTAPHYLOS PUNGENS SSP. MONTANA	
LE	ARCTOSTAPHYLOS HOOKERI SSP. RAVENII	ERICACEAE	MANZANITA, RAVEN'S (PRESIDIO)	CA
1	ARCTOSTAPHYLOS IMBRICATA	ERICACEAE	MANZANITA, SAN BRUNO MOUNTAIN	CA
3B	ARCTOSTAPHYLOS INTRICATA VAR. OBLONGIFOLIA	ERICACEAE		CA OR
2	ARCTOSTAPHYLOS KLAMATHENSIS	ERICACEAE		CA
3C	ARCTOSTAPHYLOS LUCIANA	ERICACEAE	MANZANITA, SANTA LUCIA	CA
S	ARCTOSTAPHYLOS MONTANA	*** SEE ***	ARCTOSTAPHYLOS PUNGENS SSP. MONTANA	
2	ARCTOSTAPHYLOS MONTARAENSIS	ERICACEAE	MANZANITA, MONTARA	CA
3C	ARCTOSTAPHYLOS MONTEREYENSIS	ERICACEAE	MANZANITA, MONTERREY	CA
1	ARCTOSTAPHYLOS MORROENSIS	ERICACEAE	MANZANITA, MORRO	CA
S	ARCTOSTAPHYLOS MYRTIFOLIA	*** SEE ***	ARCTOSTAPHYLOS UVA-URSI SSP. MYRTIFOLIA	
3C	ARCTOSTAPHYLOS NISSENANA	ERICACEAE		CA
2	ARCTOSTAPHYLOS OTAYENSIS	ERICACEAE	MANZANITA, OTAY	CA
S	ARCTOSTAPHYLOS PACIFICA	*** SEE ***	ARCTOSTAPHYLOS UVA-URSI VAR. SAXICOLA	
2	ARCTOSTAPHYLOS PALLIDA	ERICACEAE	MANZANITA, ALAMEDA	CA
3C	ARCTOSTAPHYLOS PECHOENSIS	ERICACEAE	MANZANITA, PECHO	CA
2	ARCTOSTAPHYLOS PILOSULA SSP. PILOSULA	ERICACEAE	MANZANITA, SANTA MARGARITA	CA
S	ARCTOSTAPHYLOS PUMILA	*** SEE ***	ARCTOSTAPHYLOS UVA-URSI SSP. PUMILA	
2	ARCTOSTAPHYLOS PUNGENS SSP. BAKERI	ERICACEAE	MANZANITA, BAKER'S	CA
2	ARCTOSTAPHYLOS PUNGENS SSP. MONTANA	ERICACEAE	MANZANITA, TAMALPAIS	CA
3C	ARCTOSTAPHYLOS REFUGIOENSIS	ERICACEAE	MANZANITA, REFUGIO	CA
2	ARCTOSTAPHYLOS RUDIS	ERICACEAE		CA
2	ARCTOSTAPHYLOS SILVICOLA	ERICACEAE	MANZANITA, SILVER-LEAVED	CA

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
5	ARCTOSTAPHYLOS STANFORDIANA SSP. HISPIDULA	*** SEE ***	ARCTOSTAPHYLOS HISPIDULA	
2	ARCTOSTAPHYLOS UVA-URSI SSP. EDMUNDSII	ERICACEAE	MANZANITA, LITTLE SUR	CA
1	ARCTOSTAPHYLOS UVA-URSI SSP. MYRTIFOLIA	ERICACEAE	MANZANITA, IONE	CA
2	ARCTOSTAPHYLOS UVA-URSI SSP. PUMILA	ERICACEAE	MANZANITA, SANDMAT	CA
1	ARCTOSTAPHYLOS UVA-URSI VAR. DENSIFLORA	ERICACEAE	MANZANITA, VINE HILL	CA
1	** ARCTOSTAPHYLOS UVA-URSI VAR. FRANCISCANA	ERICACEAE	MANZANITA, SAN FRANCISCO	CA
2	ARCTOSTAPHYLOS UVA-URSI VAR. HEARSTIORUM	ERICACEAE	MANZANITA, HEARST'S	CA
1	** ARCTOSTAPHYLOS UVA-URSI VAR. LEOBREWERI	ERICACEAE	MANZANITA, LEO BREWER'S	CA
2	ARCTOSTAPHYLOS UVA-URSI VAR. MARINENSIS	ERICACEAE	MANZANITA, PT. REYES	CA
2	ARCTOSTAPHYLOS UVA-URSI VAR. MONOENSIS	ERICACEAE	MANZANITA, MONO	CA
3C	ARCTOSTAPHYLOS UVA-URSI VAR. PARVIFOLIA	ERICACEAE	MANZANITA, HANGING GARDENS	CA
1	ARCTOSTAPHYLOS UVA-URSI VAR. SAXICOLA	ERICACEAE	MANZANITA, PACIFIC	CA
-	ARCTOSTAPHYLOS UVA-URSI VAR. SUBORBICULATA	ERICACEAE	MANZANITA, KAMCHATKA POINT	CA
2	ARCTOSTAPHYLOS VIRGATA	ERICACEAE	MANZANITA, BOLINAS	CA
2	ARENARIA ALABAMENSIS	CARYOPHYLLACEAE	SANDWORT, ALABAMA	AL NC
1	ARENARIA CUMBERLANDENSIS	CARYOPHYLLACEAE		TN
5	ARENARIA DECUMBENS	*** SEE ***	MINUARTIA DECUMBENS	
1	ARENARIA FONTINALIS	CARYOPHYLLACEAE	STITCHWORT, WATER	KY TN
2	ARENARIA FRANKLINII VAR. THOMPSONII	CARYOPHYLLACEAE		OR WA
5	ARENARIA GODFREYI	*** SEE ***	MINUARTIA GODFREYI	
3C	ARENARIA HOWELLII	CARYOPHYLLACEAE	SANDWORT, HOWELL'S	CA OR
1	ARENARIA KINGII SSP. ROSEA	CARYOPHYLLACEAE		NV
2	ARENARIA LIVERMORENSIS	CARYOPHYLLACEAE	SANDWORT, LIVERMORE	TX
2	ARENARIA MACRADENIA VAR. KUSCHEI	CARYOPHYLLACEAE	SANDWORT, FOREST CAMP	CA
5	ARENARIA MARCESCENS	*** SEE ***	MINUARTIA MARCESCENS	
2	ARENARIA PALUDICOLA	CARYOPHYLLACEAE		CA WA
5	ARENARIA ROSEI	*** SEE ***	MINUARTIA ROSEI	
3C	ARENARIA STENOMERES	CARYOPHYLLACEAE		NV
5	ARENARIA UNIFLORA	*** SEE ***	MINUARTIA UNIFLORA	
2	ARENARIA URSINA	CARYOPHYLLACEAE	SANDWORT, BEAR VALLEY	CA
2	ARGEMONE ARIZONICA	PAPAVERACEAE		AZ
2	ARGEMONE GLAUCA VAR. INERMIS	PAPAVERACEAE		HI
2	ARGEMONE MUNITA SSP. ROBUSTA	PAPAVERACEAE	PRICKLY-POPPY, ROBUST	CA
1	ARGEMONE PLEIACANTHA SSP. PINNATISECTA	PAPAVERACEAE	PRICKLY-POPPY,	NM
1	ARGYROXIPHIUM KAUENSE	ASTERACEAE	SILVERSWORD, KA'U	HI
5	ARGYROXIPHIUM MACROCEPHALUM	*** SEE ***	ARGYROXIPHIUM SANDWICENSE VAR. MACROCEPHALUM	HI
PE	ARGYROXIPHIUM SANDWICENSE VAR. SANDWICENSE	ASTERACEAE	'AHINAHINA (MAUNA KEA SILVERSWORD)	HI
1	ARGYROXIPHIUM VIRESCENS VAR. VIRESCENS	ASTERACEAE	GREENSWORD	HI
2	ARGYTHAMNIA APHOROIDES	EUPHORBIACEAE	MERCURY, WILD,	TX
3C	ARGYTHAMNIA ARGYRAEA	EUPHORBIACEAE	MERCURY, WILD,	TX
2	ARGYTHAMNIA BLODGETTII	EUPHORBIACEAE		FL
1	ARISTIDA FLORIDANA	POACEAE	TRIPLE-AWNED GRASS,	FL
2	ARISTIDA PORTORICENSIS	POACEAE	PELOS DEL DIABLO	PR
2	ARISTIDA SIMPLICIFLORA	POACEAE		AL FL MS
3C	ARNICA AMPLEXICAULIS VAR. PIPERI	ASTERACEAE		OR WA
2	ARNICA PANICULATA	ASTERACEAE		WY
3C	ARNICA VENOSA	ASTERACEAE	ARNICA, VEINY	CA
3C	ARNICA VISCOSA	ASTERACEAE	ARNICA, SHASTA	CA OR
2	ARTEMISIA ALEUTICA	ASTERACEAE	WORMWOOD, ALEUTIAN	AK
5	ARTEMISIA ANDROSACEA	*** SEE ***	ARTEMISIA SENJAVINENSIS	
2	ARTEMISIA ARGILOSA	ASTERACEAE	SAGEBRUSH, COALTOWN	CO
2	ARTEMISIA CAMPESTRIS VAR. WORMSKIOLDII	ASTFRACFAF		OR WA
3C	ARTEMISIA CANA SSP. BOLANDERI	ASTERACEAE		CA
2	ARTEMISIA LUDOVICIANA SSP. ESTESII	ASTERACEAE		OR
3C	ARTEMISIA PAPPOSA	ASTERACEAE		ID NV OR
3C	ARTEMISIA PORTERI	ASTERACEAE	SAGEBRUSH, PORTER	WY
2	ARTEMISIA SENJAVINENSIS	ASTERACEAE	SAGE, ARCTIC	AK, U.S.S.R.
3B	ARTEMISIA SP.	ASTERACEAE	ARTEMISIA (KAIHOLENA GULCH, LANAI)	HI
2	ARTEMISIA UNALASKENSIS VAR. ALEUTICA	ASTERACEAE		AK
5	ASARUM CONTRACTA	*** SEE ***	HEXASTYLIS CONTRACTA	
5	ASARUM LEWISII	*** SEE ***	HEXASTYLIS LEWISII	
5	ASARUM NANIFLORA	*** SEE ***	HEXASTYLIS NANIFLORA	
2	ASCLEPIAS CUTLERI	ASCLEPIADACEAE	MILKWEED, CUTLER	AZ UT
2	ASCLEPIAS EASTWOODIANA	ASCLEPIADACEAE	MILKWEED, EASTWOOD'S	NV
2	ASCLEPIAS MEADII	ASCLEPIADACEAE	MILKWEED, MEAD'S	IL IN IA KS MO WI
2	ASCLEPIAS PROSTRATA	ASCLEPIADACEAE		TX MEXICO
3C	ASCLEPIAS RUTHIAE	ASCLEPIADACEAE	MILKWEED, RUTH	UT
2	ASCLEPIAS VIRIDULA	ASCLEPIADACEAE		FL
PE	ASCLEPIAS WELSHII	ASCLEPIADACEAE		UT
5	ASIMINA PULCHELLA	*** SEE ***	DEERINGOTHAMNUS PULCHELLUS	
5	ASIMINA RUGELII	*** SEE ***	DEERINGOTHAMNUS RUGELII	
1	ASIMINA TETRAMERA	ANNONACEAE	PAWPAW,	FL
2	ASPLENIUM ANDREWSII	POLYPODIACEAE		AZ CO UT
3B	ASPLENIUM EBENOIDES	POLYPODIACEAE	SPLEENWORT, SCOTT'S	AL CT IL NJ OH PA TN VA WV
1	* ASPLENIUM FRAGILE VAR. INSULARIS	POLYPODIACEAE		HI
2	ASPLENIUM HETERORESILIENS	POLYPODIACEAE		FL GA NC SC
3B	ASPLENIUM KENTUCKIENSE	POLYPODIACEAE	SPLEENWORT,	AR IL IN KY OH VA
1	* ASPLENIUM LEUCOSTEGIOIDES	POLYPODIACEAE		HI

STATUS		SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
2		ASPLENIUM PLENUM	POLYPODIACEAE		FL
S		ASPLENIUM RHOMBOIDEUM	*** SEE ***	ASPLENIUM FRAGILE VAR. INSULARIS	
S		ASPLENOSORUS EBENOIDES	*** SEE ***	ASPLENIUM EBENOIDES	
3C		ASTELIA VERATROIDES SSP. MACROSPERMA	LILIACEAE	PA 'INIU	HI
3C		ASTELIA VERATROIDES VAR. GRACILIS	LILIACEAE	PA 'INIU	HI
3C		ASTELIA VERATROIDES VAR. VERATROIDES	LILIACEAE	PA 'INIU	HI
1		ASTER AVITUS	ASTERACEAE		GA SC
2		ASTER BLEPHAROPHYLLUS	ASTERACEAE		NM
3B		ASTER BRACHYPHOLIS	ASTERACEAE		FL
3C		ASTER BRICKELLIOIDES	ASTERACEAE		CA OR
3B		ASTER CHASEI	ASTERACEAE		IL
S		ASTER CHILENSIS SSP. HALLII	*** SEE ***	ASTER HALLII	
2		ASTER CHILENSIS VAR. LENTUS	ASTERACEAE	ASTER, SUISUN	CA
2		ASTER CURTUS	ASTERACEAE	ASTER, CURTUS	OR WA, Canada (B.C.)
2		ASTER DEPAUPERATUS	ASTERACEAE	ASTER, SERPENTINE	MD PA
S		ASTER ERICOIDES VAR. DEPAUPERATUS	*** SEE ***	ASTER DEPAUPERATUS	
2		ASTER FURCATUS	ASTERACEAE		AR IL IN IA MO WI
3C		ASTER GLAUCESCENS	ASTERACEAE		WA
2		ASTER GORMANII	ASTERACEAE	ASTER, GORMAN	OR
3C		ASTER GREATAI	ASTERACEAE		CA
3C		ASTER HALLII	ASTERACEAE		OR WA
2		ASTER JESSICAE	ASTERACEAE		ID WA
1		ASTER LEMMONII	ASTERACEAE		AZ
2		ASTER MOLLIS	ASTERACEAE		MT WY
3B		ASTER PALUDICOLA	ASTERACEAE		CA OR
3C		ASTER PEIRSONII	ASTERACEAE	ASTER, PEIRSON'S	CA
3B		ASTER PINIFOLIUS	ASTERACEAE	ASTER,	AL FL
3B		ASTER PLUMOSUS	ASTERACEAE		FL
3C		ASTER PUNICEUS VAR. SCABRICAULIS	ASTERACEAE		TX
3B		ASTER SANDWICENSIS	ASTERACEAE	ASTER, HAWAIIAN	HI
S		ASTER SCABRICAULIS	*** SEE ***	ASTER PUNICEUS VAR. SCABRICAULIS	
2		ASTER SCHISTOSUS	ASTERACEAE		VA
2		ASTER SPINULOSUS	ASTERACEAE		FL
3B		ASTER VERUTIFOLIUS	ASTERACEAE		MS
2		ASTER VIALIS	ASTERACEAE		OR
2		ASTER YUKONENSIS	ASTERACEAE	ASTER, YUKON	AK, Canada (Yukon)
1	*	ASTILBE CRENATILOBA	SAXIFRAGACEAE		TN
3C		ASTRAGALUS ACCUMBENS	FABACEAE		NM
3C		ASTRAGALUS ACKERMANNII	FABACEAE		NV
2		ASTRAGALUS AEQUALIS	FABACEAE		NV
1	*	ASTRAGALUS AGNICIDUS	FABACEAE		CA
3C		ASTRAGALUS ALTUS	FABACEAE		NM
3C		ASTRAGALUS ALVORDENSIS	FABACEAE		NV OR
3C		ASTRAGALUS AMNIS-AMISSI	FABACEAE	MILK-VETCH,	ID
2		ASTRAGALUS AMPULLARIUS	FABACEAE	MILK-VETCH, GUMBO	AZ UT
2		ASTRAGALUS ANSERINUS	FABACEAE		NV UT
2		ASTRAGALUS APPLEGATEI	FABACEAE		OR
2		ASTRAGALUS ATRATUS VAR. INSEPTUS	FABACEAE	MILK-VETCH,	ID
3C		ASTRAGALUS BARNEBYI	FABACEAE	MILK-VETCH, BARNEBY	AZ UT
2		ASTRAGALUS BARRII	FABACEAE		SD
3C		ASTRAGALUS BEATHII	FABACEAE	MILK-VETCH, BEATH	AZ
1		ASTRAGALUS BEATLEYAE	FABACEAE	MILK-VETCH, BEATLEY	NV
2		ASTRAGALUS BRAUNTONII	FABACEAE	MILK-VETCH, BRAUNTON'S	CA
3C		ASTRAGALUS CALLITHRIX	FABACEAE	MILK-VETCH, CALLOWAY	NV UT
3C		ASTRAGALUS CALYCOSUS VAR. MONOPHYLLIDIUS	FABACEAE		NV
2		ASTRAGALUS CAMPTOPUS	FABACEAE		ID
S		ASTRAGALUS CASTANEIFORMIS VAR. CONSOBRINUS	*** SEE ***	ASTRAGALUS CONSOBRINUS	
3C		ASTRAGALUS CASTETTERI	FABACEAE	MILK-VETCH, CASTETTER	NM
3C		ASTRAGALUS CERAMICUS VAR. APUS	FABACEAE		ID
3C		ASTRAGALUS CHLOODES	FABACEAE	MILK-VETCH, GRASS	UT
3C		ASTRAGALUS CIMAE VAR. CIMAE	FABACEAE	RATTLEWEED, CIMA	CA NV
3C		ASTRAGALUS CIMAE VAR. SUFFLATUS	FABACEAE		CA
2		ASTRAGALUS CLARIANUS	FABACEAE	MILK-VETCH, NAPA	CA
2		ASTRAGALUS COLLINUS VAR. LAURENTII	FABACEAE		OR
2		ASTRAGALUS COLUMBIANUS	FABACEAE	MILK-VETCH, COLUMBIA	WA
3C		ASTRAGALUS CONSOBRINUS	FABACEAE		UT
3C		ASTRAGALUS CONVALLARIUS VAR. FINITIMUS	FABACEAE		UT NV
3C		ASTRAGALUS COTTAMII	FABACEAE	MILK-VETCH, COTTAM	AZ UT
2		ASTRAGALUS COTTONII	FABACEAE		WA
1		ASTRAGALUS CREMNOPHYLAX VAR. CREMNOPHYLAX	FABACEAE	MILK-VETCH, SENTRY	AZ
3C		ASTRAGALUS CREMNOPHYLAX VAR. MYRIORRHAPHIS	FABACEAE		AZ
2		ASTRAGALUS CRONQUISTII	FABACEAE	MILK-VETCH, CRONQUIST	CO UT
2		ASTRAGALUS DEANEI	FABACEAE	MILK-VETCH, DEANE	CA
2		ASTRAGALUS DEBEQUAEUS	FABACEAE		CO
2		ASTRAGALUS DESERETICUS	FABACEAE	MILK-VETCH, DESERET	UT
S		ASTRAGALUS DESPERATUS VAR. CONSPECTUS	*** SEE ***	ASTRAGALUS BARNEBYI	
3C		ASTRAGALUS DETERIOR	FABACEAE	MILK-VETCH, CLIFF-PALACE	CO
3C		ASTRAGALUS DETRITALIS	FABACEAE	MILK-VETCH, DEBRIS	CO UT
2		ASTRAGALUS DIAPHANUS	FABACEAE		OR WA
2		ASTRAGALUS DOUGLASII VAR. PERSTRICTUS	FABACEAE	MILK-VETCH, ROUND-PODDED	CA

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3C		ASTRAGALUS DRABELLIFORMIS	FABACEAE		WY
3C		ASTRAGALUS DUCHESNENSIS	FABACEAE	MILK-VETCH, DUCHESNE	CO UT
3C		ASTRAGALUS ENSIFORMIS	FABACEAE		AZ UT
1		ASTRAGALUS EQUISOLENSIS	FABACEAE	MILK-VETCH,	UT
2		ASTRAGALUS FUNEREUS	FABACEAE	WOOLY POD, BLACK	CA NV
2		ASTRAGALUS GEYERI VAR. TRIQUETRUS	FABACEAE		AZ NV
2		ASTRAGALUS GYPSODES	FABACEAE	MILK-VETCH, GYPSUM	NM TX
2		ASTRAGALUS HAMILTONII	FABACEAE	MILK-VETCH, HAMILTON	UT
2		ASTRAGALUS HARRISONII	FABACEAE	MILK-VETCH, HARRISON	UT
3C		ASTRAGALUS HENRIMONTANENSIS	FABACEAE		UT
2		ASTRAGALUS HOLMGRENIORUM	FABACEAE		AZ UT
3C		ASTRAGALUS HOODIANUS	FABACEAE		OR WA
LE		ASTRAGALUS HUMILLIMUS	FABACEAE	MILK-VETCH, MANCOS	CO NM
3C		ASTRAGALUS ISELYI	FABACEAE	MILK-VETCH, ISELY	UT
2		ASTRAGALUS JAEGERIANUS	FABACEAE	MILK-VETCH, COOLGARDIE	CA
2		ASTRAGALUS JEJUNUS SSP. NOV. /INED.	FABACEAE		WY
3C		ASTRAGALUS JOHANNIS-HOWELLII	FABACEAE		CA NV
2	*	ASTRAGALUS KENTROPHYTA VAR. DOUGLASII	FABACEAE	MILK-VETCH, THISTLE, DOUGLAS	OR WA
2		ASTRAGALUS KNIGHTII	FABACEAE		NM
3C		ASTRAGALUS LANCEARIUS	FABACEAE		AZ UT
5		ASTRAGALUS LAURENTII	*** SEE ***	ASTRAGALUS COLLINUS VAR. LAURENTII	
3C		ASTRAGALUS LENTIFORMIS	FABACEAE		CA
2		ASTRAGALUS LENTIGINOSUS VAR. AMBIGUUS	FABACEAE		AZ
2		ASTRAGALUS LENTIGINOSUS VAR. ANTONIUS	FABACEAE		CA
3B		ASTRAGALUS LENTIGINOSUS VAR. CHARTACEUS	FABACEAE		CA CO ID OR UT WY
1		ASTRAGALUS LENTIGINOSUS VAR. COACHELLAE	FABACEAE		CA
3C		ASTRAGALUS LENTIGINOSUS VAR. LATUS	FABACEAE		NV
3C		ASTRAGALUS LENTIGINOSUS VAR. MARICOPAE	FABACEAE		AZ
1		ASTRAGALUS LENTIGINOSUS VAR. MICANS	FABACEAE	MILK-VETCH, SHINY	CA
1		ASTRAGALUS LENTIGINOSUS VAR. PISCINENSIS	FABACEAE		CA
3C		ASTRAGALUS LENTIGINOSUS VAR. POHLII	FABACEAE	MILK-VETCH,	UT
1		ASTRAGALUS LENTIGINOSUS VAR. SESQUIMETRALIS	FABACEAE		CA NV
2		ASTRAGALUS LENTIGINOSUS VAR. SIERRAE	FABACEAE		CA
2	*	ASTRAGALUS LENTIGINOSUS VAR. URSINUS	FABACEAE	MILK-VETCH, BEAR VALLEY	UT
3C		ASTRAGALUS LIMNOCHARIS VAR. LIMNOCHARIS	FABACEAE		UT
2		ASTRAGALUS LINIFOLIUS	FABACEAE	MILK-VETCH, GRAND JUNCTION	CO UT
3C		ASTRAGALUS LOANUS	FABACEAE	MILK-VETCH,	UT
3C		ASTRAGALUS LUTOSUS	FABACEAE		CO UT
2		ASTRAGALUS MAGDALENAE VAR. PEIRSONII	FABACEAE	MILK-VETCH, PEIRSON'S	CA
3C		ASTRAGALUS MALACOIDES	FABACEAE	MILK-VETCH, KAIPAROWITS	UT
2		ASTRAGALUS MICROCYMBUS	FABACEAE	MILK-VETCH,	CO
3B		ASTRAGALUS MINTHORNIAE VAR. GRACILIOR	FABACEAE		UT
2		ASTRAGALUS MISELLUS VAR. PAUPER	FABACEAE	MILK-VETCH, PAUPER	WA
2		ASTRAGALUS MOHAVENSIS VAR. HEMIGYRUS	FABACEAE	MILK-VETCH, DARWIN MESA	CA NV
2		ASTRAGALUS MOLLISSIMUS VAR. MARCIDUS	FABACEAE		TX
1		ASTRAGALUS MONOENSIS	FABACEAE		CA
PE		ASTRAGALUS MONTII	FABACEAE	MILK-VETCH, HELIOTROPE	UT
3C		ASTRAGALUS MONUMENTALIS	FABACEAE		AZ NM UT
2		ASTRAGALUS MULFORDIAE	FABACEAE		ID OR
2		ASTRAGALUS MUSIMONUM	FABACEAE		AZ NV
3C		ASTRAGALUS NATURITENSIS	FABACEAE	MILK-VETCH, NATURITA	CO
2		ASTRAGALUS NEVINII	FABACEAE	MILK-VETCH, SAN CLEMENTE ISLAND	CA
3C		ASTRAGALUS NIDULARIUS	FABACEAE		UT
3C		ASTRAGALUS NYENSIS	FABACEAE	MILK-VETCH,	NV
2		ASTRAGALUS ONICIFORMIS	FABACEAE		ID
3C		ASTRAGALUS OOCALYCIS	FABACEAE		CO NM
2		ASTRAGALUS OOCARPUS	FABACEAE	MILK-VETCH, DESCANSO	CA
1		ASTRAGALUS OOPHORUS VAR. CLOKEYANUS	FABACEAE		NV
3C		ASTRAGALUS OOPHORUS VAR. LONCHOCALYX	FABACEAE		NV UT
2		ASTRAGALUS OSTERHOUTII	FABACEAE	MILK-VETCH, OSTERHOUT	CO
3C		ASTRAGALUS PANAMINTENSIS	FABACEAE		CA
3C		ASTRAGALUS PARDALINUS	FABACEAE		UT
3C		ASTRAGALUS PAUPERCULUS	FABACEAE		CA
3C		ASTRAGALUS PAYSONII	FABACEAE		ID WY
2		ASTRAGALUS PECKII	FABACEAE		OR
LT		ASTRAGALUS PERIANUS	FABACEAE	MILK-VETCH, RYDBERG	UT
LT		ASTRAGALUS PHOENIX	FABACEAE	MILK-VETCH, ASH MEADOWS	NV
3C		ASTRAGALUS PORRECTUS	FABACEAE	MILK-VETCH,	NV
3C		ASTRAGALUS PROIMANTHUS	FABACEAE	MILK-VETCH,	WY
3C		ASTRAGALUS PSEUDIODANTHUS	FABACEAE		CA NV
3C		ASTRAGALUS PTEROCARPUS	FABACEAE		NV
3B		ASTRAGALUS PUNICEUS VAR. GERTRUDIS	FABACEAE		NM
3C		ASTRAGALUS PURSHII VAR. OPHIOGENES	FABACEAE	MILK-VETCH,	ID
1	*	ASTRAGALUS PYCNOSTACHYUS VAR. LANOSISSIMUS	FABACEAE	MILK-VETCH, VENTURA MARSH	CA
3C		ASTRAGALUS RAFAELENSIS	FABACEAE	MILK-VETCH, SAN RAFAEL	UT
2		ASTRAGALUS RAVENII	FABACEAE		CA
2		ASTRAGALUS REMOTUS	FABACEAE	MILK-VETCH, SPRING MOUNTAIN	NV
2		ASTRAGALUS ROBBINSII VAR. ALPINIFORMIS	FABACEAE	MILK-VETCH, ROBBINS,	OR
1		ASTRAGALUS ROBBINSII VAR. JESUPI	FABACEAE	MILK-VETCH, ROBBINS,	NH VT
2		ASTRAGALUS ROBBINSII VAR. OCCIDENTALIS	FABACEAE	MILK-VETCH, ROBBINS,	NV
3A		ASTRAGALUS ROBBINSII VAR. ROBBINSII	FABACEAE	MILK-VETCH, ROBBINS,	VT

STATUS		SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
2		ASTRAGALUS SABULOSUS	FABACEAE		UT
3C		ASTRAGALUS SAURINUS	FABACEAE	MILK-VETCH, DINOSAUR	UT
2		ASTRAGALUS SCHMOLLIAE	FABACEAE	MILK-VETCH, SCHMOLL	CO
3C		ASTRAGALUS SERENOI VAR. SORDESCENS	FABACEAE	MILK-VETCH,	NV
3C		ASTRAGALUS SERPENS	FABACEAE	MILK-VETCH,	UT
2		ASTRAGALUS SHEVOCKII	FABACEAE		CA
2		ASTRAGALUS SHULTZIORUM	FABACEAE		WY
3C		ASTRAGALUS SILICEUS	FABACEAE	MILK-VETCH,	NM
2		ASTRAGALUS SINUATUS	FABACEAE	MILK-VETCH, WHITED	WA
2		ASTRAGALUS SOLITARIUS	FABACEAE		NV OR
S		ASTRAGALUS SPALDINGII VAR. TYGHENSIS	*** SEE ***	ASTRAGALUS TYGHENSIS	
2		ASTRAGALUS STERILIS	FABACEAE	MILK-VETCH,	ID OR
S		ASTRAGALUS STOCKSII	*** SEE ***	ASTRAGALUS HENRIMONTANENSIS	
3C		ASTRAGALUS STRIATIFLORUS	FABACEAE	MILK-VETCH, ESCARPMENT	AZ UT
2		ASTRAGALUS SUBCINEREUS VAR. BASALTICUS	FABACEAE		UT
3C		ASTRAGALUS SUBVESTITUS	FABACEAE		CA
2		ASTRAGALUS TEGETARIOIDES	FABACEAE		CA OR
2		ASTRAGALUS TENER VAR. TITI	FABACEAE	RATTLEWEED, COASTAL DUNES	CA
2		ASTRAGALUS TENNESSEENSIS	FABACEAE		AL IL IN TN
2		ASTRAGALUS TEPHRODES VAR. EURYLOBUS	FABACEAE		NV
2		ASTRAGALUS TITANOPHILUS	FABACEAE		AZ
3C		ASTRAGALUS TOQUIMANUS	FABACEAE		NV
2		ASTRAGALUS TRASKIAE	FABACEAE	MILK-VETCH, TRASK'S	CA
3C		ASTRAGALUS TROGLODYTUS	FABACEAE		AZ
3C		ASTRAGALUS TWEEDYI	FABACEAE		OR WA
2		ASTRAGALUS TYGHENSIS	FABACEAE		OR
2		ASTRAGALUS UNCIALIS	FABACEAE	MILK-VETCH,	NV UT
2		ASTRAGALUS VEXILLIFLEXUS VAR. NUBILUS	FABACEAE		ID
3C		ASTRAGALUS WETHERILLII	FABACEAE		CO UT
3C		ASTRAGALUS WITTMANNII	FABACEAE		NM
3C		ASTRAGALUS WOODRUFFII	FABACEAE		UT
1		ASTRAGALUS XIPHOIDES	FABACEAE	MILK-VETCH, GLADIATOR	AZ
2		ASTRAGALUS YODER-WILLIAMSII	FABACEAE	MILK-VETCH, OSGOOD MOUNTAINS	ID NV
3C		ASTRANTHIUM ROBUSTUM	ASTERACEAE		TX
2		ATRIPLEX CANESCENS VAR. GIGANTEA	CHENOPODIACEAE		UT
3C		ATRIPLEX GRIFFITHSII	CHENOPODIACEAE	SALTBUSH, GRIFFITH'S	AZ NM
3C		ATRIPLEX KLEBERGORUM	CHENOPODIACEAE	SALTBUSH, KLEBERG'S	TX
2		ATRIPLEX PATULA SSP. SPICATA	CHENOPODIACEAE		CA
2		ATRIPLEX PLEIANTHA	CHENOPODIACEAE	SALTBUSH,	CO NM UT
1		ATRIPLEX TULARENSIS	CHENOPODIACEAE	SALTBUSH, BAKERSFIELD	CA
2		ATRIPLEX VALLICOLA	CHENOPODIACEAE	SALTBUSH, LOST HILLS	CA
3C		ATRIPLEX WELSHII	CHENOPODIACEAE	SALTBUSH, WELSH	UT
1		AUREOLARIA PATULA	SCROPHULARIACEAE		AL GA KY TN
2		AYENIA LIMITARIS	STERCULIACEAE		TX MEXICO
S		AZALEA AUSTRINUM	*** SEE ***	RHODODENDRON AUSTRINUM	
2		BACCHARIS PLUMMERAE SSP. GLABRATA	ASTERACEAE	BACCHARIS, HOOVER'S	CA
2		BACCHARIS VANESSAE	ASTERACEAE	BACCHARIS, ENCINITIS	CA
2	*	BACOPA SIMULANS	SCROPHULARIACEAE	WATER-HYSSOP, CHICKAHOMINY	VA
2		BACOPA STRAGULA	SCROPHULARIACEAE	WATER-HYSSOP, MAT-FORMING	MD VA
3C		BAHIA BIGELOVII	ASTERACEAE		TX
3C		BALDUINA ATROPURPUREA	ASTERACEAE		FL GA MS SC
3C		BALSAMORHIZA ROSEA	ASTERACEAE		OR WA
2		BALSAMORHIZA SERICEA /SP. NOV. INED.	ASTERACEAE		OR
1		BANARA VANDERBILTII	FLACOURTIACEAE		PR
LE		BAPTISIA ARACHNIFERA	FABACEAE	RATTLEWEED, HAIRY	GA
2		BAPTISIA CALYCOSA VAR. CALYCOSA	FABACEAF		FL
2		BAPTISIA CALYCOSA VAR. HIRSUTA	FABACEAE	WILD INDIGO, HAIRY	FL
S		BAPTISIA HIRSUTA	*** SEE ***	BAPTISIA CALYCOSA VAR. HIRSUTA	
3C		BAPTISIA MEGACARPA	FABACEAE	WILD INDIGO, APALACHICOLA	AL FL
3B		BAPTISIA RIPARIA	FABACEAE	WILD INDIGO,	FL
2		BAPTISIA SIMPLICIFOLIA	FABACEAE		FL
3C		BARTONIA TEXANA	GENTIANACEAE	SCREWSTEM, TEXAS	TX
2		BASIPHYLLAEA ANGUSTIFOLIA	ORCHIDACEAE		PR, Cuba, Hispaniola
2		BATESIMALVA VIOLACEA	MALVACEAE	GAY-MALLOW, PURPLE	TX, Mexico
3C		BENITOA OCCIDENTALIS	ASTERACEAE	BENITOA	CA
2		BENSONIELLA OREGONA	SAXIFRAGACEAE	BENSONIELLA	CA OR
3C		BERBERIS HARRISONIANA	BERBERIDACEAE	BARBERRY,	AZ
3C		BERBERIS HIGGINSIAE	BERBERIDACEAE	BARBERRY, HIGGIN'S	CA
S		BERBERIS NERVOSA VAR. MENDOCINENSIS	*** SEE ***	MAHONIA NERVOSA VAR. MENDOCINENSIS	
S		BERBERIS NEVINII	*** SEE ***	MAHONIA NEVINII	
S		BERBERIS PINNATA SSP. INSULARIS	*** SEE ***	MAHONIA PINNATA SSP. INSULARIS	
S		BERBERIS SONNEI	*** SEE ***	MAHONIA SONNEI	
3C		BERBERIS SWASEYI	BERBERIDACEAE		TX
2		BESSEYA BULLII	SCROPHULARIACEAE		IL IN IA MI MN OH WI
S		BETULA LENTA VAR. UBER	*** SEE ***	BETULA UBER	
LE		BETULA UBER	BETULACEAE	BIRCH, ROUND-LEAF, VIRGINIA	VA
3B		BIDENS ASPLENIOIDES	ASTERACEAE	KO'OKO'OLAU	HI
3C		BIDENS BIDENTOIDES VAR. BIDENTOIDES	ASTERACEAE	BUR-MARIGOLD,	DE NJ NY PA
2		BIDENS BIDENTOIDES VAR. MARIANA	ASTERACEAE	BUR-MARIGOLD, MARYLAND	MD
1		BIDENS CAMPYLOTHECA	ASTERACEAE	BIDENS, CAMPYLOTHECA	HI
3C		BIDENS CERVICATA	ASTERACEAE	KO'OKO'OLAU	HI
3B		BIDENS COARCTATA	ASTERACEAE	BIDENS, MANOA	HI
3C		BIDENS CONJUNCTATA	ASTERACEAE	KO'OKO'OLAU	HI

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5		BIDENS CTENOPHYLLA	*** SEE ***	BIDENS MICRANTHA SSP. CTENOPHYLLA	
LE		BIDENS CUNEATA	ASTERACEAE	BIDENS, CUNEATE	HI
3B		BIDENS DEGENERI	ASTERACEAE	KO'OKO'OLAU	HI
3B		BIDENS DISTANS	ASTERACEAE	BIDENS, LANAI	HI
3C		BIDENS FORBESII	ASTERACEAE	BIDENS, FORBES	HI
3B		BIDENS GRACILOIDES	ASTERACEAE	BIDENS, MAUNALUA	HI
3C		BIDENS HAWAIIENSIS	ASTERACEAE	KO'OKO'OLAU	HI
3B		BIDENS MACROCARPA VAR. OVATIFOLIA	ASTERACEAE	KO'OKO'OLAU	HI
3B		BIDENS MAGNIDISCA	ASTERACEAE	KO'OKO'OLAU	HI
3C		BIDENS MAUIENSIS	ASTERACEAE	KO'OKO'OLAU	HI
3C		BIDENS MENIIESII VAR. LEPTODONTA	ASTERACEAE	KO'OKO'OLAU	HI
2		BIDENS MICRANTHA SSP. CTENOPHYLLA	ASTERACEAE		HI
1		BIDENS MICRANTHA SSP. KALEALAHA	ASTERACEAE	KO'OKO'OLAU	HI
3B		BIDENS MICRANTHA VAR. CADUCA	ASTERACEAE	KO'OKO'OLAU	HI
3B		BIDENS MICRANTHA VAR. KAALANA	ASTERACEAE	KO'OKO'OLAU	HI
3B		BIDENS MICRANTHA VAR. LACINIATA	ASTERACEAE	KO'OKO'OLAU	HI
1		BIDENS MOLOKAIENSIS	ASTERACEAE	KO'OKO'OLAU	HI
3C		BIDENS NAPALIENSIS	ASTERACEAE	KO'OKO'OLAU	HI
5		BIDENS NEMATOCERA	*** SEE ***	BIDENS WIEBKEI	HI
3B		BIDENS OBTUSILOBA	ASTERACEAE	KO'OKO'OLAU	HI
3C		BIDENS POPULIFOLIA	ASTERACEAE	BIDENS, POPLAR-LEAVED	HI
3B		BIDENS PULCHELLA	ASTERACEAE	KO'OKO'OLAU	HI
3B		BIDENS SALICOIDES	ASTERACEAE	BIDENS, WILLOW-LEAVED	HI
3B		BIDENS SANDWICENSIS VAR. SETOSA	ASTERACEAE	KO'OKO'OLAU	HI
3B		BIDENS SKOTTSBERGII	ASTERACEAE	KO'OKO'OLAU	HI
3B		BIDENS STOKESII	ASTERACEAE	BIDENS, STOKES	HI
3C		BIDENS VALIDA	ASTERACEAE	BIDENS, ROBUST	HI
3C		BIDENS WAIMEANA	ASTERACEAE	KO'OKO'OLAU	HI
1		BIDENS WIEBKEI	ASTERACEAE	KO'OKO'OLAU	HI
2		BLENNOSPERMA BAKERI	ASTERACEAE	BLENNOSPERMA, BAKER'S	CA
2		BLENNOSPERMA NANUM VAR. ROBUSTUM	ASTERACEAE	BLENNOSPERMA, POINT REYES	CA
2		BLOOMERIA HUMILIS	LILIACEAE	GOLDENSTAR, DWARF	CA
3C		BOBEA SANDWICENSIS	RUBIACEAE	'AHAKEA	HI
1		BOBEA TIMONIOIDES	RUBIACEAE	'AHAKEA	HI
1		BOERHAVIA MATHISIANA	NYCTAGINACEAE		TX
5		BOLOPHYTA ALPINA	*** SEE ***	PARTHENIUM ALPINUM	
5		BOLOPHYTA LIGULATA	*** SEE ***	PARTHENIUM LIGULATUM	
5		BOLOPHYTA TETRANEURIS	*** SEE ***	PARTHENIUM TETRANEURIS	
2		BOLTONIA ASTEROIDES VAR. DECURRENS	ASTERACEAE		IL MO
1		BONAMIA GRANDIFLORA	CONVOLVULACEAE		FL
1		BONAMIA MENIIESII	CONVOLVULACEAE		HI
2		BONAMIA OVALIFOLIA	CONVOLVULACEAE		TX MEXICO
3C		BOTHRIOCHLOA EXARISTATA	POACEAE		LA TX
2		BOTRYCHIUM CRENULATUM	OPHIOGLOSSACEAE		CA
2		BOTRYCHIUM PARADOXUM	OPHIOGLOSSACEAE		MT, Canada (Alta.)
2		BOTRYCHIUM PUMICOLA	OPHIOGLOSSACEAE	GRAPE FERN, CRATER LAKE	CA OR
1	•	BOTRYCHIUM SUBBIFOLIATUM	OPHIOGLOSSACEAE	MAKOU	HI
1		BRACHIONIDIUM CILIOLATUM	ORCHIDACEAE		PR
2		BRASSIA CAUDATA	ORCHIDACEAE		FL, Mexico, West Indies, Central America, South America
2		BRAYA HUMILIS SSP. VENTOSA	BRASSICACEAE		CO
3C		BRAYA HUMILIS VAR. LEIOCARPA	BRASSICACEAE		MI VT, Canada
3C		BRAZORIA PULCHERRIMA	LAMIACEAE	BROZOS-MINT, CENTERVILLE	TX
5		BREWERIA GRANDIFLORA	*** SEE ***	BONAMIA GRANDIFLORA	
2		BRICKELLIA BRACHYPHYLLA VAR. HINCKLEYI	ASTERACEAE		TX
2		BRICKELLIA BRACHYPHYLLA VAR. TERLINGUENSIS	ASTERACEAE		TX
2		BRICKELLIA CORDIFOLIA	ASTERACEAE	NEMESIS, FLYR'S	AL FL GA
3C		BRICKELLIA DENTATA	ASTERACEAE		TX
5		BRICKELLIA EUPATORIOIDES VAR. FLORIDANA	*** SEE ***	BRICKELLIA MOSIERI	
3C		BRICKELLIA KNAPPIANA	ASTERACEAE	BRICKELLIA, KNAPP'S	CA NV
3C		BRICKELLIA LEPTOPHYLLA	ASTERACEAE		TX, MEXICO
2		BRICKELLIA MOSIERI	ASTERACEAE		AL FL GA SC
3C		BRICKELLIA SHINERI	ASTERACEAE		TX, Mexico
2		BRICKELLIA VIEJENSIS	ASTERACEAE	BRICKELLIA, SIERRA VIEJA	TX
1		BRIGHAMIA CITRINA	CAMPANULACEAE	ALULA	HI
1		BRIGHAMIA INSIGNIS	CAMPANULACEAE	ALULA	HI
1	•	BRIGHAMIA REMYI	CAMPANULACEAE		HI
1		BRIGHAMIA ROCKII	CAMPANULACEAE	PUA 'ALA	HI
2		BRODIAEA CORONARIA SSP. ROSEA	LILIACEAE	BRODIAEA, INDIAN VALLEY	CA
2		BRODIAEA FILIFOLIA	LILIACEAE	BRODIAEA, THREAD-LEAVED	CA
1		BRODIAEA INSIGNIS	LILIACEAE		CA
2		BRODIAEA KINKIENSIS	LILIACEAE	BRODIAEA, SAN CLEMENTE ISLAND	CA
2		BRODIAEA ORCUTTII	LILIACEAE	BRODIAEA, ORCUTT'S	CA, Mexico
1		BRODIAEA PALLIDA	LILIACEAE	BRODIAEA, CHINESE CAMP	CA
3C		BROMUS TEXENSIS	POACEAE		TX, Mexico
2		BRONGNIARTIA MINUTIFOLIA	FABACEAE	BRONGNIARTIA, LITTLE-LEAF	TX, Mexico
1		BRUNFELSIA PORTORICENSIS	SOLANACEAE		PR
2		BUCKLEYA DISTICHOPHYLLA	SANTALACEAE		NC TN VA
2		BUMELIA THORNEI	SAPOTACEAE	BUCKTHORN,	GA
LE		BUXUS VAHLII	BUXACEAE	BOXWOOD,	PR
2		BYRSONIMA HORNEANA	MALPIGHIACEAE	MARICAO CIMARRON	PR

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2	BYRSONIMA OPHITICOLA	MALPIGHIACEAE	MARICAO CIMARRON	PR
2	CACALIA DIVERSIFOLIA	ASTERACEAE		AL FL GA
1	CACALIA RUGELIA	ASTERACEAE		NC TN
3C	CAESALPINIA BRACHYCARPA	FABACEAE		TX
2	CAESALPINIA CULEBRAE	FABACEAE	MATO AMARILLO (SMOOTH YELLOW NICKER)	PR
3C	CAESALPINIA DRUMMONDII	FABACEAE		TX, Mexico
3B	CAESALPINIA MONENSIS	FABACEAE	MATO NEGRO (BLACK NICKER)	PR
1	CAESALPINIA PORTORICENSIS	FABACEAE	MATO (BROWN NICKER)	PR
1	CALAMAGROSTIS CAINII	POACEAE		TN
2	CALAMAGROSTIS CRASSIGLUMIS	POACEAE	REED GRASS, THURBER'S	AK CA WA, Canada (B.C.)
2	CALAMAGROSTIS DENSA	POACEAE	REED GRASS, DENSE	CA, Mexico
2	CALAMAGROSTIS FOLIOSA	POACEAE	REED GRASS, LEAFY	CA
3B	CALAMAGROSTIS INEXPANSA VAR. NOVAE-ANGLIAE	POACEAE	REED GRASS, NORTHERN, NEW ENGLAND	ME NH VT
S	CALAMAGROSTIS INSPERATA	*** SEE ***	CALAMAGROSTIS PORTERI SSP. INSPERATA	
3B	CALAMAGROSTIS NUBILA	POACEAE	REED GRASS,	NH
2	CALAMAGROSTIS PERPLEXA	POACEAE	REED GRASS, WOOD	NY
3C	CALAMAGROSTIS PORTERI	POACEAE	REED GRASS,	KY NY NC PA VA WV
2	CALAMAGROSTIS PORTERI SSP. INSPERATA	POACEAE	REED GRASS, OFER HOLLOW	AR MO OH
2	CALAMAGROSTIS TWEEDYI	POACEAE	REED GRASS,	ID MT WA
1	CALAMINTHA ASHEI	LAMIACEAE		FL GA
2	CALAMINTHA DENTATA	LAMIACEAE		FL GA
1	CALAMOVILFA ARCUATA	POACEAE	SAND GRASS,	OK TN
2	CALAMOVILFA BREVIPILIS	POACEAE	SAND GRASS,	NJ NC VA
3B	CALAMOVILFA BREVIPILIS VAR. CALVIPES	POACEAE	SAND GRASS,	VA
2	CALAMOVILFA CURTISSII	POACEAE	SAND GRASS,	FL
3C	CALLIANDRA BIFLORA	FABACEAE	STICK-PEA, TWO-FLOWERED	TX, Mexico
1	CALLICARPA AMPLA	VERBENACEAE	CAPA ROSA	PR VI
2	CALLIRHOE BUSHII	MALVACEAE	POPPY-MALLOW,	AR KS MO OK
S	CALLIRHOE PAPAVER VAR. BUSHII	*** SEE ***	CALLIRHOE BUSHII	
LE	CALLIRHOE SCABRIUSCULA	MALVACEAE	POPPY-MALLOW, TEXAS	TX
2	CALOCHORTUS CLAVATUS SSP. RECURVIFOLIUS	LILIACEAE	MARIPOSA, CRUZ	CA
1	CALOCHORTUS CLAVATUS VAR. AVIUS	LILIACEAE	MARIPOSA, PLEASANT VALLEY	CA
1	CALOCHORTUS COERULEUS VAR. WESTONII	LILIACEAE	MARIPOSA, SHIRLEY MEADOWS	CA
2	CALOCHORTUS DUNNII	LILIACEAE	MARIPOSA, DUNN'S	CA, Mexico
2	CALOCHORTUS EXCAVATUS	LILIACEAE	MARIPOSA, INYO	CA
2	CALOCHORTUS GREENEI	LILIACEAE	MARIPOSA, GREENE'S	CA OR
2	CALOCHORTUS HOWELLII	LILIACEAE		OR
2	* CALOCHORTUS INDECORUS	LILIACEAE	MARIPOSA,	OR
2	CALOCHORTUS LONGEBARBATUS VAR. LONGEBARBATUS	LILIACEAE	STAR-TULIP, LONG-HAIRED	CA OR WA
2	CALOCHORTUS LONGEBARBATUS VAR. PECKII	LILIACEAE	MARIPOSA-LILY, LONG-BEARDED, PECK'S	OR
1	* CALOCHORTUS MONANTHUS	LILIACEAE	MARIPOSA, SHASTA RIVER	CA
2	CALOCHORTUS NITIDUS	LILIACEAE		ID WA
2	CALOCHORTUS OBISPOENSIS	LILIACEAE	MARIPOSA, SAN LUIS	CA
2	CALOCHORTUS PERSISTENS	LILIACEAE	MARIPOSA, SISKIYOU	CA
3C	CALOCHORTUS SIMULANS	LILIACEAE		CA
2	CALOCHORTUS STRIATUS	LILIACEAE	MARIPOSA, ALKALI	CA NV
1	CALOCHORTUS TIBURONENSIS	LILIACEAE	MARIPOSA, TIBURON	CA
3C	CALOCHORTUS VENUSTUS VAR. SANGUINEUS	LILIACEAE		CA
2	CALYCADENIA FREMONTII	ASTERACEAE	ROSINWEED, FREMONT'S	CA
2	CALYCADENIA HOOVERI	ASTERACEAE		CA
1	CALYPTRANTHES LUQUILLENSIS	MYRTACEAE		PR
1	CALYPTRANTHES PEDUNCULARIS	MYRTACEAE		PR
2	CALYPTRANTHES THOMASIANA	MYRTACEAE		PR VI
1	CALYPTRANTHES TRIFLORUM	MYRTACEAE		PR
2	CALYPTRIDIUM PULCHELLUM	PORTULACACEAE	PUSSY PAWS, MARIPOSA	CA
1	CALYPTRONOMA RIVALIS	ARECACEAE	PALMA MANACA	PR
2	CALYSTEGIA COLLINA SSP. OXYPHYLLA	CONVOLVULACEAE		CA
2	CALYSTEGIA COLLINA SSP. VENUSTA	CONVOLVULACEAE		CA
2	CALYSTEGIA MACROSTEGIA SSP. AMPLISSIMA	CONVOLVULACEAE		CA
2	CALYSTEGIA PEIRSONII	CONVOLVULACEAE	MORNING-GLORY, PEIRSON'S	CA
2	CALYSTEGIA STEBBINSII	CONVOLVULACEAE	MORNING-GLORY, STEBBINS'	CA
2	CALYSTEGIA SUBACAULIS SSP. EPISCOPALIS	CONVOLVULACEAE		CA
3C	CAMASSIA CUSICKII	LILIACEAE		ID OR
3C	CAMASSIA LEICHTLINII VAR. LEICHTLINII	LILIACEAE	CAMASSIA, LEICHTLIN	OR
LT	CAMISSONIA BENITENSIS	ONAGRACEAE	EVENING-PRIMROSE, SAN BENITO	CA
2	CAMISSONIA CONFERTIFLORA	ONAGRACEAE		AZ
2	CAMISSONIA EXILIS	ONAGRACEAE		AZ UT
3C	CAMISSONIA GOULDII	ONAGRACEAE		AZ UT
2	CAMISSONIA GUADALUPENSIS SSP. CLEMENTINA	ONAGRACEAE	EVENING-PRIMROSE, SAN CLEMENTE I.	CA
2	CAMISSONIA HARDHAMIAE	ONAGRACEAE	EVENING-PRIMROSE, HARDHAM'S	CA
2	CAMISSONIA MEGALANTHA	ONAGRACEAE		NV
3C	CAMISSONIA NEVADENSIS	ONAGRACEAE		NV
3C	CAMISSONIA PARRYI	ONAGRACEAE		AZ UT
2	CAMISSONIA SIERRAE SSP. ALTICOLA	ONAGRACEAE		CA
2	CAMISSONIA SPECUICOLA SSP. HESPERIA	ONAGRACEAE		AZ
3C	CAMISSONIA SPECUICOLA SSP. SPECUICOLA	ONAGRACEAE		AZ
2	CAMISSONIA TANACETIFOLIA SSP. QUADRIPERFORATA	ONAGRACEAE		CA
2	CAMPANULA CALIFORNICA	CAMPANULACEAE	HAREBELL, SWAMP	CA
3C	CAMPANULA PIPERI	CAMPANULACEAE	HAREBELL, OLYMPIC	WA
3C	CAMPANULA REVERCHONII	CAMPANULACEAE		TX

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
2	CAMPANULA ROBINSIAE	CAMPANULACEAE	BELLFLOWER, ROBINS	FL
3B	CAMPANULA ROTUNDIFOLIA VAR. SACAJAWEANA	CAMPANULACEAE		OR
2	CAMPANULA SHARSMITHIAE	CAMPANULACEAE	HAREBELL, MT. HAMILTON	CA
3C	CAMPANULA SHETLERI	CAMPANULACEAE	CAMPANULA, CASTLE	CA
3C	CAMPANULA WILKINSIANA	CAMPANULACEAE	HAREBELL, WILKIN'S	CA
1	CANAVALIA CENTRALIS	FABACEAE	JACK-BEAN,	HI
1	CANAVALIA FORBESII	FABACEAE	JACK-BEAN,	HI
1	CANAVALIA HALEAKALAENSIS	FABACEAE	JACK-BEAN,	HI
3C	CANAVALIA IAOENSIS	FABACEAE	JACK-BEAN,	HI
1	CANAVALIA KAUAIENSIS	FABACEAE	JACK-BEAN,	HI
1	CANAVALIA KAUENSIS	FABACEAE	JACK-BEAN,	HI
1	CANAVALIA LANAIENSIS	FABACEAE	JACK-BEAN, LANAI	HI
1	CANAVALIA MAKAHAENSIS	FABACEAE	JACK-BEAN,	HI
1	CANAVALIA MOLOKAIENSIS	FABACEAE	JACK-BEAN, MOLOKAI	HI
1	CANAVALIA MUNROI	FABACEAE	JACK-BEAN,	HI
3C	CANAVALIA NAPALIENSIS	FABACEAE	JACK-BEAN,	HI
1	CANAVALIA NUALOLOENSIS	FABACEAE	JACK-BEAN,	HI
1	CANAVALIA PENINSULARIS	FABACEAE	JACK-BEAN,	HI
1	CANAVALIA PUBESCENS	FABACEAE	JACK-BEAN,	HI
1	CANAVALIA ROCKII	FABACEAE	PUA-KAUHI	HI
3C	CANAVALIA SANGUINEA	FABACEAE	JACK-BEAN,	HI
1	CANAVALIA STENOPHYLLA	FABACEAE	JACK-BEAN,	HI
2	CANNA PERTUSA	CANNACEAE	MARACA	FL PR
1	CAPPARIS SANDWICHIANA VAR. SANDWICHIANA	CAPPARACEAE	PUA PILO (CAPER, NATIVE)	HI
3C	CARDAMINE CONSTANCEI	BRASSICACEAE	BITTER CRESS, CONSTANCE'S	ID
5	CARDAMINE GAMBELII	*** SEE ***	NASTURTIUM GAMBELII	
3C	CARDAMINE KONAENSIS	BRASSICACEAE	BITTER CRESS, KONA	HI
2	CARDAMINE LONGII	BRASSICACEAE	BITTER CRESS, LONG'S	MA ME MD NC NH NJ NY VA
1	* CARDAMINE MICRANTHERA	BRASSICACEAE	BITTER CRESS,	NC
2	CARDAMINE PATTERSONII	BRASSICACEAE	BITTER CRESS, SADDLE MOUNTAIN	OR
3C	CARDAMINE PENDULIFLORA	BRASSICACEAE		OR
3C	CARDAMINE RUPICOLA	BRASSICACEAE	BITTER CRESS,	MT
2	* CAREX ABORIGINUM	CYPERACEAE	SEDGE, INDIAN VALLEY	ID
1	CAREX ALBIDA	CYPERACEAE	SEDGE, WHITE	CA
1	CAREX AMPLISQUAMA	CYPERACEAE		GA
3B	CAREX ARAPAHOENSIS	CYPERACEAE		CO MT UT WY
3C	CAREX AUSTROCAROLINIANA	CYPERACEAE		GA NC SC TN
2	CAREX BALTZELLII	CYPERACEAE		AL FL
2	CAREX BARRATTII	CYPERACEAE	SEDGE, BARRATT'S	AL CT DE MD NC NJ NY PA TN VA
2	CAREX BILTMOREANA	CYPERACEAE	SEDGE, BILTMORE	GA NC SC
2	CAREX CHAPMANII	CYPERACEAE	SEDGE,	FL NC SC VA
5	CAREX CURATORUM	*** SEE ***	CAREX SCIRPOIDEA VAR. CURATORUM	
2	CAREX DECOMPOSITA	CYPERACEAE		AL FL GA IL IN MI MO SC VA
3B	CAREX ELACHYCARPA	CYPERACEAE	SEDGE, AROOSTOOK	ME
2	CAREX FISSA	CYPERACEAE		OK
3C	CAREX INTERRUPTA	CYPERACEAE		OR WA
3B	CAREX JACOBI-PETERI	CYPERACEAE	SEDGE, ANDERSON	AK
3B	CAREX JOSSELYNII	CYPERACEAE	SEDGE, JOSSELYN'S	ME
1	CAREX LATEBRACTEATA	CYPERACEAE	SEDGE, WATERFALL'S	AR OK
2	CAREX LENTICULARIS VAR. DOLIA	CYPERACEAE		AK MT, Canada (Alta., B.C., Yukon)
3C	CAREX MICROPTERA VAR. CRASSINERVIA	CYPERACEAE	SEDGE, SMALL-LIVING, THICK-NERVED	CO MT NV WY
3C	CAREX MISERA	CYPERACEAE		GA NC TN
3C	CAREX OBISPOENSIS	CYPERACEAE	SEDGE, SAN LUIS	CA
3B	CAREX ONUSTA	CYPERACEAE		TX
2	CAREX ORONENSIS	CYPERACEAE	SEDGE,	ME
3C	CAREX PARRYANA SSP. IDAHOA	CYPERACEAE		ID MT
2	CAREX PAUCIFRUCTUS	CYPERACEAE	SEDGE, SIERRA	CA
5	CAREX PLECTOCARPA	*** SEE ***	CAREX LENTICULARIS VAR. DOLIA	
2	CAREX POLYMORPHA	CYPERACEAE	SEDGE, VARIABLE	CT DE MA MD ME NH NJ NY PA RI VA WV
3C	CAREX PURPURIFERA	CYPERACEAE		AL GA KY NC TN
1	* CAREX ROANENSIS	CYPERACEAE		TN
3C	CAREX SCIRPOIDEA VAR. CURATORUM	CYPERACEAE		AZ UT
3C	CAREX SOCIALIS	CYPERACEAE		AR IL IN KY MO TN
LT	CAREX SPECUICOLA	CYPERACEAE	SEDGE,	AZ
3C	CAREX TOMPKINSII	CYPERACEAE	SEDGE, THOMPKINS	CA
3C	CAREX WHITNEYI	CYPERACEAE	SEDGE, WHITNEY	CA
1	CARPENTERIA CALIFORNICA	SAXIFRAGACEAE	CARPENTERIA	CA
3B	CASSIA EXUNGUIS	FABACEAE	TAMARINDILLO	PR
2	CASSIA FASCICULATA VAR. MACROSPERMA	FABACEAE		MD VA
1	CASSIA KEYENSIS	FABACEAE	SENNA, FLORIDA KEYS	FL
1	CASSIA MIRABILIS	FABACEAE		PR
3C	CASSIA RIPLEYANA	FABACEAE		TX
5	CASTANEA OZARKENSIS	*** SEE ***	CASTANEA PUMILA VAR. OZARKENSIS	
2	CASTANEA PUMILA VAR. OZARKENSIS	FAGACEAE	CHINQUAPIN, OZARK	AL AR MO OK
2	CASTILLEJA ANNUA	SCROPHULARIACEAE		AK
1	CASTILLEJA AQUARIENSIS	SCROPHULARIACEAE	INDIAN PAINTBRUSH, AQUARIUS	UT
3C	CASTILLEJA BREVILOBATA	SCROPHULARIACEAE	INDIAN PAINTBRUSH, SHORT-LOBED RED	CA OR
2	CASTILLEJA CHLOROTICA	SCROPHULARIACEAE	INDIAN PAINTBRUSH, GREEN-TINGED	OR
1	CASTILLEJA CHRISTII	SCROPHULARIACEAE	INDIAN PAINTBRUSH, CHRIST'S	ID

STATUS		SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
3C		CASTILLEJA CHRYSANTHA	SCROPHULARIACEAE		OR
2		CASTILLEJA CILIATA	SCROPHULARIACEAE	INDIAN PAINTBRUSH,	TX
2		CASTILLEJA CINEREA	SCROPHULARIACEAE	INDIAN PAINTBRUSH, ASH GREY	CA
3A		CASTILLEJA CRUENTA	SCROPHULARIACEAE	INDIAN PAINTBRUSH,	AZ
2		CASTILLEJA CRYPTANTHA	SCROPHULARIACEAE		WA
3C		CASTILLEJA CULBERTSONII	SCROPHULARIACEAE	INDIAN PAINTBRUSH, CULBERTSON	CA
3C		CASTILLEJA ELATA	SCROPHULARIACEAE		CA OR
2		CASTILLEJA ELONGATA	SCROPHULARIACEAE		TX
5		CASTILLEJA EWANII	*** SEE ***	CASTILLEJA MARTINII VAR. EWANII	
2		CASTILLEJA FRATERNA	SCROPHULARIACEAE		OR
3C		CASTILLEJA GLANDULIFERA	SCROPHULARIACEAE		OR
2		CASTILLEJA GLEASONII	SCROPHULARIACEAE	PAINTBRUSH, MT. GLEASON	CA
LE		CASTILLEJA GRISEA	SCROPHULARIACEAE	INDIAN PAINTBRUSH, SAN CLEMENTE IS.	CA
2		CASTILLEJA HOLOLEUCA	SCROPHULARIACEAE		CA
5		CASTILLEJA HOLOLEUCA SSP. GRISEA	*** SEE ***	CASTILLEJA GRISEA	
2		CASTILLEJA KAIBABENSIS	SCROPHULARIACEAE		AZ
3C		CASTILLEJA LASSENENSIS	SCROPHULARIACEAE	INDIAN PAINTBRUSH, LEMMON'S	CA
2		CASTILLEJA LATIFOLIA SSP. MENDOCINENSIS	SCROPHULARIACEAE	INDIAN PAINTBRUSH, MENDOCINO COAST	CA
1	*	CASTILLEJA LESCHKEANA	SCROPHULARIACEAE	INDIAN PAINTBRUSH, POINT REYES	CA
2		CASTILLEJA LEVISECTA	SCROPHULARIACEAE		OR WA, CANADA (B.C.)
3B		CASTILLEJA LINOIDES	SCROPHULARIACEAE		NV
3B		CASTILLEJA LUDOVICIANA	SCROPHULARIACEAE	INDIAN PAINTBRUSH, JEFF DAVIS PARISH	LA
3C		CASTILLEJA MARTINII VAR. EWANII	SCROPHULARIACEAE	INDIAN PAINTBRUSH, EWAN'S	CA
5		CASTILLEJA MINIATA SSP. ELATA	*** SEE ***	CASTILLEJA ELATA	
2		CASTILLEJA MOLLIS	SCROPHULARIACEAE	INDIAN PAINTBRUSH, SOFT-LEAVED	CA
1		CASTILLEJA NEGLECTA	SCROPHULARIACEAE		CA
3C		CASTILLEJA ORESBIA	SCROPHULARIACEAE		ID OR
3B		CASTILLEJA OWNBEYANA	SCROPHULARIACEAE	INDIAN PAINTBRUSH, COMMON WALLOWA	OR
3C		CASTILLEJA PARVIFLORA VAR. OLYMPICA	SCROPHULARIACEAE		WA
3C		CASTILLEJA PARVULA	SCROPHULARIACEAE	INDIAN PAINTBRUSH, TUSHAR	UT
2		CASTILLEJA REVEALII	SCROPHULARIACEAE	INDIAN PAINTBRUSH, REVEAL	UT
2		CASTILLEJA SALSUGINOSA	SCROPHULARIACEAE	INDIAN PAINTBRUSH,	NV
3C		CASTILLEJA SCABRIDA	SCROPHULARIACEAE	INDIAN PAINTBRUSH,	UT
2		CASTILLEJA STEENENSIS	SCROPHULARIACEAE		OR
1		CASTILLEJA ULIGINOSA	SCROPHULARIACEAE	INDIAN PAINTBRUSH, PITKIN MARSH	CA
2		CASTILLEJA XANTHOTRICHA	SCROPHULARIACEAE		OR
2		CAULANTHUS AMPLEXICAULIS VAR. BARBARAE	BRASSICACEAE	CAULANTHUS, SANTA BARBARA	CA
2		CAULANTHUS CALIFORNICUS	BRASSICACEAE	JEWELFLOWER, CALIFORNIA	CA
5		CAULANTHUS LEMMONII	*** SEE ***	STREPTANTHUS LEMMONII	
2		CAULANTHUS SIMULANS	BRASSICACEAE		CA
2		CAULANTHUS STENOCARPUS	BRASSICACEAE	CAULANTHUS, SLENDER-POD	CA, Mexico
2		CAULOSTRAMINA JAEGERI	BRASSICACEAE	CAULOSTRAMINA, JAEGER'S	CA
3C		CEANOTHUS ARBOREUS	RHAMNACEAE		CA
2		CEANOTHUS CONFUSUS	RHAMNACEAE	CEANOTHUS, RINCON	CA
2		CEANOTHUS CYANEUS	RHAMNACEAE	CEANOTHUS, LAKESIDE	CA
2		CEANOTHUS DIVERGENS	RHAMNACEAE	CEANOTHUS, CALISTOGA	CA
2		CEANOTHUS FERRISAE	RHAMNACEAE	CALIFORNIA-LILAC, COYOTE	CA
2		CEANOTHUS GLORIOSUS VAR. PORRECTUS	RHAMNACEAE	CEANOTHUS, MOUNT VISION	CA
2		CEANOTHUS HEARSTIORUM	RHAMNACEAE	CEANOTHUS, HEARST'S	CA
2		CEANOTHUS IMPRESSUS VAR. NIPOMENSIS	RHAMNACEAE		CA
2		CEANOTHUS MARITIMUS	RHAMNACEAE	CALIFORNIA-LILAC, MARITIME	CA
2		CEANOTHUS MASONII	RHAMNACEAE	CEANOTHUS, BOLINAS	CA
3C		CEANOTHUS PROSTRATUS VAR. LAXUS	RHAMNACEAE	SQUAW CARPET	CA
2		CEANOTHUS RIGIDUS	RHAMNACEAE	CEANOTHUS, MONTERREY	CA
2		CEANOTHUS RODERICKII	RHAMNACEAE	CEANOTHUS, PINE HILL	CA
3C		CENCHRUS AGRIMONIOIDES VAR. AGRIMONIOIDES	POACEAE	SANDBUR, AGRIMONY,	HI
1	*	CENCHRUS AGRIMONIOIDES VAR. LAYSANENSIS	POACEAE	SANDBUR, AGRIMONY, LAYSAN	HI
3B		CENCHRUS PENDUNCULATUS	POACEAE	SANDBUR, WOOLY WAIANAE	HI
LT		CENTAURIUM NAMOPHILUM VAR. NAMOPHILUM	GENTIANACEAE	CENTAURY, SPRING-LOVING	CA NV
3C		CENTROSEMA ARENICOLA	FABACEAE	BUTTERFLY-PEA,	FL
1		CENTROSTEGIA LEPTOCERAS	POLYGONACEAE	SPINEFLOWER, SLENDER-HORNED	CA
5		CEPHALOCEREUS DEERINGII	*** SEE ***	CEREUS ROBINII	
3C		CERASTIUM ALEUTICUM	CARYOPHYLLACEAE	CHICKWEED, ALEUTIAN	AK
2		CERASTIUM ARVENSE VAR. VILLOSISSIMUM	CARYOPHYLLACEAE	CHICKWEED, FIELD, LONG-HAIRY	PA
5		CERASTIUM BEERINGIANUM VAR. ALEUTICUM	*** SEE ***	CERASTIUM ALEUTICUM	
3B		CERASTIUM CLAWSONII	CARYOPHYLLACEAE	CHICKWEED, MOUSE-EAR	TX
3B		CERATOPHYLLUM FLORIDANUM	CERATOPHYLLACEAE	HORNWORT, FLORIDA	FL
1		CERCOCARPUS TRASKIAE	ROSACEAE	MOUNTAIN-MAHOGANY, CATALINA	CA
PE		CEREUS ERIOPHORUS VAR. FRAGRANS	CACTACEAE	PRICKLY-APPLE, FRAGRANT	FL
2		CEREUS GRACILIS VAR. ABORIGINUM	CACTACEAE	PRICKLY-APPLE, ABORIGINAL	FL
2		CEREUS GRACILIS VAR. SIMPSONII	CACTACEAE	PRICKLY-APPLE, SIMPSON'S	FL
2		CEREUS GREGGII	CACTACEAE		AZ CA NM TX, Mexico
1		CEREUS PORTORICENSIS	CACTACEAE	HIGO CHUMBO	PR
2		CEREUS QUADRICOSTATUS	CACTACEAE	SEBUCAN	PR
LE		CEREUS ROBINII	CACTACEAE	TREE CACTUS, KEY	FL, Cuba
5		CEREUS ROBINII VAR. DEERINGII	*** SEE ***	CEREUS ROBINII	
5		CEREUS ROBINII VAR. ROBINII	*** SEE ***	CEREUS ROBINII	
3C		CHAENACTIS EVERMANNII	ASTERACEAE		ID
3C		CHAENACTIS NEVII	ASTERACEAE		OR
2		CHAENACTIS PARISHII	ASTERACEAE	CHAENACTIS, PARISH'S	CA, Mexico (Baja California)
3C		CHAENACTIS RAMOSA	ASTERACEAE		WA

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
3C	CHAENACTIS THOMPSONII	ASTERACEAE		WA
2	CHAETOPAPPA ELEGANS	ASTERACEAE		NM
2	CHAETOPAPPA HERSHEYI	ASTERACEAE		NM TX
3C	CHAMAECHAENACTIS SCAPOSA	ASTERACEAE		CO ID UT
5	CHAMAECRISTA KEYENSIS	*** SEE ***	CASSIA KEYENSIS	
5	CHAMAECRISTA MIRABILIS	*** SEE ***	CASSIA MIRABILIS	
5	CHAMAESYCE ATROCOCCA VAR. ATROCOCCA	*** SEE ***	EUPHORBIA ATROCOCCA	
5	CHAMAESYCE CUMULICOLA	*** SEE ***	EUPHORBIA CUMULICOLA	
5	CHAMAESYCE DELTOIDEA SSP. DELTOIDEA	*** SEE ***	EUPHORBIA DELTOIDEA SSP. DELTOIDEA	
5	CHAMAESYCE DELTOIDEA SSP. SERPYLLUM	*** SEE ***	EUPHORBIA DELTOIDEA SSP. SERPYLLUM	
5	CHAMAESYCE GARBERI	*** SEE ***	EUPHORBIA GARBERI	
5	CHAMAESYCE PORTERANA VAR. KEYENSIS	*** SEE ***	EUPHORBIA PORTERANA VAR. KEYENSIS	
5	CHAMAESYCE PORTERANA VAR. PORTERANA	*** SEE ***	EUPHORBIA PORTERANA VAR. PORTERANA	
5	CHAMAESYCE PORTERANA VAR. SCOPARIA	*** SEE ***	EUPWORBIA PORTERANA VAR. SCOPARIA	
5	CHAMAESYCE REMYI	*** SEE ***	EUPHORBIA REMYI	
2	CHARPENTIERA DENSIFLORA	AMARANTHACEAE	PAPALA	HI
2	CHEILANTHES ARIZONICA	POLYPODIACEAE		AZ
2	CHEILANTHES FIBRILLOSA	POLYPODIACEAE	LIP FERN, FIBRILLOSE	CA
2	CHEILANTHES PRINGLEI	POLYPODIACEAE		AZ
5	CHEILANTHES PYRAMIDALIS VAR. ARIZONICA	*** SEE ***	CHEILANTHES ARIZONICA	
2	CHEIRODENDRON HELLERI VAR. HELLERI	ARALIACEAE		HI
2	CHEIRODENDRON HELLERI VAR. MICROCARPUM	ARALIACEAE		HI
2	CHEIRODENDRON HELLERI VAR. SODALIUM	ARALIACEAE		HI
1	* CHEIRODENDRON TRIGYNUM VAR. ROCKII	ARALIACEAE	OLAPA,	HI
1	* CHEIRODENDRON TRIGYNUM VAR. SUBCORDATUM	ARALIACEAE	OLAPA,	HI
5	CHEIROGLOSSA PALMATA	*** SEE ***	OPHIOGLOSSUM PALMATUM	
3C	CHELONE OBLIQUA VAR. SPECIOSA	SCROPHULARIACEAE		AR IA IL IN KY MI MO
2	CHENOPODIUM OAHUENSE VAR. DISCOSPERMUM	CHENOPODIACEAE		HI
1	CHENOPODIUM PEKELOI	CHENOPODIACEAE	GOOSEFOOT, MOLOKAI	HI
1	CHIONANTHUS PYGMAEUS	OLEACEAE	FRINGE-TREE, PYGMY	FL
2	CHLORIS TEXENSIS	POACEAE		TX
2	CHLOROGALUM GRANDIFLORUM	LILIACEAE	SOAPROOT, RED HILLS	CA
1	CHLOROGALUM PURPUREUM VAR. PURPUREUM	LILIACEAE	AMOLE, PURPLE	CA
1	CHLOROGALUM PURPUREUM VAR. REDUCTUM	LILIACEAE	AMOLE, CAMMATTA CANYON	CA
3C	CHOISYA ARIZONICA	RUTACEAE		AZ
2	CHOISYA MOLLIS	RUTACEAE		AZ
3C	CHORIZANTHE BLAKLEYI	POLYGONACEAE		CA
2	CHORIZANTHE BREWERI	POLYGONACEAE	CHORIZANTHE, BREWER'S	CA
2	CHORIZANTHE HOWELLII	POLYGONACEAE	CHORIZANTHE, HOWELL	CA
3C	CHORIZANTHE INSIGNIS	POLYGONACEAE	CHORIZANTHE, INDIAN VALLEY	CA
5	CHORIZANTHE LEPTOCERAS	*** SEE ***	CENTROSTEGIA LEPTOCERAS	
2	CHORIZANTHE ORCUTTIANA	POLYGONACEAE	SPINEFLOWER, ORCUTT'S	CA
1	* CHORIZANTHE PARRYI VAR. FERNANDINA	POLYGONACEAE	CHORIZANTHE, SAN FERNANDO VALLEY	CA
2	CHORIZANTHE PUNGENS VAR. PUNGENS	POLYGONACEAE		CA
2	CHORIZANTHE RECTISPINA	POLYGONACEAE	CHORIZANTHE, SAN FERNANDO VALLEY	CA
3C	CHORIZANTHE SPINOSA	POLYGONACEAE	SPINEFLOWER, MOJAVE	CA
2	CHORIZANTHE STATICOIDES SSP. CHRYSACANTHA	POLYGONACEAE	TURKISH RUGGING, ORANGE COUNTY	CA
2	CHORIZANTHE VALIDA	POLYGONACEAE	SPINEFLOWER, SONOMA	CA
5	CHROMOLAENA BORINQUENSIS	*** SEE ***	EUPATORIUM BORINQUENSE	
5	CHROMOLAENA OTEROI	*** SEE ***	EUPATORIUM OTEROI	
1	CHRYSOPSIS CRUISEANA	ASTERACEAE		FL
PE	CHRYSOPSIS FLORIDANA	ASTERACEAE		FL
2	CHRYSOSPLENIUM IOWENSE	SAXIFRAGACEAE		IA MN
2	CHRYSOTHAMNUS MOLESTUS	ASTERACEAE		AZ
3C	CHRYSOTHAMNUS NAUSEOSUS SSP. NANUS	ASTERACEAE		ID OR WA
3B	CHRYSOTHAMNUS NAUSEOSUS SSP. TEXENSIS	ASTERACEAE		NM TX
5	CHRYSOTHAMNUS VISCIDIFLORUS VAR. MODESTUS	*** SEE ***	CHRYSOTHAMNUS MOLESTUS	
3C	CICUTA BOLANDERI	APIACEAE	WATER-HEMLOCK, BOLANDER	CA
1	CIMICIFUGA ARIZONICA	RANUNCULACEAE		AZ
3C	CIMICIFUGA LACINIATA	RANUNCULACEAE		OR WA
2	CIMICIFUGA RUBIFOLIA	RANUNCULACEAE		AL IL KY TN VA
3C	CIRSIUM BREVIFOLIUM	ASTERACEAE		ID OR WA
2	CIRSIUM CAMPYLON	ASTERACEAE	THISTLE, MT. HAMILTON	CA
2	CIRSIUM CILIOLATUM	ASTERACEAE	THISTLE, ASHLAND	CA OR
3C	CIRSIUM CLOKEYI	ASTERACEAE	THISTLE, CLOKEY'S	NV
2	CIRSIUM CRASSICAULE	ASTERACEAE	THISTLE, SLOUGH	CA
3C	CIRSIUM DAVISII	ASTERACEAE		ID
2	CIRSIUM FONTINALE VAR. FONTINALE	ASTERACEAE	THISTLE, FOUNTAIN	CA
2	CIRSIUM FONTINALE VAR. OBISPOENSE	ASTERACEAE	THISTLE, BOG, CHORRO CREEK	CA
3C	CIRSIUM HALLII	ASTERACEAE		CA NV OR UT
2	CIRSIUM HILLII	ASTERACEAE		IA IL IN MI MN OH PA WI, Canada (Ontario)
1	CIRSIUM HYDROPHILUM VAR. HYDROPHILUM	ASTERACEAE	THISTLE, SUISUN	CA
2	CIRSIUM HYDROPHILUM VAR. VASEYI	ASTERACEAE	THISTLE, MT. TAMALPAIS	CA
2	CIRSIUM LONCHOLEPIS	ASTERACEAE	THISTLE, LA GRACIOSA	CA
2	CIRSIUM OCCIDENTALE VAR. COMPACTUM	ASTERACEAE	THISTLE, COBWEB, COMPACT	CA
2	CIRSIUM OWNBEYI	ASTERACEAE	THISTLE, OWNBEY'S	UT
1	CIRSIUM PITCHERI	ASTERACEAE	THISTLE, DUNE	IL IN MI WI, Canada
2	CIRSIUM RHOTHOPHILUM	ASTERACEAE	THISTLE, SURF	CA
3C	CIRSIUM RYDBERGII	ASTERACEAE		AZ UT
3C	CIRSIUM TURNERI	ASTERACEAE		TX

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
PT	CIRSIUM VINACEUM	ASTERACEAE		NM
2	CIRSIUM VIRGINENSIS	ASTERACEAE		AZ UT
1	CLADOCARPA ATOLLENSIS	CUCURBITACEAE		HI
1	CLADOCARPA CAUMII	CUCURBITACEAE		HI
1	CLADOCARPA LAMOUREUXII	CUCURBITACEAE		HI
1	CLADOCARPA MAXIMOWICZII	CUCURBITACEAE		HI
1	CLADOCARPA NIIHAUENSIS	CUCURBITACEAE	PUA O KAMA	HI
1	CLADOCARPA SEMITONSUS	CUCURBITACEAE		HI
3C	CLADRASTIS KENTUKEA	FABACEAE		AL AR GA IL IN KY MS MO NC OK SC TN
5	CLADRASTIS LUTEA	*** SEE ***	CLADRASTIS KENTUKEA	
2	CLAOXYLON SANDWICENSE VAR. SANDWICENSE	EUPHORBIACEAE	POOLA	HI
3C	CLARKIA AMOENA SSP. WHITNEYI	ONAGRACEAE		CA
3B	CLARKIA AMOENA VAR. PACIFICA	ONAGRACEAE		OR WA
3C	CLARKIA AUSTRALIS	ONAGRACEAE		CA
3C	CLARKIA BILOBA SSP. AUSTRALIS	ONAGRACEAE		CA
2	CLARKIA BOREALIS SSP. ARIDA	ONAGRACEAE		CA
2	CLARKIA CALIENTENSIS	ONAGRACEAE		CA
1	CLARKIA FRANCISCANA	ONAGRACEAE	CLARKIA, PRESIDIO	CA
1	CLARKIA IMBRICATA	ONAGRACEAE		CA
1	CLARKIA LINGULATA	ONAGRACEAE	CLARKIA, MERCED	CA
2	CLARKIA MOSQUINII SSP. MOSQUINII	ONAGRACEAE		CA
3A	CLARKIA MOSQUINII SSP. XEROPHILA	ONAGRACEAE		CA
2	CLARKIA ROSTRATA	ONAGRACEAE	CLARKIA, BEAKED	CA
2	CLARKIA SPECIOSA SSP. IMMACULATA	ONAGRACEAE	CLARKIA, SAN LUIS OBISPO	CA
2	CLARKIA SPRINGVILLENSIS	ONAGRACEAE	CLARKIA, SPRINGVILLE	CA
3C	CLAYTONIA BELLIDIFOLIA	PORTULACACEAE		CA OR
5	CLAYTONIA BOSTOCKII	*** SEE ***	MONTIA BOSTOCKII	
5	CLAYTONIA FLAVA	*** SEE ***	CLAYTONIA LANCEOLATA VAR. FLAVA	
3B	CLAYTONIA LANCEOLATA VAR. CHRYSANTHA	PORTULACACEAE		WA
2	CLAYTONIA LANCEOLATA VAR. FLAVA	PORTULACACEAE		ID MT
1	CLAYTONIA LANCEOLATA VAR. PEIRSONII	PORTULACACEAE	SPRING BEAUTY, PEIRSON'S	CA
3C	CLAYTONIA MEGARHIZA VAR. NIVALIS	PORTULACACEAE		WA
3C	CLEMATIS ADDISONII	RANUNCULACEAE	VIRGIN'S BOWER, ADDISON'S	VA
3C	CLEMATIS ALBICOMA	RANUNCULACEAE	LEATHERFLOWER, WHITE-HAIRED	VA WV
3B	CLEMATIS GATTINGERI	RANUNCULACEAE	VIRGIN'S BOWER,	AL TN
2	CLEMATIS HIRSUTISSIMA VAR. ARIZONICA	RANUNCULACEAE		AZ
3B	CLEMATIS MICRANTHA	RANUNCULACEAE	OLD MAN'S BEARD	FL
3C	CLEMATIS OCCIDENTALIS VAR. DISSECTA	RANUNCULACEAE		WA
1	CLEMATIS SOCIALIS	RANUNCULACEAE		AL
2	CLEMATIS VITICAULIS	RANUNCULACEAE	LEATHERFLOWER, MILLBORO	VA
2	CLEOME MULTICAULIS	CAPPARACEAE		AZ CO NM TX WY, Mexico
5	CLEOME SANDWICENSIS	*** SEE ***	CLEOME SPINOSA SSP. NOV. /INED.	
1	CLEOME SPINOSA SSP. NOV. /INED.	CAPPARACEAE	SPIDERFLOWER, WILD	HI
3B	CLEOMELLA MONTROSAE	CAPPARACEAE		CO
1	CLERMONTIA DREPANOMORPHA	CAMPANULACEAE	CLERMONTIA, KOHALA	HI
3A	CLERMONTIA HALEAKALENSIS	CAMPANULACEAE		HI
3C	CLERMONTIA HAWAIIENSIS VAR. HAWAIIENSIS	CAMPANULACEAE		HI
3A	CLERMONTIA KONAENSIS	CAMPANULACEAE		HI
1	CLERMONTIA LINDSEYANA	CAMPANULACEAE		HI
1	CLERMONTIA LOYANA	CAMPANULACEAE		HI
1	CLERMONTIA MUNROI	CAMPANULACEAE		HI
3C	CLERMONTIA PELEANA	CAMPANULACEAE		HI
1	CLERMONTIA PYRULARIA	CAMPANULACEAE		HI
1	CLITORIA FRAGRANS	FABACEAE		FL
3C	CLUSIA FLAVA	HYPERICACEAE		West Indies
2	COCCULUS INTEGER	MENISPERMACEAE		HI
2	COCCULUS LONCHOPHYLLUS	MENISPERMACEAE		HI
2	COCCULUS VIRGATUS	MENISPERMACEAE		HI
5	COCHISEIA ROBBINSORUM	*** SEE ***	CORYPHANTHA ROBBINSORUM	
2	COELORACHIS TUBERCULOSA	POACEAE		AL FL
2	COLLINSIA ANTONINA	SCROPHULARIACEAE		CA
3C	COLLOMIA LARSENII	POLEMONIACEAE		CA OR,WA
2	COLLOMIA MACROCALYX	POLEMONIACEAE	COLLOMIA, BRISTLE-FLOWERED	OR
2	COLLOMIA MAZAMA	POLEMONIACEAE		OR
1	COLLOMIA RAWSONIANA	POLEMONIACEAE	TRUMPET, FLAMING	CA
5	COLORADOA MESAE-VERDAE	*** SEE ***	SCLEROCACTUS MESAE-VERDAE	
3C	COLUBRINA CALIFORNICA	RHAMNACEAE		AZ CA
1	COLUBRINA OPPOSITIFOLIA	RHAMNACEAE	KAUILA	HI
2	COLUBRINA STRICTA	RHAMNACEAE	SNAKEWOOD, COMAL	TX, Mexico
1	COMMELINA GIGAS	COMMELINACEAE	DAYFLOWER, CLIMBING	FL
2	CONDALIA HOOKERI VAR. EDWARDSIANA	RHAMNACEAE	BRASIL, EDWARDS'	TX
2	CONRADINA BREVIFOLIA	LAMIACEAE	ROSEMARY, SHORT-LEAVED	FL
2	CONRADINA GLABRA	LAMIACEAE	ROSEMARY, APALACHICOLA	FL
2	CONRADINA GRANDIFLORA	LAMIACEAE		FL
1	CONRADINA VERTICILLATA	LAMIACEAE		KY TN
5	CONYZA ERIOPHYLLA	*** SEE ***	ERIGERON ERIOPHYLLUS	
1	COPROSMA FAUREI VAR. LANAIENSIS	RUBIACEAE		HI
1	COPROSMA MONTANA VAR. ORBICULARIS	RUBIACEAE		HI
1	COPROSMA OCHRACEA VAR. KAALAE	RUBIACEAE		HI
3C	COPROSMA PUBENS VAR. SESSILIFLORA	RUBIACEAE		HI
1	COPROSMA SERRATA	RUBIACEAE		HI
2	CORDIA BELLONIS	BORAGINACEAE		PR

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
2	CORDIA RUPICOLA	BORAGINACEAE		PR, British V.I.
1	CORDIA WAGNERORUM	BORAGINACEAE		PR
5	CORDYLANTHUS BRUNNEUS VAR. CAPILLARIS	*** SEE ***	CORDYLANTHUS TENUIS SSP. CAPILLARIS /INED.	
2	CORDYLANTHUS EREMICUS SSP. BERNARDINUS /INED.	SCROPHULARIACEAE	BIRD'S-BEAK, SAN BERNARDINO	CA
2	CORDYLANTHUS EREMICUS SSP. EREMICUS	SCROPHULARIACEAE	BIRD'S-BEAK, DESERT	CA
5	CORDYLANTHUS LITTORALIS	*** SEE ***	CORDYLANTHUS RIGIDUS SSP. LITTORALIS /INED.	
LE	CORDYLANTHUS MARITIMUS SSP. MARITIMUS	SCROPHULARIACEAE	BIRD'S-BEAK, SALT MARSH	CA, Mexico (Baja California)
2	CORDYLANTHUS MARITIMUS SSP. PALUSTRIS	SCROPHULARIACEAE	BIRD'S-BEAK, NORTH COAST	CA OR
2	CORDYLANTHUS MOLLIS SSP. HISPIDUS	SCROPHULARIACEAE	BIRD'S-BEAK, HISPID	CA
1	CORDYLANTHUS MOLLIS SSP. MOLLIS	SCROPHULARIACEAE	BIRD'S-BEAK, SOFT	CA
2	CORDYLANTHUS NIDULARIUS	SCROPHULARIACEAE	BIRDS-ON-NEST	CA
PE	CORDYLANTHUS PALMATUS	SCROPHULARIACEAE	BIRD'S-BEAK, PALMATE-BRACTED	CA
5	CORDYLANTHUS RAMOSUS SSP. EREMICUS	*** SEE ***	CORDYLANTHUS EREMICUS SSP. EREMICUS	
1	CORDYLANTHUS RIGIDUS SSP. LITTORALIS /INED.	SCROPHULARIACEAE	BIRD'S-BEAK, SEASIDE	CA
2	CORDYLANTHUS TECOPENSIS	SCROPHULARIACEAE	BIRD'S-BEAK, TECOPA	CA NV
2	CORDYLANTHUS TENUIS SSP. CAPILLARIS /INED.	SCROPHULARIACEAE	BIRD'S-BEAK, PENNELL	CA
2	CORDYLANTHUS TENUIS SSP. PALLESCENS /INED.	SCROPHULARIACEAE	BIRD'S-BEAK, PALLID	CA
2	COREOPSIS HAMILTONII	ASTERACEAE	COREOPSIS, MT. HAMILTON	CA
3B	COREOPSIS HETEROLEPIS	ASTERACEAE		AR
3C	COREOPSIS INTERMEDIA	ASTERACEAE	TICKSEED, GOLDEN WAVE	LA TX
2	COREOPSIS LATIFOLIA	ASTERACEAE		GA NC SC TN
3C	COREOPSIS PULCHRA	ASTERACEAE		AL
3B	COREOPSIS TRIPTERIS VAR. SUBRHOMBOIDEA	ASTERACEAE		TX
2	CORETHROGYNE FILAGINIFOLIA VAR. LINIFOLIA	ASTERACEAE		CA
1	CORNUTIA OBOVATA	VERBENACEAE	NIGUA	PR
2	CORYDALIS AQUAE-GELIDAE	FUMARIACEAE		OR WA
3C	CORYDALIS CASEANA SSP. BRACHYCARPA	FUMARIACEAE		UT
3C	CORYDALIS CASEANA SSP. CASEANA	FUMARIACEAE		CA
3C	CORYDALIS CASEANA SSP. HASTATA	FUMARIACEAE		ID
2	CORYPHANTHA DASYACANTHA VAR. DASYACANTHA	CACTACEAE		TX MEXICO
3C	CORYPHANTHA DASYACANTHA VAR. VARICOLOR	CACTACEAE		TX
3C	CORYPHANTHA DUNCANII	CACTACEAE		NM TX
3C	CORYPHANTHA HESTERI	CACTACEAE		TX
LE	CORYPHANTHA MINIMA	CACTACEAE	CORY CACTUS, NELLIE	TX
2	CORYPHANTHA MISSOURIENSIS VAR. MARSTONII	CACTACEAE		AZ UT
5	CORYPHANTHA NELLIEAE	*** SEE ***	CORYPHANTHA MINIMA	
LT	CORYPHANTHA RAMILLOSA	CACTACEAE	CORY CACTUS, BUNCHED	TX, Mexico (Coahuila)
2	CORYPHANTHA RECURVATA	CACTACEAE		AZ, Mexico
PT	CORYPHANTHA ROBBINSORUM	CACTACEAE	CACTUS, COCHISE PINCUSHION	AZ
1	CORYPHANTHA SCHEERI VAR. ROBUSTISPINA	CACTACEAE		AZ, Mexico
3C	CORYPHANTHA SCHEERI VAR. UNCINATA	CACTACEAE		TX
LT	CORYPHANTHA SNEEDII VAR. LEEI	CACTACEAE	PINCUSHION CACTUS, LEE	NM
LE	CORYPHANTHA SNEEDII VAR. SNEEDII	CACTACEAE	PINCUSHION CACTUS, SNEED	NM TX
2	CORYPHANTHA STROBILIFORMIS VAR. DURISPINA	CACTACEAE		TX, Mexico
2	CORYPHANTHA SULCATA VAR. NICKELSIAE	CACTACEAE		TX, Mexico
2	CORYPHANTHA VIVIPARA VAR. ALVERSONII	CACTACEAE	PINCUSHION CACTUS, ALVERSON'S	CA
3B	CORYPHANTHA VIVIPARA VAR. BUOFLAMA	CACTACEAE		AZ
3C	CORYPHANTHA VIVIPARA VAR. ROSEA	CACTACEAE		AZ CA NV UT
3C	COURSETIA AXILLARIS	FABACEAE		TX, Mexico
LE	COWANIA SUBINTEGRA	ROSACEAE	CLIFF-ROSE, ARIZONA	AZ
2	CRATAEGUS BERBERIFOLIA	ROSACEAE		LA TX
2	CRATAEGUS HARBISONII	ROSACEAE	HAW,	AL GA TN
3B	CRATAEGUS STENOSEPALA	ROSACEAE		TX
3B	CRATAEGUS SUTHERLANDENSIS	ROSACEAE		TX
2	CRATAEGUS WARNERI	ROSACEAE		TX
1	CRESCENTIA PORTORICENSIS	BIGNONIACEAE	HIGUERO DE SIERRA	PR
2	CROOMIA PAUCIFLORA	STEMONACEAE	CROOMIA	AL FL GA LA
3C	CROSSOSOMA CALIFORNICUM	CROSSOSOMATACEAE		CA, Mexico (Baja California)
3C	CROSSOSOMA PARVIFLORUM	CROSSOSOMATACEAE		AZ
2	CROTON ALABAMENSIS	EUPHORBIACEAE		AL TN
2	CROTON ELLIOTTII	EUPHORBIACEAE		AL FL GA SC
3B	CROTON GLANDULOSUS VAR. SIMPSONII	EUPHORBIACEAE		FL
2	CROTON IMPRESSUS	EUPHORBIACEAE		PR, Hispaniola
2	CROTON NUMMULARIIFOLIUS	EUPHORBIACEAE		PR, Cuba, Hispaniola
3C	CROTON WIGGINSII	EUPHORBIACEAE		AZ CA, Mexico (Baja California, Sonora)
2	• CRYPTANTHA APERTA	BORAGINACEAE	CATSEYE,	CO
3C	CRYPTANTHA ATWOODII	BORAGINACEAE	CATSEYE, ATWOOD'S	AZ
1	CRYPTANTHA BARNEBYI	BORAGINACEAE	CATSEYE, BARNEBY	UT
3C	CRYPTANTHA BREVIFLORA	BORAGINACEAE	CATSEYE,	ID UT
2	CRYPTANTHA COMPACTA	BORAGINACEAE	CATSEYE, COMPACT	UT
2	CRYPTANTHA CRASSIPES	BORAGINACEAE		TX
2	CRYPTANTHA CREUTIFELDTII	BORAGINACEAE		UT

STATUS		SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
2		CRYPTANTHA CRINITA	BORAGINACEAE	CRYPTANTHA, SILKY	CA
3C		CRYPTANTHA CRYMOPHILA	BORAGINACEAE		CA
3C		CRYPTANTHA ELATA	BORAGINACEAE	CATSEYE, CLIFFDWELLER'S CANDLESTICK	CO UT
2		CRYPTANTHA GANDERI	BORAGINACEAE	CRYPTANTHA, GANDER'S	CA, Mexico
3C		CRYPTANTHA GRAHAMII	BORAGINACEAE	CATSEYE, GRAHAM	UT
2		CRYPTANTHA HOFFMANNII	BORAGINACEAE		CA NV
3C		CRYPTANTHA HYPSOPHILA	BORAGINACEAE		CA ID OR
2	*	CRYPTANTHA INSOLITA	BORAGINACEAE	CATSEYE,	NV
3C		CRYPTANTHA INTERRUPTA	BORAGINACEAE		ID MT NV OR, Canada (B.C.)
3C		CRYPTANTHA JOHNSTONII	BORAGINACEAE	CATSEYE, JOHNSTON	UT
3C		CRYPTANTHA JONESIANA	BORAGINACEAE	CATSEYE, JONES	UT
3C		CRYPTANTHA MENSANA	BORAGINACEAE		UT
S		CRYPTANTHA NUBIGENA	*** SEE ***	CRYPTANTHA HYPSOPHILA	
2		CRYPTANTHA OCHROLEUCA	BORAGINACEAE	CATSEYE, YELLOW-WHITE	UT
3C		CRYPTANTHA PARADOXA	BORAGINACEAE		CO NM UT
2		CRYPTANTHA ROOSIORUM	BORAGINACEAE	CATSEYE, BRISTLE-CONE	CA
3C		CRYPTANTHA SEMIGLABRA	BORAGINACEAE	CATSEYE,	AZ
2		CRYPTANTHA SHACKLETTEANA	BORAGINACEAE	CATSEYE,	AK
3C		CRYPTANTHA SOBOLIFERA	BORAGINACEAE	CATSEYE,	MT
3C		CRYPTANTHA STRICTA	BORAGINACEAE	CATSEYE,	CO UT WY
2		CRYPTANTHA SUBCAPITATA	BORAGINACEAE		WY
3C		CRYPTANTHA THOMPSONII	BORAGINACEAE		OR WA
2		CRYPTANTHA TRASKIAE	BORAGINACEAE		CA
3C		CRYPTANTHA TUMULOSA	BORAGINACEAE		CA NV
3C		CRYPTANTHA WEBERI	BORAGINACEAE	CATSEYE, WEBER'S	CO
1		CRYPTOCARYA OAHUENSIS	LAURACEAE		HI
1	*	CTENITIS SQUAMIGERA	POLYPODIACEAE		HI
3C		CTENIUM FLORIDANUM	POACEAE		FL GA
2		CUCURBITA OKEECHOBEENSIS	CUCURBITACEAE	GOURD, OKEECHOBEE	FL
3C		CUCURBITA TEXANA	CUCURBITACEAE		TX
2		CUPHEA ASPERA	LYTHRACEAE		FL
PE		CUPRESSUS ABRAMSIANA	CUPRESSACEAE	CYPRESS, SANTA CRUZ	CA
S		CUPRESSUS ARIZONICA VAR. NEVADENSIS	*** SEE ***	CUPRESSUS NEVADENSIS	
S		CUPRESSUS ARIZONICA VAR. STEPHENSONII	*** SEE ***	CUPRESSUS STEPHENSONII	
2		CUPRESSUS GOVENIANA	CUPRESSACEAE	CYPRESS, GOWEN	CA
S		CUPRESSUS GOVENIANA VAR. ABRAMSIANA	*** SEE ***	CUPRESSUS ABRAMSIANA	
2		CUPRESSUS MACROCARPA	CUPRESSACEAE	CYPRESS, MONTEREY	CA
3C		CUPRESSUS NEVADENSIS	CUPRESSACEAE		CA
1		CUPRESSUS STEPHENSONII	CUPRESSACEAE	CYPRESS, CUYAMACA	CA
1		CUSCUTA ATTENUATA	CUSCUTACEAE	DODDER,	OK
3C		CUSCUTA HARPERI	CUSCUTACEAE		AL GA
3C		CUSCUTA HOWELLIANA	CUSCUTACEAE	DODDER, BOGG'S LAKE	CA
2	*	CUSCUTA WARNERI	CUSCUTACEAE	DODDER, WARNER'S	UT
1		CYANEA ANGUSTIFOLIA VAR. LANAIENSIS	CAMPANULACEAE		HI
3C		CYANEA ANGUSTIFOLIA VAR. RACEMOSA	CAMPANULACEAE		HI
1	*	CYANEA ARBOREA	CAMPANULACEAE		HI
S		CYANEA ARBOREA VAR. PYCNOCARPA	*** SEE ***	CYANEA PYCNOCARPA	
1	*	CYANEA ASPLENIIFOLIA	CAMPANULACEAE		HI
1		CYANEA BALDWINII	CAMPANULACEAE	CYANEA, BALDWIN	HI
1		CYANEA BRYANII	CAMPANULACEAE	CYANEA, BRYAN	HI
1		CYANEA CARLSONII	CAMPANULACEAE		HI
1		CYANEA CHOCKII	CAMPANULACEAE		HI
3A		CYANEA COMATA	CAMPANULACEAE		HI
1		CYANEA FERNALDII	CAMPANULACEAE		HI
1		CYANEA GIBSONII	CAMPANULACEAE		HI
1	*	CYANEA GIFFARDII	CAMPANULACEAE		HI
3C		CYANEA GRIMESIANA VAR. GRIMESIANA	CAMPANULACEAE		HI
2		CYANEA GRIMESIANA VAR. HIRSUTIFOLIA	CAMPANULACEAE		HI
1	*	CYANEA GRIMESIANA VAR. LYDGATEI	CAMPANULACEAE		HI
1		CYANEA GRIMESIANA VAR. MAUIENSIS	CAMPANULACEAE		HI
1		CYANEA GRIMESIANA VAR. MUNROI	CAMPANULACEAE		HI
2		CYANEA KUNTHIANA	CAMPANULACEAE		HI
1		CYANEA LEPTOSTEGIA	CAMPANULACEAE	CYANEA, GIANT KOKE'E	HI
S		CYANEA LINDSEYANA	*** SEE ***	CLERMONTIA LINDSEYANA	
1		CYANEA LINEARIFOLIA	CAMPANULACEAE		HI
1		CYANEA MARKSII	CAMPANULACEAE		HI
1		CYANEA MCELDOWNEYI	CAMPANULACEAE		HI
2		CYANEA NELSONII	CAMPANULACEAE		HI
S		CYANEA PINNATIFIDA	*** SEE ***	ROLLANDIA PINNATIFIDA	
1		CYANEA PLATYPHYLLA	CAMPANULACEAE		HI
1		CYANEA PYCNOCARPA	CAMPANULACEAE		HI
1	*	CYANEA REGINA	CAMPANULACEAE		HI
S		CYANEA RIVULARIS	*** SEE ***	DELISSEA RIVULARIS	
1		CYANEA ROLLANDIOIDES	CAMPANULACEAE		HI
2		CYANEA SCABRA	CAMPANULACEAE		HI
1		CYANEA SHIPMANII	CAMPANULACEAE		HI
1		CYANEA SOLANACEA	CAMPANULACEAE	POPOLO	HI
1		CYANEA SOLENOCALYX	CAMPANULACEAE	CYANEA, MOLOKAI	HI
1		CYANEA STICTOPHYLLA	CAMPANULACEAE		HI
1		CYANEA SUBMURICATA	CAMPANULACEAE		HI
1		CYANEA SUPERBA	CAMPANULACEAE		HI
1		CYANEA TRITOMANTHA	CAMPANULACEAE	AKU 'AKU	HI

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
S	CYATHEA BROOKSII	*** SEE ***	ALSOPHILA BROOKSII	
S	CYATHEA DRYOPTEROIDES	*** SEE ***	ALSOPHILA DRYOPTEROIDES	
PE	CYCLADENIA HUMILIS VAR. JONESII	APOCYNACEAE	CYCLADENIA, JONES	UT
S	CYCLODON ALABAMENSIS	*** SEE ***	MATELEA ALABAMENSIS	
3C	CYMOPHYLLUS FRASERI	CYPERACEAE		KY MD NC PA SC TN VA WV
3C	CYMOPTERUS BASALTICUS	APIACEAE		NV UT
2	CYMOPTERUS BECKII	APIACEAE		UT
3C	CYMOPTERUS CORRUGATUS	APIACEAE		ID NV OR
3C	CYMOPTERUS COULTERI	APIACEAE	BISCUITROOT, COULTER	UT
2	CYMOPTERUS DESERTICOLA	APIACEAE	CYMOPTERUS, DESERT	CA
3C	CYMOPTERUS DUCHESNENSIS	APIACEAE	BISCUITROOT, DUCHESNE	CO UT
2	CYMOPTERUS GOODRICHII	APIACEAE		NV
2	CYMOPTERUS HIGGINSII	APIACEAE	BISCUITROOT, HIGGINS	UT
3C	CYMOPTERUS IBAPENSIS	APIACEAE		ID
2	CYMOPTERUS MINIMUS	APIACEAE	BISCUITROOT, CEDAR BREAKS	UT
3C	CYMOPTERUS NEWBERRYI	APIACEAE		AZ UT
2	CYMOPTERUS NIVALIS	APIACEAE		NV
2	CYMOPTERUS RIPLEYI VAR. SANICULOIDES	APIACEAE		NV
3C	CYMOPTERUS ROSEI	APIACEAE		UT
2	CYMOPTERUS SP. NOV. /INED.	APIACEAE		WY
S	CYMOPTERUS SP. NOV. /INED. (CUSTER CO.)	*** SEE ***	CYMOPTERUS IBAPENSIS	ID
2	CYMOPTERUS SP. NOV. /INED. (CUSTER, LEMHI COS.)	APIACEAE		ID
2	CYNANCHUM MONENSE	ASCLEPIADACEAE		PR
2	CYNANCHUM WIGGINSII	ASCLEPIADACEAE		AZ
3C	CYPERUS GRANITOPHILUS	CYPERACEAE		AL GA NC SC
2	CYPERUS GRAYIOIDES	CYPERACEAE	SEDGE, UMBRELLA.	IL
2	CYPERUS ONEROSUS	CYPERACEAE		TX
1	CYPERUS PENNATIFORMIS VAR. BRYANII	CYPERACEAE		HI
S	CYPERUS URBANII	*** SEE ***	MARISCUS URBANII	
3C	CYPRIPEDIUM ARIETINUM	ORCHIDACEAE	LADY'S-SLIPPER, RAM'S-HEAD	CT ME MA MI MN NH NY VT WI
3C	CYPRIPEDIUM CALIFORNICUM	ORCHIDACEAE	LADY'S-SLIPPER, CALIFORNIA	CA OR
3C	CYPRIPEDIUM CANDIDUM	ORCHIDACEAE	LADY'S-SLIPPER, SMALL WHITE	IL IN IA KY MI MN MO NE NJ NY ND OH PA SD WI
3C	CYPRIPEDIUM FASCICULATUM	ORCHIDACEAE		CA CO ID MT OR UT WA WY. Canada
2	CYPRIPEDIUM KENTUCKIENSE	ORCHIDACEAE		AL AR KY LA MS TN
3C	CYPRIPEDIUM MONTANUM	ORCHIDACEAE		AK CA MT OR WA WY. Canada (Alta., B.C.)
1	CYRTANDRA ALATA	GESNERIACEAE		HI
1	CYRTANDRA ALNEA	GESNERIACEAE		HI
1	CYRTANDRA AMBIGUA	GESNERIACEAE		HI
1	CYRTANDRA AXILLIFLORA	GESNERIACEAE		HI
1	CYRTANDRA BASIPARTITA	GESNERIACEAE		HI
3A	CYRTANDRA BEGONIIFOLIA	GESNERIACEAE	'ILIHIA	HI
2	CYRTANDRA BISERRATA	GESNERIACEAE		HI
1	CYRTANDRA BREVICORNUTA	GESNERIACEAE		HI
1	CYRTANDRA BRYANII	GESNERIACEAE		HI
1	CYRTANDRA CAMPANIFORMIS	GESNERIACEAE		HI
1	* CYRTANDRA CARINATA	GESNERIACEAE		HI
1	CYRTANDRA CAUDATISEPALA	GESNERIACEAE		HI
1	CYRTANDRA CHARTACEA	GESNERIACEAE		HI
1	CYRTANDRA CHRISTOPHERSENII	GESNERIACEAE		HI
1	* CYRTANDRA COLLARIFERA	GESNERIACEAE		HI
2	CYRTANDRA CONRADTII	GESNERIACEAE		HI
1	* CYRTANDRA CORDIFOLIA VAR. BREVIPILITA	GESNERIACEAE		HI
2	CYRTANDRA CORDIFOLIA VAR. GYNOGLABRA	GESNERIACEAE		HI
1	* CYRTANDRA CRASSIOR	GESNERIACEAE		HI
1	CYRTANDRA CRENATA	GESNERIACEAE		HI
1	CYRTANDRA CUPULIFORMIS	GESNERIACEAE		HI
1	CYRTANDRA DENTATA	GESNERIACEAE		HI
1	CYRTANDRA ELLIPTICIFOLIA	GESNERIACEAE		HI
1	CYRTANDRA ELLIPTISEPALA	GESNERIACEAE		HI
1	CYRTANDRA FERRICOLORATA	GESNERIACEAE		HI
1	* CYRTANDRA FERRUGINOSA	GESNERIACEAE		HI
2	CYRTANDRA FILIPES	GESNERIACEAE		HI
1	CYRTANDRA FORBESII	GESNERIACEAE		HI
1	CYRTANDRA FOSBERGII	GESNERIACEAE		HI
1	CYRTANDRA FREDERICKII	GESNERIACEAE		HI
1	CYRTANDRA FUSIFORMIS	GESNERIACEAE		HI
1	* CYRTANDRA GARBERI	GESNERIACEAE		HI
2	CYRTANDRA GEORGIANA	GESNERIACEAE		HI
2	CYRTANDRA GIFFARDII	GESNERIACEAE		HI
2	CYRTANDRA GLAUCA	GESNERIACEAE		HI
3A	CYRTANDRA GRACILIS	GESNERIACEAE		HI
2	CYRTANDRA GRAYANA VAR. LANAIENSIS	GESNERIACEAE		HI
3A	CYRTANDRA GROSSECRENATA	GESNERIACEAE		HI
2	CYRTANDRA HALAWENSIS	GESNERIACEAE		HI
2	CYRTANDRA HAWAIENSIS	GESNERIACEAE		HI
2	CYRTANDRA HIRSUTULA	GESNERIACEAE		HI
1	CYRTANDRA HOBDYI	GESNERIACEAE		HI
3A	CYRTANDRA HONOLULENSIS	GESNERIACEAE		HI

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
2	CYRTANDRA HOSAKAE	GESNERIACEAE		HI
1	CYRTANDRA INFRAPALLIDA	GESNERIACEAE		HI
1	CYRTANDRA INTONSA	GESNERIACEAE		HI
2	CYRTANDRA INTRAPILOSA	GESNERIACEAE		HI
2	CYRTANDRA INTRAVILLOSA	GESNERIACEAE		HI
1	CYRTANDRA KAALAE	GESNERIACEAE		HI
1	CYRTANDRA KAHANENSIS	GESNERIACEAE		HI
1	CYRTANDRA KAHUKUENSIS	GESNERIACEAE		HI
1	CYRTANDRA KALUANUIENSIS	GESNERIACEAE		HI
1	CYRTANDRA KANEOHEENSIS	GESNERIACEAE		HI
1	* CYRTANDRA KAUAIENSIS	GESNERIACEAE	ULUNAHELE	HI
2	CYRTANDRA KAULANTHA	GESNERIACEAE		HI
1	CYRTANDRA KOOLAUENSIS	GESNERIACEAE		HI
1	* CYRTANDRA LAEVIS	GESNERIACEAE		HI
3A	CYRTANDRA LAXIFLORA	GESNERIACEAE		HI
3A	CYRTANDRA LESSONIANA VAR. ANGUSTIFOLIA	GESNERIACEAE		HI
1	CYRTANDRA LESSONIANA VAR. INTRAPUBENS	GESNERIACEAE		HI
2	CYRTANDRA LIMOSIFLORA	GESNERIACEAE		HI
3A	CYRTANDRA LINEARIS	GESNERIACEAE		HI
2	CYRTANDRA LONGICALYX	GESNERIACEAE		HI
1	* CYRTANDRA LONGIFOLIA VAR. LONGIFOLIA	GESNERIACEAE		HI
3A	CYRTANDRA LONGIFOLIA VAR. PARALLELA	GESNERIACEAE		HI
1	CYRTANDRA LONGILOBA	GESNERIACEAE		HI
2	CYRTANDRA LYSIOSEPALA VAR. GRAYI	GESNERIACEAE		HI
2	CYRTANDRA LYSIOSEPALA VAR. HALEAKALENSIS	GESNERIACEAE		HI
2	CYRTANDRA LYSIOSEPALA VAR. LYSIOSEPALA	GESNERIACEAE		HI
2	CYRTANDRA MACRANTHA	GESNERIACEAE		HI
2	CYRTANDRA MALACOPHYLLA VAR. MALACOPHYLLA	GESNERIACEAE		HI
1	* CYRTANDRA MANNII	GESNERIACEAE		HI
1	CYRTANDRA MEGASTIGMATA	GESNERIACEAE		HI
1	CYRTANDRA MENZIESII	GESNERIACEAE	HA'I WALE	HI
2	CYRTANDRA MUNROI	GESNERIACEAE		HI
1	CYRTANDRA NIUENSIS	GESNERIACEAE		HI
1	CYRTANDRA NUBINCOLENS	GESNERIACEAE		HI
2	CYRTANDRA OENOBARBA	GESNERIACEAE		HI
1	CYRTANDRA OLIVACEA	GESNERIACEAE		HI
1	CYRTANDRA PALOLOENSIS	GESNERIACEAE		HI
2	CYRTANDRA PALUDOSA VAR. HAUPUENSIS	GESNERIACEAE		HI
1	* CYRTANDRA PARTITA	GESNERIACEAE		HI
1	CYRTANDRA PEARSALLII	GESNERIACEAE		HI
1	CYRTANDRA PERSTAMINODICA	GESNERIACEAE		HI
1	* CYRTANDRA PICKERINGII	GESNERIACEAE		HI
1	* CYRTANDRA PILIGYNA	GESNERIACEAE		HI
2	CYRTANDRA PLATYPHYLLA VAR. HILOENSIS	GESNERIACEAE		HI
1	CYRTANDRA PLURIFOLIA	GESNERIACEAE		HI
1	CYRTANDRA POLYANTHA	GESNERIACEAE		HI
1	CYRTANDRA PRUINOSA	GESNERIACEAE		HI
1	CYRTANDRA PUBENS	GESNERIACEAE		HI
2	CYRTANDRA RAMOSISSIMA	GESNERIACEAE		HI
1	CYRTANDRA ROCKII	GESNERIACEAE		HI
1	CYRTANDRA SANDWICENSIS	GESNERIACEAE		HI
3A	CYRTANDRA SCABRELLA	GESNERIACEAE		HI
1	* CYRTANDRA SKOTTSBERGII	GESNERIACEAE		HI
1	CYRTANDRA SUBCORDATA	GESNERIACEAE		HI
3A	CYRTANDRA SUBINTEGRA	GESNERIACEAE		HI
1	CYRTANDRA SUBRECTA	GESNERIACEAE		HI
1	CYRTANDRA SUBUMBELLATA VAR. INTOSA	GESNERIACEAE		HI
1	CYRTANDRA TERNATA	GESNERIACEAE		HI
3A	CYRTANDRA TRIFLORA	GESNERIACEAE		HI
1	CYRTANDRA TURBINIFORMIS	GESNERIACEAE		HI
1	* CYRTANDRA VANIOTA	GESNERIACEAE		HI
1	CYRTANDRA VILLICALYX VAR. PUBENTIGYNA	GESNERIACEAE		HI
1	CYRTANDRA VILLOSA	GESNERIACEAE		HI
1	CYRTANDRA VILLOSIFLORA	GESNERIACEAE		HI
1	* CYRTANDRA WAIANUENSIS	GESNERIACEAE		HI
1	* CYRTANDRA WAIOLANI	GESNERIACEAE		HI
1	CYRTANDRA WAIOMAOENSIS	GESNERIACEAE		HI
5	DALEA ARBORESCENS	*** SEE ***	PSOROTHAMNUS ARBORESCENS	
2	DALEA BARTONII	FABACEAE		TX
2	DALEA EPICA	FABACEAE	PRAIRIE-CLOVER, HOLE-IN-THE-ROCK	UT
2	DALEA FOLIOSA	FABACEAE	PRAIRIE-CLOVER,	AL IL TN
3C	DALEA GATTINGERI	FABACEAE		AL GA TN
5	DALEA KINGII	*** SEE ***	PSOROTHAMNUS KINGII	
2	DALEA REVERCHONII	FABACEAE	PRAIRIE-CLOVER, COMANCHE-PEAK	TX
2	DALEA SABINALIS	FABACEAE	PRAIRIE-CLOVER, SABINAL	TX
3C	DALEA SCARIOSA	FABACEAE	PRAIRIE-CLOVER,	NM
1	DALEA TENTACULOIDES	FABACEAE	INDIGOBUSH, GENTRY'S	AZ
5	DALEA THOMPSONAE	*** SEE ***	PSOROTHAMNUS THOMPSONAE	
1	DAPHNOPSIS HELLERANA	THYMELAEACEAE		PR
3C	DARLINGTONIA CALIFORNICA	SARRACENIACEAE	PITCHERPLANT, CALIFORNIA	CA OR
3C	DASYNOTUS DAUBENMIREI	BORAGINACEAE		ID
2	DEDECKERA EUREKENSIS	POLYGONACEAE	JULY GOLD	CA
1	DEERINGOTHAMNUS PULCHELLUS	ANNONACEAE	SQUIRREL-BANANA, WHITE	FL

STATUS		SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
1		DEERINGOTHAMNUS RUGELII	ANNONACEAE	SQUIRREL-BANANA, YELLOW	FL
1	*	DELISSEA FALLAX	CAMPANULACEAE		HI
1	*	DELISSEA LACINIATA	CAMPANULACEAE		HI
1	*	DELISSEA NIIHAUENSIS	CAMPANULACEAE		HI
1	*	DELISSEA PARVIFLORA	CAMPANULACEAE		HI
1		DELISSEA RHYTIDOSPERMA	CAMPANULACEAE		HI
2		DELISSEA RIVULARIS	CAMPANULACEAE		HI
1	*	DELISSEA SINUATA	CAMPANULACEAE		HI
3C		DELISSEA SUBCORDATA VAR. OBTUSIFOLIA	CAMPANULACEAE		HI
3C		DELISSEA SUBCORDATA VAR. SUBCORDATA	CAMPANULACEAE		HI
1	*	DELISSEA UNDULATA	CAMPANULACEAE		HI
3C		DELPHINIUM ALABAMICUM	RANUNCULACEAE		AL
2		DELPHINIUM BAKERI	RANUNCULACEAE	LARKSPUR, BAKER'S	CA
2		DELPHINIUM CALIFORNICUM SSP. INTERIUS	RANUNCULACEAE		CA
2		DELPHINIUM HESPERIUM SSP. CUYAMACAE	RANUNCULACEAE	LARKSPUR, CUYAMACA	CA
2		DELPHINIUM HUTCHINSONAE	RANUNCULACEAE	DELPHINIUM, HUTCHINSON'S	CA
2		DELPHINIUM INOPINUM	RANUNCULACEAE		CA
LE		DELPHINIUM KINKIENSE	RANUNCULACEAE	LARKSPUR, SAN CLEMENTE ISLAND	CA
2		DELPHINIUM LEUCOPHAEUM	RANUNCULACEAE		OR
2		DELPHINIUM LUTEUM	RANUNCULACEAE	LARKSPUR, YELLOW	CA
3C		DELPHINIUM MULTIPLEX	RANUNCULACEAE	LARKSPUR,	WA
3C		DELPHINIUM NEWTONIANUM	RANUNCULACEAE		AR
3C		DELPHINIUM NUTTALLIANUM VAR. LINEAPETALUM	RANUNCULACEAE	LARKSPUR.	WA
3C		DELPHINIUM PARISHII SSP. PURPUREUM	RANUNCULACEAE		CA
2		DELPHINIUM PAVONACEUM	RANUNCULACEAE		OR
3C		DELPHINIUM TRELEASEI	RANUNCULACEAE		AR MO
1		DELPHINIUM VARIEGATUM SSP. THORNEI	RANUNCULACEAE	LARKSPUR, ROYAL, THORNE'S	CA
1		DELPHINIUM VIRIDESCENS	RANUNCULACEAE	LARKSPUR, WENATCHEE	WA
3C		DELPHINIUM XANTHOLEUCUM	RANUNCULACEAE	LARKSPUR, NORTHWESTERN	WA
2		DENDROMECON RIGIDA SSP. RHAMNOIDES	PAPAVERACEAE		CA
2		DENDROPEMON SINTENISII	LORANTHACEAE	MICAQUILLO (MISTLETOE)	PR
3B		DENTARIA INCISA	BRASSICACEAE		TN
2		DESCURAINIA TORULOSA	BRASSICACEAE		WY
2		DESMANTHUS BICORNUTUS	FABACEAE	BUNDLEFLOWER, RUBY	Mexico
2		DESMODIUM LINDHEIMERI	FABACEAE		TX, Mexico
S		DICENTRA FORMOSA SSP. NEVADENSIS	*** SEE ***	DICENTRA NEVADENSIS	
3C		DICENTRA FORMOSA SSP. OREGANA	FUMARIACEAE	BLEEDINGHEART, PACIFIC	CA OR
3C		DICENTRA NEVADENSIS	FUMARIACEAE	BLEEDINGHEART, NEVADA	CA
3C		DICENTRA OCHROLEUCA	FUMARIACEAE	BLEEDINGHEART, YELLOW	CA
PE		DICERANDRA CORNUTISSIMA	LAMIACEAE	BALM, LONGSPURRED	FL
PE		DICERANDRA FRUTESCENS	LAMIACEAE	BALM, SCRUB	FL
LE		DICERANDRA IMMACULATA	LAMIACEAE		FL
3C		DICERANDRA ODORATISSIMA	LAMIACEAE		FL GA SC
2		DICHANTHELIUM LANUGINOSUM VAR. THERMALE	POACEAE	PANIC GRASS, HOT SPRING	CA
3C		DICHELOSTEMMA LACUNA-VERNALIS	LILIACEAE	BRODIAEA, VERNAL POOL	CA
3C		DICHONDRA DONNELLIANA	CONVOLVULACEAE	DICHONDRA, CALIFORNIA	CA
3C		DICHONDRA OCCIDENTALIS	CONVOLVULACEAE		CA
3B		DICLIPTERA KRUGII	ACANTHACEAE		PR
1		DIELLIA ERECTA	POLYPODIACEAE		HI
1		DIELLIA FALCATA	POLYPODIACEAE		HI
1		DIELLIA LACINIATA	POLYPODIACEAE		HI
1	*	DIELLIA MANNII	POLYPODIACEAE		HI
1		DIELLIA UNISORA	POLYPODIACEAE		HI
2		DIGITARIA FLORIDANA	POACEAE		FL
2		DIGITARIA GRACILLIMA	POACEAE		FL
2		DIGITARIA PAUCIFLORA	POACEAE	FINGER GRASS,	FL
3C		DIONAEA MUSCIPULA	DROSERACEAE	VENUS' FLY-TRAP	NC SC
2		DIPLACUS ARIDUS	SCROPHULARIACEAE	BUSH MONKEYFLOWER, LOW	CA, Mexico (Baja California)
1	*	DIPLAZIUM MOLOKAIENSE	POLYPODIACEAE		HI
1	*	DISSANTHELIUM CALIFORNICUM	POACEAE	DISSANTHELIUM, CALIFORNIA	CA, Mexico
3C		DISSOCHONDRUS BIFLORUS	POACEAE		HI
2		DITAXIS CALIFORNICA	EUPHORBIACEAE	DITAXIS, CALIFORNIA	CA
3B		DITAXIS DIVERSIFLORA	EUPHORBIACEAE		NV
2		DITHYREA MARITIMA	BRASSICACEAE	SPECTACLE-POD, BEACH	CA
2		DODECATHEON FRENCHII	PRIMULACEAE	SHOOTINGSTAR, FRENCH'S	AR IL IN KY MO
3C		DODECATHEON POETICUM	PRIMULACEAE		OR WA
2		DODONAEA ERIOCARPA VAR. CONFERTIOR	SAPINDACEAE		HI
2		DODONAEA ERIOCARPA VAR. COSTULATA	SAPINDACEAE		HI
2		DODONAEA ERIOCARPA VAR. FORBESII	SAPINDACEAE		HI
2		DODONAEA ERIOCARPA VAR. LANAIENSIS	SAPINDACEAE		HI
2		DODONAEA ERIOCARPA VAR. MOLOKAIENSIS	SAPINDACEAE		HI
2		DODONAEA ERIOCARPA VAR. OBLONGA	SAPINDACEAE		HI
2		DODONAEA ERIOCARPA VAR. PALLIDA	SAPINDACEAE		HI
2		DODONAEA ERIOCARPA VAR. SKOTTSBERGII	SAPINDACEAE		HI
2		DODONAEA ERIOCARPA VAR. VARIANS	SAPINDACEAE		HI
2		DODONAEA SANDWICENSIS VAR. LATIFOLIA	SAPINDACEAE		HI
2		DODONAEA SANDWICENSIS VAR. SIMULANS	SAPINDACEAE		HI
2		DODONAEA STENOPTERA VAR. FAURIEI	SAPINDACEAE		HI
1		DODONAEA STENOPTERA VAR. STENOPTERA	SAPINDACEAE		HI
2		DOUGLASIA IDAHOENSIS	PRIMULACEAE		ID
3C		DOUGLASIA LAEVIGATA VAR. LAEVIGATA	PRIMULACEAE		OR WA

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
3C	DOUGLASIA NIVALIS VAR. NIVALIS	PRIMULACEAE		WA
2	DOWNINGIA CONCOLOR VAR. BREVIOR	CAMPANULACEAE	DOWNINGIA, CUYAMACA LAKE	CA
3C	DOWNINGIA HUMILIS	CAMPANULACEAE		CA
S	DOWNINGIA PUSILLA	*** SEE ***	DOWNINGIA HUMILIS	
3C	DRABA APICULATA VAR. DAVIESIAE	BRASSICACEAE		MT
2	DRABA APRICA	BRASSICACEAE		AR GA MO OK SC
3C	DRABA ARGYRAEA	BRASSICACEAE		ID
2	DRABA ARIDA	BRASSICACEAE		NV
3C	DRABA ASPRELLA VAR. ASPRELLA	BRASSICACEAE		AZ
3B	DRABA ASPRELLA VAR. KAIBABENSIS	BRASSICACEAE		AZ
3B	DRABA ASPRELLA VAR. STELLIGERA	BRASSICACEAE		AZ
3C	DRABA ASPRELLA VAR. ZIONENSIS	BRASSICACEAE	WHITLOW-GRASS, ZION	UT
3C	DRABA ASTEROPHORA VAR. ASTEROPHORA	BRASSICACEAE	DRABA, LAKE TAHOE	CA NV
2	DRABA ASTEROPHORA VAR. MACROCARPA	BRASSICACEAE	DRABA, CUP LAKE	CA
2	DRABA CARNOSULA	BRASSICACEAE	DRABA, MT. EDDY	CA
3C	DRABA CRASSIFOLIA VAR. NEVADENSIS	BRASSICACEAE		CA NV
3C	DRABA CRUCIATA VAR. CRUCIATA	BRASSICACEAE	DRABA, MINERAL KING	CA
3C	DRABA CRUCIATA VAR. INTEGRIFOLIA	BRASSICACEAE	DRABA, WHITNEY	CA
3C	DRABA DOUGLASII VAR. CROCKERI	BRASSICACEAE		CA NV
3C	DRABA DOUGLASII VAR. DOUGLASII	BRASSICACEAE		CA ID NV OR WA
3C	DRABA EXUNGUICULATA	BRASSICACEAE	WHITLOW-WORT.	CO
S	DRABA HOWELLII VAR. CARNOSULA	*** SEE ***	DRABA CARNOSULA	
2	DRABA JAEGERI	BRASSICACEAE		NV
2	DRABA LEMMONII VAR. CYCLOMORPHA	BRASSICACEAE		OR
3C	DRABA LEMMONII VAR. INCRASSATA	BRASSICACEAE		CA
2	DRABA MAGUIREI VAR. BURKEI	BRASSICACEAE		UT
3C	DRABA MAGUIREI VAR. MAGUIREI	BRASSICACEAE		UT
3C	DRABA MOGOLLONICA	BRASSICACEAE		NM
2	DRABA MURRAYI	BRASSICACEAE		AK, Canada (Yukon)
3C	DRABA NIVALIS VAR. BREVICULA	BRASSICACEAE	DRABA, SNOW, LITTLE	WY
S	DRABA OLIGOSPERMA VAR. PECTINIPILA	*** SEE ***	DRABA PECTINIPILA	
2	DRABA PAUCIFRUCTA	BRASSICACEAE		NV
3C	DRABA PECTINIPILA	BRASSICACEAE		CO WY
3C	DRABA QUADRICOSTATA	BRASSICACEAE	DRABA, BODIE HILLS	CA NV
3C	DRABA RUAXES	BRASSICACEAE		AK WA, Canada (B.C.)
3C	DRABA SOBOLIFERA	BRASSICACEAE	WHITLOW-GRASS, STOLON	UT
3C	DRABA SPHAEROCARPA	BRASSICACEAE		ID
3C	DRABA SPHAEROIDES VAR. CUSICKII	BRASSICACEAE		NV OR UT
3C	DRABA STENOLOBA VAR. RAMOSA	BRASSICACEAE		CA NV
3C	DRABA SUBALPINA	BRASSICACEAE	WHITLOW-GRASS.	UT
S	DRABA VENTOSA VAR. RUAXES	*** SEE ***	DRABA RUAXES	
S	DRABA ZIONENSIS	*** SEE ***	DRABA ASPRELLA VAR. ZIONENSIS	
3C	DRACAENA AUREA	LILIACEAE	HALAPEPE.	HI
1	DRACAENA FORBESII	LILIACEAE	HALAPEPE,	HI
1	DRACAENA HAWAIIENSIS	LILIACEAE	HALAPEPE.	HI
S	DRYPETES PHYLLANTHOIDES	*** SEE ***	NEOWAWRAEA PHYLLANTHOIDES	
3C	DUBAUTIA ARBOREA	ASTERACEAE		HI
1	DUBAUTIA HERBSTOBATAE	ASTERACEAE		HI
3B	DUBAUTIA HILLEBRANDII	ASTERACEAE		HI
3C	DUBAUTIA KNUDSENII	ASTERACEAE	DUBAUTIA, KNUDSEN	HI
3B	DUBAUTIA KNUDSENII VAR. DEGENERI	ASTERACEAE		HI
2	DUBAUTIA LAEVIGATA VAR. PARVIFOLIA	ASTERACEAE		HI
1	DUBAUTIA LATIFOLIA	ASTERACEAE		HI
2	DUBAUTIA LAXA VAR. BLAKEI	ASTERACEAE		HI
1	DUBAUTIA LAXA VAR. WAIANENSIS	ASTERACEAE	NAENAE-PUA-MELEMELE.	HI
3B	DUBAUTIA LONCHOPHYLLA	ASTERACEAE		HI
2	DUBAUTIA MAGNIFOLIA	ASTERACEAE		HI
1	DUBAUTIA MICROCEPHALA	ASTERACEAE		HI
3B	DUBAUTIA MOLOKAIENSIS	ASTERACEAE		HI
3B	DUBAUTIA MONTANA VAR. LONGIFOLIA	ASTERACEAE		HI
3B	DUBAUTIA MONTANA VAR. ROBUSTIOR	ASTERACEAE		HI
1	DUBAUTIA PLANTAGINEA VAR. ACRIDENTATA	ASTERACEAE		HI
3C	DUBAUTIA PLANTAGINEA VAR. PLANTAGINEA	ASTERACEAE		HI
2	DUBAUTIA PLATYPHYLLA VAR. LEPTOPHYLLA	ASTERACEAE		HI
1	DUBAUTIA RETICULATA	ASTERACEAE		HI
3B	DUBAUTIA ROCKII	ASTERACEAE		HI
3C	DUBAUTIA SHERFFIANA	ASTERACEAE	RAILLIARDIA, SHERFF	HI
3B	DUBAUTIA STRUTHIOLOIDES	ASTERACEAE	NA'ENA'E	HI
3B	DUBAUTIA TERNIFOLIA	ASTERACEAE		HI
3B	DUBAUTIA THYRSIFLORA VAR. CERNUA	ASTERACEAE		HI
3B	DUBAUTIA THYRSIFLORA VAR. THYRSIFLORA	ASTERACEAE		HI
2	DUBAUTIA WAIALEALAE VAR. MEGAPHYLLA	ASTERACEAE		HI
2	DUDLEYA ABRAMSII SSP. MURINA	CRASSULACEAE	LIVEFOREVER, SAN LUIS OBISPO	CA
2	DUDLEYA BETTINAE	CRASSULACEAE	LIVEFOREVER, BETTY'S	CA
S	DUDLEYA BLOCHMANIAE SSP. BREVIFOLIA	*** SEE ***	DUDLEYA BREVIFOLIA	
2	DUDLEYA BLOCHMANIAE SSP. INSULARIS	CRASSULACEAE	DUDLEYA, SANTA ROSA ISLAND	CA
1	DUDLEYA BREVIFOLIA	CRASSULACEAE	DUDLEYA, SHORT-LEAVED	CA
2	DUDLEYA CANDELABRUM	CRASSULACEAE	LIVEFOREVER, CANDLEHOLDER	CA
S	DUDLEYA COLLOMIAE	*** SEE ***	DUDLEYA SAXOSA VAR. COLLOMIAE	
2	DUDLEYA CYMOSA SSP. MARCESCENS	CRASSULACEAE	LIVEFOREVER, SANTA MONICA MOUNTAINS	CA
2	DUDLEYA DENSIFLORA	CRASSULACEAE	DUDLEYA, SAN GABRIEL MOUNTAIN	CA
2	DUDLEYA MULTICAULIS	CRASSULACEAE	LIVEFOREVER, MANY-STEMMED	CA
2	DUDLEYA NESIOTICA	CRASSULACEAE	LIVEFOREVER, SANTA CRUZ ISLAND	CA

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1	DUDLEYA PARVA	CRASSULACEAE	DUDLEYA, SERPENTINE	CA
2	DUDLEYA SAXOSA SSP. SAXOSA	CRASSULACEAE		CA
3C	DUDLEYA SAXOSA VAR. COLLOMIAE	CRASSULACEAE		AZ
1	DUDLEYA STOLONIFERA	CRASSULACEAE	LIVEFOREVER, LAGUNA BEACH	CA
LE	DUDLEYA TRASKIAE	CRASSULACEAE	LIVEFOREVER, SANTA BARBARA ISLAND	CA
2	DUDLEYA VARIEGATA	CRASSULACEAE	DUDLEYA, VARIEGATED	CA, Mexico
2	DUDLEYA VERITYI	CRASSULACEAE		CA
2	DUDLEYA VIRENS	CRASSULACEAE	LIVEFOREVER, GREEN	CA
1	DUDLEYA VISCIDA	CRASSULACEAE	LIVEFOREVER, STICKY	CA
3C	DYSCHORISTE CRENULATA	ACANTHACEAE		TX, Mexico
LE	DYSSODIA TEPHROLEUCA	ASTERACEAE	DOGWEED, ASHY	TX
S	ECHEVERIA COLLOMIAE	*** SEE ***	DUDLEYA SAXOSA VAR. COLLOMIAE	
S	ECHEVERIA RUSBYI	*** SEE ***	GRAPTOPETALUM RUSBYI	
S	ECHINACEA ANGUSTIFOLIA VAR. TENNESSEENSIS	*** SEE ***	ECHINACEA TENNESSEENSIS	
2	ECHINACEA LAEVIGATA	ASTERACEAE	CONEFLOWER,	AL GA NC SC VA
LE	ECHINACEA TENNESSEENSIS	ASTERACEAE	CONEFLOWER, PURPLE, TENNESSEE	TN
2	ECHINOCACTUS ASTERIAS	CACTACEAE	CACTUS, STAR	TX MEXICO
S	ECHINOCACTUS GLAUCUS	*** SEE ***	SCLEROCACTUS GLAUCUS	
LE	ECHINOCACTUS HORIZONTHALONIUS VAR. NICHOLII	CACTACEAE	CACTUS, TURK'S HEAD, NICHOL'S	AZ
S	ECHINOCACTUS MARIPOSENSIS	*** SEE ***	NEOLLOYDIA MARIPOSENSIS	
S	ECHINOCACTUS MESAE-VERDAE	*** SEE ***	SCLEROCACTUS MESAE-VERDAE	
S	ECHINOCACTUS PEEBLESIANUS	*** SEE ***	PEDIOCACTUS PEEBLESIANUS VAR. PEEBLESIANUS	
S	ECHINOCACTUS SILERI	*** SEE ***	PEDIOCACTUS SILERI	
S	ECHINOCACTUS SUBGLAUCUS	*** SEE ***	SCLEROCACTUS GLAUCUS	
S	ECHINOCACTUS TOBUSCHII	*** SEE ***	ANCISTROCACTUS TOBUSCHII	
S	ECHINOCACTUS WHIPPLEI VAR. GLAUCUS	*** SEE ***	SCLEROCACTUS GLAUCUS	
S	ECHINOCEREUS ARIZONICUS	*** SEE ***	ECHINOCEREUS TRIGLOCHIDIATUS VAR. ARIZONICUS	
2	ECHINOCEREUS BERLANDIERI VAR. ANGUSTICEPS	CACTACEAE		TX
S	ECHINOCEREUS BLANKII VAR. ANGUSTICEPS	*** SEE ***	ECHINOCEREUS BERLANDIERI VAR. ANGUSTICEPS	
2	ECHINOCEREUS CHLORANTHUS VAR. NEOCAPILLUS	CACTACEAE	HEDGEHOG CACTUS,	TX
S	ECHINOCEREUS COCCINEUS VAR. INERMIS	*** SEE ***	ECHINOCEREUS TRIGLOCHIDIATUS VAR. INERMIS	
S	ECHINOCEREUS DAVISII	*** SEE ***	ECHINOCEREUS VIRIDIFLORUS VAR. DAVISII	
2	ECHINOCEREUS ENGELMANNII VAR. HOWEI	CACTACEAE	HEDGEHOG CACTUS, HOWE'S	CA
2	ECHINOCEREUS ENGELMANNII VAR. MUNZII	CACTACEAE	HEDGEHOG CACTUS, MUNZ'S	CA, Mexico
LE	ECHINOCEREUS ENGELMANNII VAR. PURPUREUS	CACTACEAE	HEDGEHOG CACTUS, PURPLE-SPINED	UT
LE	ECHINOCEREUS FENDLERI VAR. KUENZLERI	CACTACEAE	HEDGEHOG CACTUS, KUENILER	NM
S	ECHINOCEREUS HEMPELII /OF AUTHORS, NOT FOBE	*** SEE ***	ECHINOCEREUS FENDLERI VAR. KUENZLERI	
S	ECHINOCEREUS KUENZLERI	*** SEE ***	ECHINOCEREUS FENDLERI VAR. KUENZLERI	
3C	ECHINOCEREUS LEDINGII	CACTACEAE		AZ
LE	ECHINOCEREUS LLOYDII	CACTACEAE	HEDGEHOG CACTUS, LLOYD'S	TX
S	ECHINOCEREUS MELANOCENTRUS	*** SEE ***	ECHINOCEREUS REICHENBACHII VAR. ALBERTII	
S	ECHINOCEREUS PHOENICEUS VAR. INERMIS	*** SEE ***	ECHINOCEREUS TRIGLOCHIDIATUS VAR. INERMIS	
LE	ECHINOCEREUS REICHENBACHII VAR. ALBERTII	CACTACEAE	CACTUS, BLACK LACE	TX
1	ECHINOCEREUS REICHENBACHII VAR. CHISOSENSIS	CACTACEAE		TX, Mexico
2	ECHINOCEREUS REICHENBACHII VAR. FITCHII	CACTACEAE		TX
S	ECHINOCEREUS ROETTERI VAR. LLOYDII	*** SEE ***	ECHINOCEREUS LLOYDII	
3B	ECHINOCEREUS RUSSANTHUS	CACTACEAE	HEDGEHOG CACTUS, RUSTY	TX
LE	ECHINOCEREUS TRIGLOCHIDIATUS VAR. ARIZONICUS	CACTACEAE	HEDGEHOG CACTUS, ARIZONA	AZ
LE	ECHINOCEREUS TRIGLOCHIDIATUS VAR. INERMIS	CACTACEAE	HEDGEHOG CACTUS, SPINELESS	CO UT
3C	ECHINOCEREUS VIRIDIFLORUS VAR. CORRELLII	CACTACEAE		TX
LE	ECHINOCEREUS VIRIDIFLORUS VAR. DAVISII	CACTACEAE	PITAYA, GREEN, DAVIS'	TX
S	ECHINOMASTUS MARIPOSENSIS	*** SEE ***	NEOLLOYDIA MARIPOSENSIS	
S	ECTOSPERMA ALEXANDRAE	*** SEE ***	SWALLENIA ALEXANDRAE	
3C	ELEOCHARIS AUSTROTEXANA	CYPERACEAE		TX
2	ELEOCHARIS BRACHYCARPA	CYPERACEAE		TX MEXICO
2	ELEOCHARIS CYLINDRICA	CYPERACEAE	SPIKE-RUSH, CYLINDER	TX, Mexico
3C	ELLIOTTIA RACEMOSA	ERICACEAE	PLUME, GEORGIA	GA SC
3A	ELODEA BRANDEGEAE	HYDROCHARITACEAE	WATERWEED, TRUCKEE	CA
1	* ELODEA LINEARIS	HYDROCHARITACEAE	WATERWEED, NASHVILLE	TN
2	* ELODEA NEVADENSIS	HYDROCHARITACEAE	WATERWEED, NEVADA	NV
3A	ELODEA SCHWEINITZII	HYDROCHARITACEAE	WATERWEED, SCHWEINITZ'S	PA
2	ELYMUS SVENSONII	POACEAE		TN
2	ELYTRARIA CAROLINIENSIS VAR. ANGUSTIFOLIA	ACANTHACEAE		FL
3C	ELYTRARIA CAROLINIENSIS VAR. CAROLINIENSIS	ACANTHACEAE		FL GA SC
2	EMBELIA HILLEBRANDII	MYRSINACEAE		HI
3C	ENCELIA FRUTESCENS VAR. RESINOSA	ASTERACEAE		AZ
2	ENCELIOPSIS COVILLEI	ASTERACEAE	DAISY, PANAMINT	CA
LT	ENCELIOPSIS NUDICAULIS VAR. CORRUGATA	ASTERACEAE	SUNRAY, ASH MEADOWS	NV

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
2	ENCYCLIA BOOTHIANA VAR. ERYTHRONIOIDES	ORCHIDACEAE	ORCHID, DOLLAR	FL, Bahamas, Belize, Cuba, Jamaica, Mexico, Hispaniola, South America
3C	ENCYCLIA KRUGII	ORCHIDACEAE		PR
2	ENCYCLIA SINTENISII	ORCHIDACEAE		PR, Cuba, Jamaica, Hispaniola
3C	EPHEDRA FUNEREA	EPHEDRACEAE		CA NV
3B	EPIDENDRUM BRITTONIANUM	ORCHIDACEAE		PR
S	EPIDENDRUM ERYTHRONIOIDES	*** SEE ***	ENCYCLIA BOOTHIANA VAR. ERYTHRONIOIDES	
3C	EPIDENDRUM KRANZLINII	ORCHIDACEAE		PR
S	EPIDENDRUM KRUGII	*** SEE ***	ENCYCLIA KRUGII	
2	EPIDENDRUM LACERUM	ORCHIDACEAE		PR, Cuba
S	EPIDENDRUM SINTENISII	*** SEE ***	ENCYCLIA SINTENISII	
3C	EPILOBIUM NEVADENSE	ONAGRACEAE	WILLOWHERB, NEVADA	NV UT
3C	EPILOBIUM NIVIUM	ONAGRACEAE	WILLOWHERB, SNOW MOUNTAIN	CA
S	EPILOBIUM OBCORDATUM SSP. SISKIYOUENSE	*** SEE ***	EPILOBIUM SISKIYOUENSE	
2	EPILOBIUM OREGANUM	ONAGRACEAE		CA OR
3C	EPILOBIUM SISKIYOUENSE	ONAGRACEAE	ROCK-FRINGE, SISKIYOU	CA OR
3C	EPITHELANTHA BOKEI	CACTACEAE		TX, Mexico
1	ERAGROSTIS FOSBERGII	POACEAE	LOVE GRASS, FOSBERG'S	HI
1	* ERAGROSTIS MAUIENSIS	POACEAE	LOVE GRASS, MAUI	HI
1	ERAGROSTIS NIIHAUENSIS	POACEAE	LOVE GRASS, NIIHAU	HI
1	* ERAGROSTIS PAUPERA	POACEAE		HI
2	ERAGROSTIS TRACYI	POACEAE	LOVE GRASS, SANIBEL	FL
2	EREMALCHE KERNENSIS	MALVACEAE	MALLOW, KERN	CA
2	ERIASTRUM BRANDEGEAE	POLEMONIACEAE	ERIASTRUM, BRANDEGEE	CA
1	ERIASTRUM DENSIFOLIUM SSP. SANCTORUM	POLEMONIACEAE	ERIASTRUM, SANTA ANA RIVER	CA
2	ERIASTRUM HOOVERI	POLEMONIACEAE	ERIASTRUM, HOOVER'S	CA
2	ERIASTRUM TRACYI	POLEMONIACEAE	ERIASTRUM, TRACY	CA
1	ERICAMERIA FASCICULATA	ASTERACEAE	GOLDENWEED, EASTWOOD'S	CA
S	ERICAMERIA PALMERI SSP. PALMERI	*** SEE ***	HAPLOPAPPUS PALMERI SSP. PALMERI	
3C	ERIGERON ABAJOENSIS	ASTERACEAE	DAISY, ABAJO	UT
2	ERIGERON AEQUIFOLIUS	ASTERACEAE	DAISY, HALL'S	CA
3C	ERIGERON ALLOCOTUS	ASTERACEAE	FLEABANE, BRANCHED	MT WY
3C	ERIGERON ARENARIOIDES	ASTERACEAE		UT
3C	ERIGERON ARIZONICUS	ASTERACEAE		AZ
1	ERIGERON BASALTICUS	ASTERACEAE	DAISY, BASALT	WA
3C	ERIGERON BIGELOVII	ASTERACEAE		TX, Mexico
3C	ERIGERON BLOOMERI VAR. NUDATUS	ASTERACEAE		CA OR
3C	ERIGERON CALVUS	ASTERACEAE	FLEABANE,	CA
2	ERIGERON CHRYSOPSIDIS VAR. BREVIFOLIUS	ASTERACEAE		OR
2	ERIGERON CRONQUISTII	ASTERACEAE	DAISY, CRONQUIST	UT
2	ERIGERON DECUMBENS VAR. DECUMBENS	ASTERACEAE		OR
3B	ERIGERON DELICATUS	ASTERACEAE	FLEABANE, DEL NORTE	CA OR
3C	ERIGERON ERIOPHYLLUS	ASTERACEAE	FLEABANE,	AZ
S	ERIGERON FLABELLARIS VAR. TRILOBATUS	*** SEE ***	ERIGERON PROSELYTICUS	
3C	ERIGERON FLETTII	ASTERACEAE		WA
2	ERIGERON FLEXUOSUS	ASTERACEAE	DAISY, TRINITY ALPS	CA
3C	ERIGERON FOLIOSUS VAR. BLOCHMANIAE	ASTERACEAE	LEAFY-DAISY, BLOCKMAN'S	CA
3C	ERIGERON GARRETTII	ASTERACEAE		UT
S	ERIGERON GEISERI VAR. CALCICOLA	*** SEE ***	ERIGERON MIMEGLETES	
S	ERIGERON GRANDIFLORUS SSP. MUIRII	*** SEE ***	ERIGERON MUIRII	
2	ERIGERON HESSII	ASTERACEAE		NM
2	ERIGERON HOWELLII	ASTERACEAE	FLEABANE, HOWELL'S	OR WA
2	ERIGERON HULTENII	ASTERACEAE		AK
2	ERIGERON KACHINENSIS	ASTERACEAE	DAISY, KACHINA	CO UT
1	ERIGERON KUSCHEI	ASTERACEAE	FLEABANE,	AZ
2	ERIGERON LATUS	ASTERACEAE	FLEABANE,	ID NV
3C	ERIGERON LEIBERGII	ASTERACEAE		WA
2	ERIGERON LEMMONII	ASTERACEAE		AZ
3C	ERIGERON LOBATUS	ASTERACEAE		AZ
2	ERIGERON MAGUIREI VAR. HARRISONII	ASTERACEAE		UT
LE	ERIGERON MAGUIREI VAR. MAGUIREI	ASTERACEAE	DAISY, MAGUIRE	UT
2	ERIGERON MANCUS	ASTERACEAE	DAISY, DEPAUPERATE	UT
2	ERIGERON MIMEGLETES	ASTERACEAE	FLEABANE,	TX
2	ERIGERON MUIRII	ASTERACEAE		AK
2	ERIGERON MULTICEPS	ASTERACEAE	DAISY, KERN RIVER	CA
3C	ERIGERON OREGANUS	ASTERACEAE	FLEABANE, OREGON	OR WA
2	ERIGERON OVINUS	ASTERACEAE		NV
2	ERIGERON PARISHII	ASTERACEAE	DAISY, PARISH'S	CA
3A	ERIGERON PERGLABER	ASTERACEAE		AZ
3C	ERIGERON PIPERANUS	ASTERACEAE		WA
2	ERIGERON PRINGLEI	ASTERACEAE		AZ
3C	ERIGERON PROSELYTICUS	ASTERACEAE	DAISY, CLIFF	UT
2	ERIGERON PULCHELLUS VAR. TOLSTEADII	ASTERACEAE		MN
3C	ERIGERON RELIGIOSUS	ASTERACEAE	FLEABANE, CLEAR CREEK	UT
LT	ERIGERON RHIZOMATUS	ASTERACEAE	FLEABANE,	NM
2	ERIGERON SIONIS	ASTERACEAE		UT
2	ERIGERON SP. NOV. /INED.	ASTERACEAE		WY
2	ERIGERON SUPPLEX	ASTERACEAE	DAISY, SUPPLE	CA
3C	ERIGERON UNCIALIS VAR. CONJUGANS	ASTERACEAE		NV
2	ERIGERON UNTERMANNII	ASTERACEAE		UT

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2	ERIOCAULON KORNICKIANUM	ERIOCAULACEAE	PIPEWORT,	AR GA OK TX
2	ERIOCAULON PARKERI	ERIOCAULACEAE	PIPEWORT, PARKER'S	CT DC DE MA MD ME NC NJ NY PA VA, Canada (N.B., Que.)
2	ERIOCHLOA MICHAUXII VAR. SIMPSONII	POACEAE		FL
1	ERIODICTYON ALTISSIMUM	HYDROPHYLLACEAE	MOUNTAIN BALM, INDIAN KNOB	CA
1	ERIODICTYON CAPITATUM	HYDROPHYLLACEAE	LOMPOC YERBA SANTA	CA
3C	ERIOGONUM ALLENII	POLYGONACEAE		VA WV
3C	ERIOGONUM ALPINUM	POLYGONACEAE	WILD BUCKWHEAT, TRINITY	CA
1	ERIOGONUM AMMOPHILUM	POLYGONACEAE	WILD BUCKWHEAT, SAND-LOVING	UT
2	ERIOGONUM AMPULLACEUM	POLYGONACEAE	WILD BUCKWHEAT, MONO	CA
3C	ERIOGONUM ANEMOPHILUM	POLYGONACEAE	WILD BUCKWHEAT,	NV
2	ERIOGONUM APACHENSE	POLYGONACEAE		AZ
1	ERIOGONUM APRICUM VAR. APRICUM	POLYGONACEAE	WILD BUCKWHEAT, IONE	CA
1	ERIOGONUM APRICUM VAR. PROSTRATUM	POLYGONACEAE	WILD BUCKWHEAT, IRISH HILL	CA
2	ERIOGONUM ARETIOIDES	POLYGONACEAE	WILD BUCKWHEAT, WIDSTOE	UT
2	ERIOGONUM ARGOPHYLLUM	POLYGONACEAE	WILD BUCKWHEAT,	NV
3C	ERIOGONUM BEATLEYAE	POLYGONACEAE	WILD BUCKWHEAT, BEATLEY	CA NV
2	ERIOGONUM BIFURCATUM	POLYGONACEAE		CA NV
2	ERIOGONUM BRANDEGEI	POLYGONACEAE	WILD BUCKWHEAT, BRANDEGEE	CO
2	ERIOGONUM BREEDLOVEI VAR. BREEDLOVEI	POLYGONACEAE	WILD BUCKWHEAT, PIUTE	CA
2	ERIOGONUM BREEDLOVEI VAR. SHEVOCKII	POLYGONACEAE		CA
2	ERIOGONUM BUTTERWORTHIANUM	POLYGONACEAE	WILD BUCKWHEAT, BUTTERWORTH'S	CA
3C	ERIOGONUM CANINUM	POLYGONACEAE	WILD BUCKWHEAT, TIBURON	CA
2	ERIOGONUM CAPILLARE	POLYGONACEAE	WILD BUCKWHEAT,	AZ
2	ERIOGONUM CHRYSOPS	POLYGONACEAE	WILD BUCKWHEAT, GOLDEN	OR
3C	ERIOGONUM CLAVELLATUM	POLYGONACEAE	WILD BUCKWHEAT, COMB WASH	CO UT
3C	ERIOGONUM CONCINNUM	POLYGONACEAE		NV
3C	ERIOGONUM CONGDONII	POLYGONACEAE	ERIOGONUM, CONGDON	CA
3C	ERIOGONUM CONTIGUUM	POLYGONACEAE		CA NV
3C	ERIOGONUM CORRELLII	POLYGONACEAE		TX
3B	ERIOGONUM CORYMBOSUM VAR. DAVIDSEI	POLYGONACEAE	WILD BUCKWHEAT, CORYMBED, DAVIDSE	UT
3C	ERIOGONUM CORYMBOSUM VAR. MATTHEWSAE	POLYGONACEAE	WILD BUCKWHEAT, MATTHEW'S	UT
3C	ERIOGONUM CORYMBOSUM VAR. REVEALIANUM	POLYGONACEAE	WILD BUCKWHEAT, CORYMBED, REVEAL	UT
2	ERIOGONUM CROCATUM	POLYGONACEAE	WILD BUCKWHEAT, CONEJO	CA
2	ERIOGONUM CRONQUISTII	POLYGONACEAE	WILD BUCKWHEAT, CRONQUIST	UT
2	ERIOGONUM CROSBYAE	POLYGONACEAE		OR
2	ERIOGONUM CUSICKII	POLYGONACEAE		OR
3C	ERIOGONUM DARROVII	POLYGONACEAE	WILD BUCKWHEAT,	AZ NV
3B	ERIOGONUM DENSUM	POLYGONACEAE		AZ NM
3C	ERIOGONUM DESERTICOLA	POLYGONACEAE	ERIOGONUM, DESERT	AZ CA
3C	ERIOGONUM DICLINUM	POLYGONACEAE	ERIOGONUM, JAMES CANYON	CA OR
3C	ERIOGONUM EASTWOODIANUM	POLYGONACEAE		CA
3C	ERIOGONUM EPHEDROIDES	POLYGONACEAE	WILD BUCKWHEAT, EPHEDRA	CO UT
2	ERIOGONUM EREMICOLA	POLYGONACEAE	ERIOGONUM, WILD ROSE CANYON	CA
3C	ERIOGONUM EREMICUM	POLYGONACEAE	WILD BUCKWHEAT, LIMESTONE	UT
3C	ERIOGONUM ERICIFOLIUM VAR. ERICIFOLIUM	POLYGONACEAE	WILD BUCKWHEAT,	AZ
2	ERIOGONUM ERICIFOLIUM VAR. THORNEI	POLYGONACEAE	WILD BUCKWHEAT, THORNE'S	CA
2	ERIOGONUM FLAVUM VAR. AQUILINUM	POLYGONACEAE	WILD BUCKWHEAT,	AK
S	ERIOGONUM FLORIDANUM	*** SEE ***	ERIOGONUM LONGIFOLIUM VAR. GNAPHALIFOLIUM	
2	ERIOGONUM GIGANTEUM VAR. COMPACTUM	POLYGONACEAE	GIANT BUCKWHEAT, SANTA BARBARA IS.	CA
2	ERIOGONUM GIGANTEUM VAR. FORMOSUM	POLYGONACEAE	WILD BUCKWHEAT, SAN CLEMENTE IS.	CA
3C	ERIOGONUM GILMANII	POLYGONACEAE	WILD BUCKWHEAT, GILMAN'S	CA
2	ERIOGONUM GOSSYPINUM	POLYGONACEAE	ERIOGONUM, COTTON	CA
2	ERIOGONUM GRANDE VAR. DUNKLEI	POLYGONACEAE	WILD BUCKWHEAT, SAN MIGUEL IS.	CA
3C	ERIOGONUM GRANDE VAR. TIMORUM	POLYGONACEAE	WILD BUCKWHEAT, SAN NICOLAS ISLAND	CA
3B	ERIOGONUM GRAYI	POLYGONACEAE		UT
LT	ERIOGONUM GYPSOPHILUM	POLYGONACEAE	WILD-BUCKWHEAT, GYPSUM	NM
S	ERIOGONUM HARPERI	*** SEE ***	ERIOGONUM LONGIFOLIUM VAR. HARPERI	
3C	ERIOGONUM HEERMANNII VAR. FLOCCOSUM	POLYGONACEAE	ERIOGONUM, CLARK MOUNTAIN	CA NV
3C	ERIOGONUM HEERMANNII VAR. SUBRACEMOSUM	POLYGONACEAE		AZ UT
3C	ERIOGONUM HIRTELLUM	POLYGONACEAE	WILD BUCKWHEAT, KLAMATH MOUNTAIN	CA
3C	ERIOGONUM HOFFMANNII VAR. HOFFMANNII	POLYGONACEAE	ERIOGONUM, HOFFMAN,	CA
3C	ERIOGONUM HOFFMANNII VAR. ROBUSTIUS	POLYGONACEAE	ERIOGONUM, HOFFMAN, ROBUST	CA
2	ERIOGONUM HOLMGRENII	POLYGONACEAE		NV
1	ERIOGONUM HUMIVAGANS	POLYGONACEAE	WILD BUCKWHEAT, SPREADING	UT
3C	ERIOGONUM HYLOPHILUM	POLYGONACEAE	WILD BUCKWHEAT, BADLANDS	UT
3C	ERIOGONUM INTERMONTANUM	POLYGONACEAE	WILD BUCKWHEAT, DIVIDE	UT
3C	ERIOGONUM INTRAFRACTUM	POLYGONACEAE	WILD BUCKWHEAT, JOINTED	CA
3C	ERIOGONUM JAMESII VAR. RUPICOLA	POLYGONACEAE	WILD BUCKWHEAT, SANDSTONE	UT
1	ERIOGONUM KELLOGGII	POLYGONACEAE	WILD BUCKWHEAT, RED MOUNTAIN	CA
2	ERIOGONUM KENNEDYI VAR. AUSTROMONTANUM	POLYGONACEAE	WILD BUCKWHEAT, SOUTHERN MOUNTAIN	CA
2	ERIOGONUM KENNEDYI VAR. PINICOLA	POLYGONACEAE	WILD BUCKWHEAT, CACHE PEAK	CA
2	ERIOGONUM LAGOPUS	POLYGONACEAE		MT WY
3C	ERIOGONUM LANCIFOLIUM	POLYGONACEAE	WILD BUCKWHEAT, LANCE LEAF	UT
3C	ERIOGONUM LATENS	POLYGONACEAE		CA
3C	ERIOGONUM LEMMONII	POLYGONACEAE	WILD BUCKWHEAT,	NV
3C	ERIOGONUM LIBERTINI	POLYGONACEAE		CA
2	ERIOGONUM LOBBII VAR. ROBUSTUM	POLYGONACEAE		NV
3C	ERIOGONUM LOGANUM	POLYGONACEAE	WILD BUCKWHEAT, LOGAN	UT
S	ERIOGONUM LONGIFOLIUM VAR. FLORIDANUM	*** SEE ***	ERIOGONUM FLORIDANUM	
2	ERIOGONUM LONGIFOLIUM VAR. GNAPHALIFOLIUM	POLYGONACEAE	WILD BUCKWHEAT, SCRUB	FL

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
2	ERIOGONUM LONGIFOLIUM VAR. HARPERI	POLYGONACEAE		AL KY TN
2	ERIOGONUM MICROTHECUM VAR. JOHNSTONII	POLYGONACEAE	BRUSH BUCKWHEAT, JOHNSTON'S	CA
2	ERIOGONUM MICROTHECUM VAR. PANAMINTENSE	POLYGONACEAE	BRUSH BUCKWHEAT, PANAMINT MOUNTAINS	CA
2	ERIOGONUM MORTONIANUM	POLYGONACEAE	WILD BUCKWHEAT,	AZ
3B	ERIOGONUM NANUM	POLYGONACEAE	WILD BUCKWHEAT, DWARF	UT
3C	ERIOGONUM NATUM	POLYGONACEAE		UT
3C	ERIOGONUM NEALLEYI	POLYGONACEAE	WILD BUCKWHEAT, IRION COUNTY	TX
2	ERIOGONUM NERVULOSUM	POLYGONACEAE		CA
3C	ERIOGONUM NORTONII	POLYGONACEAE	WILD BUCKWHEAT, PINNACLES	CA
3C	ERIOGONUM NOVONUDUM	POLYGONACEAE		OR
2	ERIOGONUM NUDUM VAR. MURINUM	POLYGONACEAE	WILD BUCKWHEAT, MOUSE	CA
3B	ERIOGONUM NUMMULARE	POLYGONACEAE		UT
3C	ERIOGONUM OSTLUNDII	POLYGONACEAE	WILD BUCKWHEAT, OSTLUND	UT
3C	ERIOGONUM OVALIFOLIUM VAR. CAELESTINUM	POLYGONACEAE		NV
1	ERIOGONUM OVALIFOLIUM VAR. VINEUM	POLYGONACEAE	WILD BUCKWHEAT,	CA
PE	ERIOGONUM OVALIFOLIUM VAR. WILLIAMSIAE	POLYGONACEAE		NV
3C	ERIOGONUM PANGUICENSE VAR. ALPESTRE	POLYGONACEAE	WILD BUCKWHEAT, PANGUITCH	UT
3B	ERIOGONUM PARVIFOLIUM VAR. LUCIDUM	POLYGONACEAE	WILD BUCKWHEAT, POINT LOBOS	CA
3B	ERIOGONUM PARVIFOLIUM VAR. PAYNEI	POLYGONACEAE	ERIOGONUM, SANTA PAULA	CA
LE	ERIOGONUM PELINOPHILUM	POLYGONACEAE	WILD-BUCKWHEAT, CLAY-LOVING	CO
3C	ERIOGONUM PENDULUM	POLYGONACEAE	WILD BUCKWHEAT, WALDO	CA OR
2	ERIOGONUM PROCIDUUM	POLYGONACEAE	WILD BUCKWHEAT, PROSTRATE	CA NV OR
2	ERIOGONUM RIPLEYI	POLYGONACEAE		AZ
3C	ERIOGONUM RUBRICAULE	POLYGONACEAE		NV
3C	ERIOGONUM SAURINUM	POLYGONACEAE	WILD BUCKWHEAT, DINOSAUR	CO UT
2	ERIOGONUM SCOPULORUM	POLYGONACEAE		OR
3C	ERIOGONUM SISKIYOUENSE	POLYGONACEAE	ERIOGONUM, SISKIYOU	CA
2	ERIOGONUM SMITHII	POLYGONACEAE	WILD BUCKWHEAT, SMITH	UT
2	ERIOGONUM SOREDIUM	POLYGONACEAE		UT
5	ERIOGONUM SP. (LAKEVIEW CO., OR)	*** SEE ***	ERIOGONUM CROSBYAE	
5	ERIOGONUM SP. (TRINITY, TEHAMA COS., CA)	*** SEE ***	ERIOGONUM LIBERTINI	
2	ERIOGONUM SUFFRUTICOSUM	POLYGONACEAE	WILD BUCKWHEAT, BUSHY	TX
3C	ERIOGONUM TEMBLORENSE	POLYGONACEAE	WILD BUCKWHEAT, TEMBLOR	CA
3C	ERIOGONUM THOMPSONAE VAR. ALBIFLORUM	POLYGONACEAE	WILD BUCKWHEAT, THOMPSON, WHITE-FLOW	AZ UT
2	ERIOGONUM THOMPSONAE VAR. ATWOODII	POLYGONACEAE	WILD BUCKWHEAT, THOMPSON, ATWOOD'S	AZ
3C	ERIOGONUM THOMPSONAE VAR. THOMPSONAE	POLYGONACEAE	WILD BUCKWHEAT, THOMPSON, THOMPSON'S	AZ UT
3C	ERIOGONUM THYMOIDES	POLYGONACEAE		ID OR WA
2	ERIOGONUM TRUNCATUM	POLYGONACEAE	ERIOGONUM, CONTRA COSTA	CA
3C	ERIOGONUM TUMULOSUM	POLYGONACEAE		CO UT
1	ERIOGONUM TWISSELMANNII	POLYGONACEAE	ERIOGONUM, TWISSELMANN'S	CA
2	ERIOGONUM UMBELLATUM VAR. HUMISTRATUM	POLYGONACEAE	WILD BUCKWHEAT, MT. EDDY	CA
3C	ERIOGONUM UMBELLATUM VAR. HYPOLEIUM	POLYGONACEAE		WA
3C	ERIOGONUM UMBELLATUM VAR. MINUS	POLYGONACEAE	WILD BUCKWHEAT, SULFUR-FLOWERED, ALP	CA
3C	ERIOGONUM UMBELLATUM VAR. TORREYANUM	POLYGONACEAE	WILD BUCKWHEAT, SULFUR-FLOWERED, TOR	CA
3C	ERIOGONUM VESTITUM	POLYGONACEAE	ERIOGONUM, IDRIA	CA
5	ERIOGONUM VILLIFLORUM VAR. TUMULOSUM	*** SEE ***	ERIOGONUM TUMULOSUM	
3C	ERIOGONUM VIRIDULUM	POLYGONACEAE		CO UT
2	ERIOGONUM VISCIDULUM	POLYGONACEAE	WILD BUCKWHEAT,	NV
2	ERIOGONUM VISHERI	POLYGONACEAE		SD WY
2	ERIOGONUM WRIGHTII VAR. OLANCHENSE	POLYGONACEAE	WILD BUCKWHEAT, OLANCHE PEAK	CA
3C	ERIOGONUM ZIONIS VAR. COCCINEUM	POLYGONACEAE	WILD BUCKWHEAT, ZION,	AZ
3C	ERIOGONUM ZIONIS VAR. ZIONIS	POLYGONACEAE	WILD BUCKWHEAT, ZION,	AZ UT
3C	ERIOPHYLLUM CONGDONII	ASTERACEAE	ERIOPHYLLUM, CONGDON'S	CA
2	ERIOPHYLLUM LANATUM VAR. HALLII	ASTERACEAE	WOOLY-SUNFLOWER, FT. TEJON	CA
1	ERIOPHYLLUM LATILOBUM	ASTERACEAE	WOOLY-SUNFLOWER, SAN MATEO	CA
2	ERIOPHYLLUM MOHAVENSE	ASTERACEAE	WOOLY-SUNFLOWER, BARSTOW	CA
2	ERIOPHYLLUM NEVINII	ASTERACEAE		CA
2	ERIOPHYLLUM NUBIGENUM	ASTERACEAE	WOOLY-SUNFLOWER, YOSEMITE	CA
5	ERIOPHYLLUM NUBIGENUM VAR. CONGDONII	*** SEE ***	ERIOPHYLLUM CONGDONII	
2	ERITHALIS REVOLUTA	RUBIACEAE		PR
3C	ERRAZURIZIA ROTUNDATA	FABACEAE		AZ
2	ERYNGIUM ARISTULATUM VAR. HOOVERI	APIACEAE	BUTTON-CELERY, HOOVER'S	CA
1	ERYNGIUM ARISTULATUM VAR. PARISHII	APIACEAE	COYOTE-THISTLE, SAN DIEGO	CA
LE	ERYNGIUM CONSTANCEI	APIACEAE	COYOTE-THISTLE, CONSTANCE'S	CA
1	ERYNGIUM CUNEIFOLIUM	APIACEAE		FL
2	ERYNGIUM MATHIASIAE	APIACEAE	COYOTE-THISTLE, MATHIAS	CA
3C	ERYNGIUM PETIOLATUM	APIACEAE		OR WA
2	ERYNGIUM PINNATISECTUM	APIACEAE	COYOTE-THISTLE, TUOLUMNE	CA
1	ERYNGIUM RACEMOSUM	APIACEAE	COYOTE-THISTLE, DELTA	CA
2	ERYNGIUM SPINOSEPALUM	APIACEAE		CA
2	ERYSIMUM AMMOPHILUM	BRASSICACEAE	WALLFLOWER, COAST	CA
5	ERYSIMUM ANGUSTATUM /GREENE	*** SEE ***	ERYSIMUM CAPITATUM VAR. ANGUSTATUM	
5	ERYSIMUM ANGUSTATUM /P.A.RYDBERG	*** SEE ***	ERYSIMUM ASPERUM VAR. ANGUSTATUM	
2	ERYSIMUM ASPERUM VAR. ANGUSTATUM	BRASSICACEAE	WALLFLOWER,	AK, Canada (Yukon)
LE	ERYSIMUM CAPITATUM VAR. ANGUSTATUM	BRASSICACEAE	WALLFLOWER, CONTRA COSTA	CA
2	ERYSIMUM FRANCISCANUM VAR. FRANCISCANUM	BRASSICACEAE	WALLFLOWER, SAN FRANCISCO	CA
2	ERYSIMUM INSULARE	BRASSICACEAE	WALLFLOWER, ISLAND	CA, Mexico
1	ERYSIMUM MENZIESII	BRASSICACEAE	WALLFLOWER, MENZIES'	CA
1	ERYSIMUM TERETIFOLIUM	BRASSICACEAE	WALLFLOWER, BEN LOMOND	CA
3C	ERYTHRONIUM CLIFTONII /SP. NOV. INED.	LILIACEAE		CA
3C	ERYTHRONIUM GRANDIFLORUM SSP. PUSATERI	LILIACEAE	FAWN-LILY,	CA
3C	ERYTHRONIUM HELENAE	LILIACEAE		CA
3C	ERYTHRONIUM HOWELLII	LILIACEAE		CA OR

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
3C	ERYTHRONIUM OREGONUM	LILIACEAE		OR WA
PE	ERYTHRONIUM PROPULLANS	LILIACEAE		MN
2	ERYTHRONIUM SP. NOV. /INED.	LILIACEAE		OR
2	ERYTHRONIUM TUOLUMNENSE	LILIACEAE	FAWN-LILY, TUOLUMNE	CA
2	ESCHSCHOLZIA PROCERA	PAPAVERACEAE	POPPY, KERNVILLE	CA
3C	ESCHSCHOLZIA RAMOSA	PAPAVERACEAE	POPPY, ISLAND	CA
2	ESCHSCHOLZIA RHOMBIPETALA	PAPAVERACEAE	POPPY, DIAMOND-PETALED	CA
S	ESCOBARIA LEEI	*** SEE ***	CORYPHANTHA SNEEDII VAR. LEEI	
S	ESCOBARIA NELLIEAE	*** SEE ***	CORYPHANTHA MINIMA	
S	ESCOBARIA SNEEDII	*** SEE ***	CORYPHANTHA SNEEDII VAR. SNEEDII	
1	EUGENIA HAEMATOCARPA	MYRTACEAE	UVILLO	PR
2	EUGENIA MARGARETTAE	MYRTACEAE		PR
1	EUGENIA MOLOKAIANA	MYRTACEAE	NIOI	HI
1	EUGENIA UNDERWOODII	MYRTACEAE		PR
S	EULOPHIA ECRISTATA	*** SEE ***	PTEROGLOSSASPIS ECRISTATA	
2	EUPATORIUM BORINQUENSE	ASTERACEAE		PR
3A	EUPATORIUM DROSEROLEPIS	ASTERACEAE	OREGANILLO	PR
3C	EUPATORIUM LEUCOLEPIS VAR. NOVAE-ANGLIAE	ASTERACEAE	THOROUGHWORT, WHITE-BRACTED, N.E.	MA RI
1	EUPATORIUM LUCIAE-BRAUNIAE	ASTERACEAE		KY TN
2	EUPATORIUM OTEROI	ASTERACEAE		PR
2	EUPATORIUM RESINOSUM	ASTERACEAE	BONESET, PINE BARRENS	DE NJ NY NC
3B	EUPATORIUM RESINOSUM VAR. KENTUCKIENSE	ASTERACEAE	THOROUGHWORT,	KY
3B	EUPATORIUM SALTUENSE	ASTERACEAE		NC VA
3C	EUPATORIUM SHASTENSE	ASTERACEAE	EUPATORIUM, SHASTA	CA
3C	EUPHORBIA ARNOTTIANA VAR. ARNOTTIANA	EUPHORBIACEAE		HI
1	EUPHORBIA ARNOTTIANA VAR. INTEGRIFOLIA	EUPHORBIACEAE		HI
1	EUPHORBIA ATROCOCCA	EUPHORBIACEAE		HI
3B	EUPHORBIA AUSTRINA	EUPHORBIACEAE		FL
3C	EUPHORBIA CELASTROIDES VAR. HALAWANA	EUPHORBIACEAE		HI
3C	EUPHORBIA CELASTROIDES VAR. HAUPUANA	EUPHORBIACEAE		HI
3C	EUPHORBIA CELASTROIDES VAR. HUMBERTII	EUPHORBIACEAE		HI
1	EUPHORBIA CELASTROIDES VAR. KAENANA	EUPHORBIACEAE		HI
1	EUPHORBIA CELASTROIDES VAR. KEALIANA	EUPHORBIACEAE		HI
1	EUPHORBIA CELASTROIDES VAR. KOHALANA	EUPHORBIACEAE		HI
3C	EUPHORBIA CELASTROIDES VAR. MOOMOMIANA	EUPHORBIACEAE		HI
2	EUPHORBIA CELASTROIDES VAR. NELSONII	EUPHORBIACEAE		HI
1	* EUPHORBIA CELASTROIDES VAR. NEMATOPODA	EUPHORBIACEAE		HI
3C	EUPHORBIA CELASTROIDES VAR. NIUENSIS	EUPHORBIACEAE		HI
1	EUPHORBIA CELASTROIDES VAR. SAXICOLA	EUPHORBIACEAE		HI
1	EUPHORBIA CELASTROIDES VAR. STOKESII	EUPHORBIACEAE		HI
3C	EUPHORBIA CELASTROIDES VAR. WAIKOLUENSIS	EUPHORBIACEAE		HI
2	EUPHORBIA CUMULICOLA	EUPHORBIACEAE		FL
1	EUPHORBIA DEGENERI VAR. MOLOKAIENSIS	EUPHORBIACEAE		HI
LE	EUPHORBIA DELTOIDEA SSP. DELTOIDEA	EUPHORBIACEAE		FL
1	EUPHORBIA DELTOIDEA SSP. SERPYLLUM	EUPHORBIACEAE	SPURGE, WEDGE	FL
3A	EUPHORBIA DEPPEANA	EUPHORBIACEAE		HI
3C	EUPHORBIA DISCOIDALIS	EUPHORBIACEAE		FL
3C	EUPHORBIA EXSERTA	EUPHORBIACEAE		FL GA NC SC
2	EUPHORBIA FENDLERI VAR. TRILIGULATA	EUPHORBIACEAE	SPURGE,	TX
LT	EUPHORBIA GARBERI	EUPHORBIACEAE	SPURGE,	FL
2	EUPHORBIA GOLONDRINA	EUPHORBIACEAE	SPURGE,	TX
1	EUPHORBIA HAELEELEANA	EUPHORBIACEAE		HI
1	EUPHORBIA HALEMANUI	EUPHORBIACEAE		HI
2	EUPHORBIA HILLEBRANDII VAR. PALIKEANA	EUPHORBIACEAE		HI
1	EUPHORBIA HILLEBRANDII VAR. WAIMANOANA	EUPHORBIACEAE		HI
1	EUPHORBIA HOOVERI	EUPHORBIACEAE	SPURGE, HOOVER	CA
3C	EUPHORBIA INNOCUA	EUPHORBIACEAE		TX
3C	EUPHORBIA JEJUNA	EUPHORBIACEAE		TX
3C	EUPHORBIA MULTIFORMIS VAR. HALEAKALANA	EUPHORBIACEAE		HI
1	* EUPHORBIA MULTIFORMIS VAR. KAALANA	EUPHORBIACEAE		HI
1	EUPHORBIA MULTIFORMIS VAR. KAPULEIENSIS	EUPHORBIACEAE		HI
3C	EUPHORBIA MULTIFORMIS VAR. MULTIFORMIS	EUPHORBIACEAE		HI
1	EUPHORBIA MULTIFORMIS VAR. PERDITA	EUPHORBIACEAE		HI
1	EUPHORBIA MULTIFORMIS VAR. SPARSIFLORA	EUPHORBIACEAE		HI
3A	EUPHORBIA MULTIFORMIS VAR. TOMENTELLA	EUPHORBIACEAE		HI
3C	EUPHORBIA NEPHRADENIA	EUPHORBIACEAE	SPURGE, PARIA	UT
3C	EUPHORBIA OCELLATA VAR. RATTANII	EUPHORBIACEAE		CA
3C	EUPHORBIA OLOWALUANA VAR. OLOWALUANA	EUPHORBIACEAE		HI
2	EUPHORBIA PERENNANS	EUPHORBIACEAE		TX
2	EUPHORBIA PLATYSPERMA	EUPHORBIACEAE	SPURGE, FLAT-SEEDED	AZ, MEXICO
1	EUPHORBIA PORTERANA VAR. KEYENSIS	EUPHORBIACEAE	SPURGE, PORTER'S,	FL
1	EUPHORBIA PORTERANA VAR. PORTERANA	EUPHORBIACEAE		FL
1	EUPHORBIA PORTERANA VAR. SCOPARIA	EUPHORBIACEAE	SPURGE, PORTER'S.	FL
2	EUPHORBIA PURPUREA	EUPHORBIACEAE	SPURGE, DARLINGTON'S	DE MD NJ NC OH PA VA WV
1	EUPHORBIA REMYI	EUPHORBIACEAE		HI
3C	EUPHORBIA ROEMERANA	EUPHORBIACEAE		TX
1	EUPHORBIA SKOTTSBERGII VAR. AUDENS	EUPHORBIACEAE		HI
LE	EUPHORBIA SKOTTSBERGII VAR. KALAELOANA	EUPHORBIACEAE	'AKOKO, 'EWA PLAINS	HI
1	* EUPHORBIA SKOTTSBERGII VAR. SKOTTSBERGII	EUPHORBIACEAE		HI
1	* EUPHORBIA SKOTTSBERGII VAR. VACCINIOIDES	EUPHORBIACEAE		HI
3C	EUPHORBIA STRICTIOR	EUPHORBIACEAE		NM TX
2	EUPHORBIA TELEPHIOIDES	EUPHORBIACEAE		FL
1	EURYA SANDWICENSIS VAR. GRANDIFOLIA	THEACEAE		HI

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
3C	EURYTAENIA HINCKLEYI	APIACEAE		TX
3C	EUTREMA PENLANDII	BRASSICACEAE		CO
3C	EXOCARPOS GAUDICHAUDII	SANTALACEAE		HI
1	EXOCARPOS LUTEOLUS	SANTALACEAE	HEAU (EXOCARPOS, LEAFY)	HI
2	FEROCACTUS ACANTHODES VAR. ACANTHODES	CACTACEAE		AZ CA, Mexico
5	FEROCACTUS ACANTHODES VAR. EASTWOODIAE	*** SEE ***	FEROCACTUS EASTWOODIAE COMB. NOV. /INED.	
3C	FEROCACTUS EASTWOODIAE /COMB. NOV. INED.	CACTACEAE		AZ
2	FEROCACTUS VIRIDESCENS	CACTACEAE	BARREL CACTUS, SAN DIEGO	CA, Mexico
2	FESTUCA DASYCLADA	POACEAE	FESCUE, SEDGE	CO UT
2	FESTUCA HALLII	POACEAE		CO
2	FESTUCA LIGULATA	POACEAE		TX
3C	FILIPENDULA OCCIDENTALIS	ROSACEAE	QUEEN-OF-THE-FOREST	OR
1	FIMBRISTYLIS PERPUSILLA	CYPERACEAE	FIMBRISTYLIS, HARPER'S	GA MD NC SC
3C	FIMBRISTYLIS SPADICEA	CYPERACEAE		CA NV, Tropical America
1	FLAVERIA MACDOUGALLII	ASTERACEAE		AZ
1	FORESTIERA SEGREGATA VAR. PINETORUM	OLEACEAE		FL
3C	FORSELLESIA PUNGENS VAR. GLABRA	CROSSOSOMATACEAE		CA NV
2	FORSELLESIA TEXENSIS	CELASTRACEAE		TX
3C	FOTHERGILLA GARDENII	HAMAMELIDACEAE	WITCH-ALDER, DWARF	AL FL GA MS NC SC
LE	FRANKENIA JOHNSTONII	FRANKENIACEAE	FRANKENIA, JOHNSTON'S	TX, Mexico (Nuevo Leon)
1	** FRANKLINIA ALATAMAHA	THEACEAE	FRANKLIN TREE	GA
5	FRASERA ALBICAULIS VAR. IDAHOENSIS	*** SEE ***	FRASERA IDAHOENSIS	
2	FRASERA COLORADENSIS	GENTIANACEAE		CO
1	FRASERA GYPSICOLA	GENTIANACEAE	GREEN-GENTIAN,	NV UT
3C	FRASERA IDAHOENSIS	GENTIANACEAE		ID OR
2	FRASERA PAHUTENSIS	GENTIANACEAE	GREEN-GENTIAN,	NV
3C	FRASERA PUBERULENTA	GENTIANACEAE		CA
3C	FRASERA TUBULOSA	GENTIANACEAE		CA
2	FRASERA UMPQUAENSIS	GENTIANACEAE	GREEN-GENTIAN, UMPQUA	CA OR
3C	FRAXINUS ANOMALA VAR. LOWELLII	OLEACEAE	ASH,	AZ
3C	FRAXINUS CUSPIDATA VAR. MACROPETALA	OLEACEAE	ASH,	AZ CA NV NM
3C	FRAXINUS GOODDINGII	OLEACEAE	ASH, GOODDING'S	AZ, Mexico
2	FREMONTODENDRON DECUMBENS	STERCULIACEAE	FLANNELBUSH, PINE HILL	CA
2	FREMONTODENDRON MEXICANUM	STERCULIACEAE	FREMONTIA, MEXICAN	CA, Mexico
3B	FRITILLARIA ADAMANTINA	LILIACEAE	MISSION-BELLS, DIAMOND LAKE	OR
2	FRITILLARIA AGRESTIS	LILIACEAE		CA
3C	FRITILLARIA BRANDEGEI	LILIACEAE	FRITILLARY, GREENHORN	CA
2	FRITILLARIA EASTWOODIAE	LILIACEAE	FRITILLARY, BUTTE	CA
2	FRITILLARIA FALCATA	LILIACEAE	FRITILLARY, TALUS	CA
2	FRITILLARIA GENTNERI	LILIACEAE	MISSION-BELLS, GENTNER	OR
2	FRITILLARIA GRAYANA	LILIACEAE	FRITILLARY, RODERICK'S	CA
2	FRITILLARIA LILIACEA	LILIACEAE		CA
2	FRITILLARIA OJAIENSIS	LILIACEAE		CA
5	FRITILLARIA PHAEANTHERA	*** SEE ***	FRITILLARIA EASTWOODIAE	
2	FRITILLARIA PLURIFLORA	LILIACEAE		CA
5	FRITILLARIA RODERICKII	*** SEE ***	FRITILLARIA GRAYANA	
2	FRITILLARIA STRIATA	LILIACEAE	ADOBE-LILY, GREENHORN	CA
2	FRITILLARIA VIRIDEA	LILIACEAE		CA
2	FRYXELLIA PYGMAEA	MALVACEAE		TX MEXICO
1	GAHNIA LANAIENSIS	CYPERACEAE		HI
2	GAILLARDIA FLAVA	ASTERACEAE	BLANKETFLOWER, YELLOW	UT
2	GALACTIA EGGERSII	FABACEAE		VI, British V.I.
2	GALACTIA PINETORUM	FABACEAE	MILK-PEA	FL
LE	GALACTIA SMALLII	FABACEAE	MILK-PEA, SMALL'S	FL
3B	GALINSOGA SEMICALVA VAR. PERCALVA	ASTERACEAE		AZ
3C	GALIUM ANDREWSII VAR. GATENSE	RUBIACEAE		CA
2	GALIUM ANGUSTIFOLIUM SSP. BORREGOENSE	RUBIACEAE	BEDSTRAW, BORREGO	CA
2	GALIUM BUXIFOLIUM	RUBIACEAE	BEDSTRAW, ISLAND	CA
2	GALIUM CALIFORNICUM SSP. LUCIENSE	RUBIACEAE	BEDSTRAW,	CA
2	GALIUM CALIFORNICUM SSP. PRIMUM	RUBIACEAE	BEDSTRAW, SAN JACINTO	CA
2	GALIUM CALIFORNICUM SSP. SIERRAE	RUBIACEAE	BEDSTRAW, EL DORADO	CA
3C	GALIUM CALIFORNICUM VAR. MIGUELENSE	RUBIACEAE		CA
2	GALIUM CATALINENSE SSP. ACRISPUM	RUBIACEAE	BEDSTRAW, SAN CLEMENTE ISLAND	CA
3C	GALIUM CLEMENTIS	RUBIACEAE	BEDSTRAW, SANTA LUCIA	CA
3C	GALIUM COLLOMAE	RUBIACEAE	BEDSTRAW,	AZ
2	GALIUM CORRELLII	RUBIACEAE		TX
2	GALIUM GLABRESCENS SSP. MODOCENSE	RUBIACEAE	BEDSTRAW, MODOC	CA
2	GALIUM GRANDE	RUBIACEAE	BEDSTRAW,	CA
2	GALIUM HARDHAMIAE	RUBIACEAE	BEDSTRAW, HARDHAM'S	CA
2	GALIUM HILENDIAE SSP. KINGSTONENSE	RUBIACEAE	BEDSTRAW, KINGSTON	CA NV
2	GALIUM HYPOTRICHIUM VAR. TOMENTELLUM	RUBIACEAE		CA
3C	GALIUM SERPENTICUM SSP. SCOTTICUM	RUBIACEAE	BEDSTRAW,	CA
2	GALIUM SERPENTICUM SSP. WARNERENSE	RUBIACEAE		CA
2	GALVEZIA SPECIOSA	SCROPHULARIACEAE	GAMBELIA, SHOWY	CA
LE	GARDENIA BRIGHAMII	RUBIACEAE	NANU	HI
2	GARDENIA WEISSICHII	RUBIACEAE		HI
3C	GAURA DEMAREEI	ONAGRACEAE		AR
1	GAURA NEOMEXICANA SSP. COLORADENSIS	ONAGRACEAE		CO WY
5	GAYA VIOLACEA	*** SEE ***	BATESIMALVA VIOLACEA	
3C	GAYLUSSACIA BRACHYCERA	ERICACEAE	HUCKLEBERRY, BOX	DE KY MD PA TN VA WV
2	GENISTIDIUM DUMOSUM	FABACEAE		TX, Mexico
5	GENTIANA ALEUTICA	*** SEE ***	GENTIANELLA PROPINQUA SSP. ALEUTICA	
3C	GENTIANA AUSTROMONTANA	GENTIANACEAE		NC TN VA WV

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
3C	GENTIANA AUTUMNALIS	GENTIANACEAE	GENTIAN, PINE BARREN	DE NJ NC SC VA
2	GENTIANA BISETAEA	GENTIANACEAE	GENTIAN,	OR
3B	GENTIANA DELOACHII	GENTIANACEAE	GENTIAN,	GA
3C	GENTIANA FREMONTII	GENTIANACEAE	GENTIAN, MOSS	CA
3C	GENTIANA PENNELLIANA	GENTIANACEAE	GENTIAN, WIREGRASS	FL
S	GENTIANA PORPHYRIO	*** SEE ***	GENTIANA AUTUMNALIS	
3C	GENTIANELLA PROPINQUA SSP. ALEUTICA	GENTIANACEAE		AK
1	GEOCARPON MINIMUM	CARYOPHYLLACEAE		AR MO
1	GERANIUM ARBOREUM	GERANIACEAE	GERANIUM, HAWAIIAN, RED-FLOWERED	HI
1	GERANIUM CUNEATUM VAR. HOLOLEUCUM	GERANIACEAE	GERANIUM, NATIVE,	HI
3C	GERANIUM MARGINALE	GERANIACEAE		UT
1	GERANIUM MULTIFLORUM VAR. MULTIFLORUM	GERANIACEAE	GERANIUM, NATIVE,	HI
2	GERANIUM MULTIFLORUM VAR. OVATIFOLIUM	GERANIACEAE	HINA HINA, LARGE-LEAVED	HI
2	GERANIUM MULTIFLORUM VAR. SUPERBUM	GERANIACEAE	GERANIUM, NATIVE	HI
3B	GERANIUM TOQUIMENSE	GERANIACEAE	GERANIUM,	NV
S	GERARDIA ACUTA	*** SEE ***	AGALINIS ACUTA	
S	GERARDIA STENOPHYLLA	*** SEE ***	AGALINIS STENOPHYLLA	
2	GESNERIA PAUCIFLORA	GESNERIACEAE		PR
2	GEUM GENICULATUM	ROSACEAE	AVENS, BENT	NC TN
3C	GEUM PECKII	ROSACEAE	AVENS, MOUNTAIN	NH, Canada
2	GEUM RADIATUM	ROSACEAE	AVENS, SPREADING	NC TN
1	GILIA CAESPITOSA	POLEMONIACEAE	GILIA, RABBIT VALLEY	UT
2	GILIA FORMOSA	POLEMONIACEAE	GILIA, BEAUTIFUL	NM
3C	GILIA MCVICKERAE	POLEMONIACEAE		UT
3C	GILIA NYENSIS	POLEMONIACEAE		NV
3C	GILIA PENTSTEMONOIDES	POLEMONIACEAE		CO
3C	GILIA RIPLEYI	POLEMONIACEAE		CA NV
1	GILIA TENUIFLORA SSP. ARENARIA	POLEMONIACEAE		CA
2	GILIA TENUIFLORA SSP. HOFFMANNII	POLEMONIACEAE	GILIA, SLENDER-FLOWERED, HOFFMAN	CA
2	GILMANIA LUTEOLA	POLYGONACEAE	GOLDEN CARPET	CA
2	GITHOPSIS DIFFUSA SSP. FILICAULIS	CAMPANULACEAE	BLUECUP, MISSION CANYON	CA
3B	GITHOPSIS LATIFOLIA	CAMPANULACEAE	BLUECUP, LAKE ALAMANOR	CA
PE	GLAUCOCARPUM SUFFRUTESCENS	BRASSICACEAE		UT
2	GLOEOCANTHARELLUS PURPURASCENS	GOMPHACEAE	MUSHROOM, INDIAN CREEK	NC
1	GLYCERIA NUBIGENA	POACEAE	MANNA GRASS, SMOKY MOUNTAINS	NC TN
2	GNAPHALIUM OBTUSIFOLIUM VAR. SAXICOLA	ASTERACEAE	CATFOOT, ROCK,	HI
3C	GNAPHALIUM SANDWICENSIUM VAR. FLAGELLARE	ASTERACEAE	'ENA'ENA, WHIP	HI
2	GNAPHALIUM SANDWICENSIUM VAR. MOLOKAIENSE	ASTERACEAE	'ENA'ENA	HI
LE	GOETZEA ELEGANS	SOLANACEAE	MATA BUEY	PR
2	GONOCALYX CONCOLOR	ERICACEAE		PR
S	GOSSYPIUM SANDVICENSE	*** SEE ***	GOSSYPIUM TOMENTOSUM	
3C	GOSSYPIUM TOMENTOSUM	MALVACEAE	COTTON, HAWAIIAN	HI
3A	GOUANIA BISHOPII	RHAMNACEAE		HI
3A	GOUANIA CUCULLATA	RHAMNACEAE		HI
1	GOUANIA FAURIEI	RHAMNACEAE		HI
1	GOUANIA GAGNEI	RHAMNACEAE		HI
3A	GOUANIA HAWAIIENSIS	RHAMNACEAE		HI
LE	GOUANIA HILLEBRANDII	RHAMNACEAE		HI
3A	GOUANIA LYDGATEI	RHAMNACEAE		HI
3A	GOUANIA MANNII	RHAMNACEAE		HI
3A	GOUANIA MEYENII	RHAMNACEAE		HI
1	* GOUANIA OLIVERI	RHAMNACEAE		HI
3A	GOUANIA PILATA	RHAMNACEAE		HI
3A	GOUANIA REMYI	RHAMNACEAE		HI
3A	GOUANIA SANDWICHIANA	RHAMNACEAE		HI
3A	GOUANIA THINOPHILA	RHAMNACEAE		HI
1	* GOUANIA VITIFOLIA	RHAMNACEAE		HI
3B	GOULDIA SP. /SP. NOV. INED.	RUBIACEAE	(MT. KAHILI, KAUAI CO.)	HI
2	GOULDIA ST-JOHNII VAR. MUNROI	RUBIACEAE		HI
1	GOULDIA TERMINALIS VAR. BOBEOIDES	RUBIACEAE		HI
1	GOULDIA TERMINALIS VAR. CONGESTA	RUBIACEAE		HI
1	GOULDIA TERMINALIS VAR. CRASSICAULIS	RUBIACEAE		HI
1	GOULDIA TERMINALIS VAR. DEGENERI	RUBIACEAE		HI
1	GOULDIA TERMINALIS VAR. LANAI	RUBIACEAE		HI
2	GOULDIA TERMINALIS VAR. PARVIFOLIA	RUBIACEAE		HI
1	GOULDIA TERMINALIS VAR. PSEUDODICHOTOMA	RUBIACEAE		HI
1	GOULDIA TERMINALIS VAR. PUBESCENS	RUBIACEAE		HI
1	GOULDIA TERMINALIS VAR. QUADRANGULARIS	RUBIACEAE		HI
1	GOULDIA TERMINALIS VAR. ROTUNDIFOLIA	RUBIACEAE		HI
1	GOULDIA TERMINALIS VAR. SUBCORDATA	RUBIACEAE		HI
2	GRAFFENRIEDA OTTOSCHULZII	MELASTOMATACEAE	CAMASEY, PETITES GRAINES	PR, Dominican Republic, Haiti
2	GRAMMITIS NIMBATA	POLYPODIACEAE		NC, Cuba
2	GRAPTOPETALUM BARTRAMII	CRASSULACEAE		AZ
3C	GRAPTOPETALUM RUSBYI	CRASSULACEAE		AZ NM
2	GRATIOLA HETEROSEPALA	SCROPHULARIACEAE	HEDGE-HYSSOP, BOGGS LAKE	CA OR
3B	GREENELLA DISCOIDEA	ASTERACEAE		AZ
LT	GRINDELIA FRAXINO-PRATENSIS	ASTERACEAE	GUM-PLANT, ASH MEADOWS	CA NV
3C	GRINDELIA HALLII	ASTERACEAE		CA
2	GRINDELIA HOWELLII	ASTERACEAE		ID MT
2	GRINDELIA MARITIMA	ASTERACEAE	GUMPLANT, SAN FRANCISCO	CA
3C	GRINDELIA OOLEPIS	ASTERACEAE	GUMWEED, PLAINS	TX

STATUS		SCIENTIFIC NAME	FAMILY	COMMON NAMF	RANGE
2		GRINDELIA STRICTA SSP. BLAKEI	ASTERACEAE		CA
S		GROSSULARIA ECHINELLA	*** SEE ***	RIBES ECHINELLUM	
3C		GUNNERA KAALENSIS	HALORAGACEAE		HI
3C		GUNNERA MAKAHAENSIS	HALORAGACEAE		HI
3C		GUTIERREZIA CALIFORNICA	ASTERACEAE	MATCHWEED, BAY	CA
3B		GUTIERREZIA LINOIDES	ASTERACEAE		AZ
3C		GUTIERREZIA SAROTHRAE VAR. POMARIENSIS	ASTERACEAE		UT
3B		GYMNOCARPIUM HETEROSPORUM	POLYPODIACEAE		AK MI MN WI
2		GYMNOPOGON FLORIDANUS	POACEAE		FL
S		HABENARIA FLAVA	*** SEE ***	PLATANTHERA FLAVA	
S		HABENARIA GREENEI	*** SEE ***	PLATANTHERA UNALASCENSIS SSP. MARITIMA	
S		HABENARIA HOLOCHILA	*** SEE ***	PLATANTHERA HOLOCHILA	
S		HABENARIA INTEGRA	*** SEE ***	PLATANTHERA INTEGRA	
S		HABENARIA LEUCOPHAEA	*** SEE ***	PLATANTHERA LEUCOPHAEA	
S		HABENARIA MARITIMA	*** SEE ***	PLATANTHERA UNALASCENSIS SSP. MARITIMA	
S		HABENARIA PERAMOENA	*** SEE ***	PLATANTHERA PERAMOENA	
S		HABENARIA UNALASCENSIS VAR. MARITIMA	*** SEE ***	PLATANTHERA UNALASCENSIS SSP. MARITIMA	
2		HACKELIA BREVICULA	BORAGINACEAE	STICKSEED, POISON CANYON	CA
1		HACKELIA CRONQUISTII	BORAGINACEAE	STICKSEED, CRONQUIST'S	OR
3C		HACKELIA DAVISII	BORAGINACEAE	STICKSEED, DAVIS'	ID
3C		HACKELIA HISPIDA	BORAGINACEAE		ID OR WA
2		HACKELIA IBAPENSIS	BORAGINACEAE	STICKSEED,	UT
3C		HACKELIA OPHIOBIA	BORAGINACEAE	STICKSEED,	NV OR
S		HACKELIA PATENS VAR. SEMIGLABRA	*** SEE ***	HACKELIA CRONQUISTII	
3C		HACKELIA SHARSMITHII	BORAGINACEAE		CA NV
2		HACKELIA VENUSTA	BORAGINACEAE	STICKSEED, SHOWY	WA
3C		HALIMOLOBOS PERPLEXA VAR. LEMHIENSIS	BRASSICACEAE		ID
2		HALIMOLOBOS PERPLEXA VAR. PERPLEXA	BRASSICACEAE		ID
3C		HALIMOLOBOS VIRGATA	BRASSICACEAE	HALIMOLOBUS, VIRGATE	CA
3C		HAPLOPAPPUS ABERRANS	ASTERACEAE		ID
2		HAPLOPAPPUS ALPINUS	ASTERACEAE		NV
S		HAPLOPAPPUS BRICKELLIOIDES	*** SEE ***	HAZARDIA BRICKELLIOIDES	
S		HAPLOPAPPUS CANUS	*** SEE ***	HAZARDIA CANA	
S		HAPLOPAPPUS CARTHAMOIDES VAR. MAXIMUS	*** SEE ***	HAPLOPAPPUS RADIATUS	
3C		HAPLOPAPPUS CERVINUS	ASTERACEAE		AZ NV UT
3B		HAPLOPAPPUS CONTRACTUS	ASTERACEAE	GOLDENWEED,	WY
S		HAPLOPAPPUS EASTWOODIAE	*** SEE ***	ERICAMERIA FASCICULATA	
3C		HAPLOPAPPUS EXIMIUS	ASTERACEAE		CA NV
2		HAPLOPAPPUS FREMONTII SSP. MONOCEPHALUS	ASTERACEAE	GOLDENWEED,	CO
3C		HAPLOPAPPUS HALLII	ASTERACEAE		OR WA
2		HAPLOPAPPUS INSECTICRURIS	ASTERACEAE		ID
S		HAPLOPAPPUS INTEGRIFOLIUS SSP. INSECTICRURIS	*** SEE ***	HAPLOPAPPUS INSECTICRURIS	
2		HAPLOPAPPUS LIATRIFORMIS	ASTERACEAE		ID WA
3C		HAPLOPAPPUS OPHITIDIS	ASTERACEAE	MACRONEMA, SERPENTINE	CA
2		HAPLOPAPPUS PALMERI SSP. PALMERI	ASTERACEAE		CA, Mexico
3C		HAPLOPAPPUS RACEMOSUS SSP. CONGESTUS	ASTERACEAE		CA OR
2		HAPLOPAPPUS RADIATUS	ASTERACEAE	GOLDENWEED,	ID OR
3C		HAPLOPAPPUS SALICINUS	ASTERACEAE	GOLDENWEED,	AZ
3C		HAPLOPAPPUS SCOPULORUM	ASTERACEAE		AZ UT
3C		HAPLOPAPPUS SPINULOSUS SSP. LAEVIS	ASTERACEAE		NM
2		HAPLOPAPPUS UNIFLORUS SSP. GOSSYPINUS	ASTERACEAE	GOLDEN-ASTER, BEAR VALLEY	CA
3C		HAPLOPAPPUS WATSONII	ASTERACEAE		NV
1	*	HAPLOSTACHYS BRYANII	LAMIACEAE		HI
LE		HAPLOSTACHYS HAPLOSTACHYA VAR. ANGUSTIFOLIA	LAMIACEAE		HI
1	*	HAPLOSTACHYS HAPLOSTACHYA VAR. HAPLOSTACHYA	LAMIACEAE		HI
1	*	HAPLOSTACHYS HAPLOSTACHYA VAR. LEPTOSTACHYA	LAMIACEAE		HI
1	*	HAPLOSTACHYS LINEARIFOLIA	LAMIACEAE		HI
1	*	HAPLOSTACHYS MUNROI	LAMIACEAE		HI
1	*	HAPLOSTACHYS TRUNCATA	LAMIACEAE		HI
LE		HARPEROCALLIS FLAVA	LILIACEAE	HARPER'S BEAUTY	FL
S		HARRISIA PORTORICENSIS	*** SEE ***	CEREUS PORTORICENSIS	
2		HARTWRIGHTIA FLORIDANA	ASTERACEAE	HARTWRIGHTIA	FL GA
1		HASTINGSIA BRACTEOSA	LILIACEAE		OR
3C		HAZARDIA BRICKELLIOIDES	ASTERACEAE		CA NV
2		HAZARDIA CANA	ASTERACEAE	HAZARDIA, ISLAND	CA, Mexico (Baja California Norte)
2		HAZARDIA ORCUTTII	ASTERACEAE	HAZARDIA, ORCUTT'S	CA, Mexico
3B		HECHTIA TEXENSIS	BROMELIACEAE		TX
LT		HEDEOMA APICULATUM	LAMIACEAE	PENNYROYAL, MCKITTRICK	NM TX
3C		HEDEOMA DENTATUM	LAMIACEAE		AZ
3C		HEDEOMA DIFFUSUM	LAMIACEAE	PENNYROYAL, FLAGSTAFF	AZ
1		HEDEOMA GRAVEOLENS	LAMIACEAE	PENNYROYAL, MOCK	FL
3C		HEDEOMA MOLLE	LAMIACEAE		TX
3C		HEDEOMA NANUM VAR. CALIFORNICUM	LAMIACEAE		AZ CA NV
2		HEDEOMA PILOSUM	LAMIACEAE	PENNYROYAL, OLD BLUE	TX
3C		HEDEOMA PULCHERRIMUM	LAMIACEAE		NM
LE		HEDEOMA TODSENII	LAMIACEAE	PENNYROYAL, TODSEN'S	NM
1	*	HEDYOTIS ANGUSTA VAR. ANGUSTA	RUBIACEAE		HI
1		HEDYOTIS ANGUSTA VAR. UMBROSA	RUBIACEAE	HEDYOTIS, NARROW-LEAVED	HI

STATUS		SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
3A		HEDYOTIS COOKIANA	RUBIACEAE		HI
1	*	HEDYOTIS CORIACEA	RUBIACEAE	KIO'ELE	HI
1		HEDYOTIS DEGENERI	RUBIACEAE		HI
2		HEDYOTIS ELATIOR VAR. ELATIOR	RUBIACEAE		HI
1		HEDYOTIS ELATIOR VAR. HERBACEA	RUBIACEAE		HI
2		HEDYOTIS FLUVIATILIS VAR. KAUAIENSIS	RUBIACEAE		HI
1	*	HEDYOTIS FOLIOSA	RUBIACEAE		HI
2		HEDYOTIS FORMOSA	RUBIACEAE		HI
2		HEDYOTIS GLAUCIFOLIA VAR. HELLERI	RUBIACEAE		HI
1		HEDYOTIS LITTORALIS	RUBIACEAE		HI
2		HEDYOTIS MANNII	RUBIACEAE		HI
2		HEDYOTIS NIGRICANS VAR. PULVINATA	RUBIACEAE	DIAMONDFLOWERS,	FL
1		HEDYOTIS PARVULA	RUBIACEAE		HI
2		HEDYOTIS PURPUREA VAR. MONTANA	RUBIACEAE		NC
1	*	HEDYOTIS REMYI VAR. NUTTALLII	RUBIACEAE		HI
1		HEDYOTIS REMYI VAR. PLANA	RUBIACEAE		HI
S		HEDYOTIS SCHLECHTENDAHLIANA VAR. NUTTALLII	*** SEE ***	HEDYOTIS REMYI VAR. NUTTALLII	
S		HEDYOTIS SCHLECHTENDAHLIANA VAR. PLANA	*** SEE ***	HEDYOTIS REMYI VAR. PLANA	
1		HEDYOTIS SCHLECHTENDAHLIANA VAR. RETICULATA	RUBIACEAE		HI
1		HEDYOTIS ST.-JOHNII	RUBIACEAE	HEDYOTIS, NA PALI BEACH	HI
1	*	HEDYOTIS THYRSOIDEA	RUBIACEAE		HI
3C		HEDYSARUM BOREALE VAR. GREMIALE	FABACEAE		UT
2		HEDYSARUM OCCIDENTALE VAR. CANONE	FABACEAE		UT
3C		HEIMIA LONGIPES	LYTHRACEAE		TX
3C		HELENIUM ARIZONICUM	ASTERACEAE		AZ
2		HELENIUM VIRGINICUM	ASTERACEAE		VA
2		HELIANTHELLA CASTANEA	ASTERACEAE	ROCK-ROSE, DIABLO	CA
3C		HELIANTHEMUM DUMOSUM	CISTACEAE	ROCK-ROSE, BUSHY	CT MA NY RI
2		HELIANTHEMUM GREENEI	CISTACEAE	RUSH-ROSE, ISLAND	CA
2		HELIANTHEMUM SUFFRUTESCENS	CISTACEAE	RUSH-ROSE, AMADOR	CA
2		HELIANTHUS CARNOSUS	ASTERACEAE		FL
1		HELIANTHUS DEBILIS SSP. VESTITUS	ASTERACEAE		FL
3C		HELIANTHUS DESERTICOLA	ASTERACEAE		AZ NV UT
2		HELIANTHUS EGGERTII	ASTERACEAE		AL KY NC TN
3C		HELIANTHUS EXILIS	ASTERACEAE	SUNFLOWER, SERPENTINE	CA
2		HELIANTHUS GLAUCOPHYLLUS	ASTERACEAE		NC TN
3B		HELIANTHUS LACINIATUS SSP. CRENATUS	ASTERACEAE		NM
3B		HELIANTHUS LUDENS	ASTERACEAE	GOLDENEYE, FIELD	TX
2		HELIANTHUS NIVEUS SSP. TEPHRODES	ASTERACEAE	SUNFLOWER, DESERT	CA, Mexico
1	*	HELIANTHUS NUTTALLII SSP. PARISHII	ASTERACEAE	SUNFLOWER, LOS ANGELES	CA
1		HELIANTHUS PARADOXUS	ASTERACEAE	SUNFLOWER,	NM TX
2		HELIANTHUS PRAECOX SSP. HIRTUS	ASTERACEAE		TX
3A		HELIANTHUS PRAETERMISSUS	ASTERACEAE		NM
1		HELIANTHUS SCHWEINITZII	ASTERACEAE		NC SC
2		HELIANTHUS SMITHII	ASTERACEAE		AL GA
S		HELIOMERIS SOLICEPS	*** SEE ***	VIGUIERA SOLICEPS	
1		HELIOTROPIUM GUANICENSE	BORAGINACEAE	COTORRILLA	PR
2		HELIOTROPIUM POLYPHYLLUM VAR. HORIZONTALE	BORAGINACEAE		FL
2		HELONIAS BULLATA	LILIACEAE	SWAMP-PINK,	DE GA MD NJ NY NC SC VA
1		HEMIZONIA ARIDA	ASTERACEAE	TARWEED, RED ROCK	CA
2		HEMIZONIA CONJUGENS	ASTERACEAE	TARWEED, OTAY	CA
2		HEMIZONIA FLORIBUNDA	ASTERACEAE	TARWEED, TECATE	CA, Mexico
3C		HEMIZONIA HALLIANA	ASTERACEAE	TARWEED, HALL'S	CA
2		HEMIZONIA MINTHORNII	ASTERACEAE	TARWEED, SANTA SUSANA	CA
1	*	HEMIZONIA MOHAVENSIS	ASTERACEAE	TARWEED, MAJAVE	CA
2		HEMIZONIA MULTICAULIS SSP. MULTICAULIS	ASTERACEAE		CA
2		HEMIZONIA MULTICAULIS SSP. VERNALIS	ASTERACEAE		CA
2		HERITIERA LONGIPETIOLATA	STERCULIACEAE	UFA-HALONTANO	GU, Rota, Saipan
3B		HERMIDIUM ALIPES VAR. PALLIDUM	NYCTAGINACEAE		CO UT
1		HESPEROCNIDE SANDWICENSIS	URTICACEAE		HI
3C		HESPEROLINON ADENOPHYLLUM	LINACEAE	DWARF-FLAX, GLANDULAR	CA
3C		HESPEROLINON BICARPELLATUM	LINACEAE	DWARF-FLAX, TWO CARPEL	CA
2		HESPEROLINON BREWERI	LINACEAE	DWARF-FLAX, BREWER'S	CA
1		HESPEROLINON CONGESTUM	LINACEAE	DWARF-FLAX, MARIN	CA
2		HESPEROLINON DIDYMOCARPUM	LINACEAE	DWARF-FLAX, LAKE COUNTY	CA
3C		HESPEROLINON DRYMARIOIDES	LINACEAE	DWARF-FLAX, DRYMARIA	CA
1		HESPEROMANNIA ARBORESCENS	ASTERACEAE	HESPEROMANNIA, LANAI	HI
1		HESPEROMANNIA ARBUSCULA	ASTERACEAE	HESPEROMANNIA, MAUI	HI
1		HESPEROMANNIA LYDGATEI	ASTERACEAE	HESPEROMANNIA, KAUAI	HI
S		HETEROTHECA FLEXUOSA	*** SEE ***	PITYOPSIS FLEXUOSA	
S		HETEROTHECA FLORIDANA	*** SEE ***	CHRYSOPSIS FLORIDANA	
2		HETEROTHECA JONESII	ASTERACEAE	GOLDEN-ASTER, JONES	UT
S		HETEROTHECA RUTHII	*** SEE ***	PITYOPSIS RUTHII	
3C		HEUCHERA AMERICANA VAR. HISPIDA	SAXIFRAGACEAE	ALUMROOT,	VA WV
S		HEUCHERA ARKANSANA	*** SEE ***	HEUCHERA VILLOSA VAR. ARKANSANA	
2		HEUCHERA BREVISTAMINEA	SAXIFRAGACEAE	HEUCHERA, LAGUNA	CA
3C		HEUCHERA DURANII	SAXIFRAGACEAE	HEUCHERA, DURAN'S	CA NV
S		HEUCHERA HISPIDA	*** SEE ***	HEUCHERA AMERICANA VAR. HISPIDA	
2		HEUCHERA MAXIMA	SAXIFRAGACEAE	ALUMROOT, ISLAND	CA
2		HEUCHERA MISSOURIENSIS	SAXIFRAGACEAE		MO

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
2	HEUCHERA VILLOSA VAR. ARKANSANA	SAXIFRAGACEAE		AR
3C	HEXALECTRIS GRANDIFLORA	ORCHIDACEAE	HEXALECTRIS, GREENMAN'S	TX, Mexico
2	HEXALECTRIS NITIDA	ORCHIDACEAE		TX
2	HEXALECTRIS REVOLUTA	ORCHIDACEAE		TX, Mexico
2	HEXASTYLIS CONTRACTA	ARISTOLOCHIACEAE		KY NC TN
2	HEXASTYLIS LEWISII	ARISTOLOCHIACEAE	HEARTLEAF,	NC VA
1	HEXASTYLIS NANIFLORA	ARISTOLOCHIACEAE	HEARTLEAF, DWARF-FLOWERED	NC SC
3C	HEXASTYLIS SPECIOSA	ARISTOLOCHIACEAE	HEARTLEAF, HARPER'S	AL
3A	HIBISCADELPHUS BOMBYCINUS	MALVACEAE		HI
PE	HIBISCADELPHUS DISTANS	MALVACEAE	HAU KUAHIWI, KAUAI	HI
1	** HIBISCADELPHUS GIFFARDIANUS	MALVACEAE	HAU KUAHIWI, KILAUEA	HI
1	HIBISCADELPHUS HUALALAIENSIS	MALVACEAE	HAU KUAHIWI, HUALALAI	HI
3A	HIBISCADELPHUS WILDERANUS	MALVACEAE		HI
1	HIBISCUS BRACKENRIDGEI	MALVACEAE	HIBISCUS, NATIVE YELLOW	HI
2	HIBISCUS CALIFORNICUS	MALVACEAE	HIBISCUS, CALIFORNIA	CA
1	HIBISCUS CLAYI	MALVACEAE	HIBISCUS, CLAY'S	HI
2	HIBISCUS DASYCALYX	MALVACEAE	ROSE-MALLOW, NECHES RIVER	TX
1	HIBISCUS IMMACULATUS	MALVACEAE	HIBISCUS, WHITE MOLOKAI	HI
3C	HIBISCUS KAHILII	MALVACEAE	HIBISCUS,	HI
2	HIBISCUS KOKIO VAR. KOKIO	MALVACEAE	PUALOALO, KOKI'O 'ULA 'ULA	HI
2	HIBISCUS KOKIO VAR. PUKOONIS	MALVACEAE		HI
1	HIBISCUS NEWHOUSEI	MALVACEAE		HI
2	HIBISCUS ROEATAE	MALVACEAE		HI
2	HIBISCUS SAINT-JOHNIANUS	MALVACEAE		HI
3C	HIBISCUS WAIMEAE	MALVACEAE		HI
3C	HIERACIUM LONGIBERBE	ASTERACEAE		OR WA
PE	HOFFMANNSEGGIA TENELLA	FABACEAE	RUSH-PEA, SLENDER	TX
1	HOLOCARPHA MACRADENIA	ASTERACEAE	TARWEED, SANTA CRUZ	CA
2	HORKELIA HENDERSONII	ROSACEAE		OR
3C	HORKELIA TRUNCATA	ROSACEAE		CA
2	HORKELIA TULARENSIS	ROSACEAE		CA
2	HORKELIA WILDERAE	ROSACEAE	HORKELIA, WILDER'S	CA
3B	HOUSTONIA CAERULEA VAR. FAXONORUM	RUBIACEAF	BLUET, ALPINE	NH, St. Pierre & Mipuelon
S	HOUSTONIA MONTANA	*** SEE ***	HEDYOTIS PURPUREA VAR. MONTANA	
S	HOUSTONIA NIGRICANS VAR. PULVINATA	*** SEE ***	HEDYOTIS NIGRICANS VAR. PULVINATA	
S	HOUSTONIA PULVINATA	*** SEE ***	HEDYOTIS NIGRICANS VAR. PULVINATA	
2	HOWELLIA AQUATILIS	CAMPANULACEAE		CA ID MT OR WA
S	HUDSONIA ERICOIDES SSP. MONTANA	*** SEE ***	HUDSONIA MONTANA	
LT	HUDSONIA MONTANA	CISTACEAE	GOLDEN-HEATHER, MOUNTAIN	NC
3C	HULSEA CALIFORNICA	ASTERACEAE		CA
S	HULSEA INYOENSIS	*** SEE ***	HULSEA VESTITA SSP. INYOENSIS	
3C	HULSEA VESTITA SSP. INYOENSIS	ASTERACEAE	HULSEA, INYO	CA NV
3C	HYDRASTIS CANADENSIS	RANUNCULACEAE	GOLDENSEAL	AL AR CT DE GA IL IN KY MD MI MN MS MO NE NY NC OH PA TN VT VA WV WI, Canada
3C	HYDROPHYLLUM CAPITATUM VAR. THOMPSONII	HYDROPHYLLACEAE	WATERLEAF, BALLHEAD, THOMPSON'S	OR WA
2	HYMENOCALLIS CORONARIA	LILIACEAE	SPIDER-LILY,	AL GA SC
3C	HYMENOCALLIS LATIFOLIA	LILIACEAE		FL, Bahamas, Cavman Islands, Cuba, Hispaniola, Jamaica
3C	HYMENOPAPPUS FILIFOLIUS VAR. IDAHOENSIS	ASTERACEAE		ID
3C	HYMENOPAPPUS FILIFOLIUS VAR. TOMENTOSUS	ASTERACEAE	HYMENOPAPPUS, COBWEB	UT
2	HYMENOPHYLLUM TUNBRIGENSE	HYMENOPHYLLACEAE		SC, Europe
1	HYMENOXYS ACAULIS VAR. GLABRA	ASTERACEAE	DAISY, LAKESIDE	IL OH, Canada (Ont.)
2	HYMENOXYS DEPRESSA	ASTERACEAE		UT
2	HYMENOXYS HELENIOIDES	ASTERACEAE		AZ CO UT
3C	HYMENOXYS QUINQUESQUAMATA	ASTERACEAE		AZ
3C	HYMENOXYS SUBINTEGRA	ASTERACEAE		AZ
PE	HYMENOXYS TEXANA	ASTERACEAE	BITTERWEED, TEXAS	TX
3C	HYMENOXYS TURNERI	ASTERACEAE		TX
1	HYPERICUM CUMULICOLA	HYPERICACEAE	ST. JOHN'S-WORT, HIGHLANDS SCRUB	FL
1	HYPERICUM EDISONIANUM	HYPERICACEAE	ASCYRUM, EDISON'S	FL
2	HYPERICUM LISSOPHLOEUS	HYPERICACEAE		FL
3C	HYPERICUM SPHAEROCARPUM VAR. TURGIDUM	HYPERICACEAE	ST.JOHN'S-WORT,	AL KY TN
S	HYPOPITYS CALIFORNICUS	*** SEE ***	PITYOPUS CALIFORNICUS	
3C	HYPOXIS LONGII	LILIACEAE	STAR-GRASS,	AR LA OK TX VA
2	HYSTRIX CALIFORNICA	POACEAE	GRASS, BOTTLEBRUSH, CALIFORNIA	CA
2	ILEX AMELANCHIER	AQUIFOLIACEAE	HOLLY,	AL FL GA LA MS NC SC
2	ILEX COLLINA	AQUIFOLIACEAE	HOLLY, LONG-STALKED	NC VA WV
1	ILEX COOKII	AQUIFOLIACEAE	TE	PR
3C	ILEX OPACA VAR. ARENICOLA	AQUIFOLIACEAE		FL
PE	ILIAMNA COREI	MALVACEAE		VA
2	ILIAMNA REMOTA	MALVACEAE	GLOBE-MALLOW, KANKAKEE	IL IN VA
S	ILIAMNA REMOTA VAR. COREI	*** SEE ***	ILIAMNA COREI	
2	ILLICIUM PARVIFLORUM	ILLICIACEAE		FL
3C	IPOMOEA CAIRICA VAR. LINEARILOBA	CONVOLVULACEAE		HI
3C	IPOMOEA CARDIOPHYLLA	CONVOLVULACEAE		TX
3B	IPOMOEA EGREGIA	CONVOLVULACEAE	MORNING-GLORY,	AZ NM
1	IPOMOEA KRUGII	CONVOLVULACEAE	MORNING-GLORY, KRUG'S WHITE	PR
2	IPOMOEA LEMMONII	CONVOLVULACEAE	MORNING-GLORY, LEMMON'S	AZ
3C	IPOMOPSIS GLOBULARIS	POLEMONIACEAE		CO

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
2	IPOMOPSIS POLYANTHA	POLEMONIACEAE		CO
2	IPOMOPSIS POLYANTHA VAR. POLYANTHA	POLEMONIACEAE		CO
1	IRIS LACUSTRIS	IRIDACEAE	IRIS,	MI WI, Canada (Ont.)
3C	IRIS TENAX SSP. KLAMATHENSIS	IRIDACEAE	IRIS,	CA
3B	IRIS TENAX VAR. GORMANII	IRIDACEAE	IRIS,	OR
3C	IRIS TENUIS	IRIDACEAE	IRIS, CLACKAMAS	OR
1	ISCHAEMUM BYRONE	POACEAE		HI
1	ISODENDRION FORBESII	VIOLACEAE	AUPAKA,	HI
3A	ISODENDRION HAWAIIENSE	VIOLACEAE	WAHINE-NOHO-KULA	HI
3A	ISODENDRION HILLEBRANDII	VIOLACEAE	AUPAKA,	HI
1	ISODENDRION HOSAKAE	VIOLACEAE	AUPAKA,	HI
3A	ISODENDRION LANAIENSE	VIOLACEAE	AUPAKA,	HI
1	ISODENDRION LAURIFOLIUM	VIOLACEAE	AUPAKA,	HI
1	ISODENDRION LONGIFOLIUM	VIOLACEAE	AUPAKA,	HI
1	* ISODENDRION LYDGATEI	VIOLACEAE	AUPAKA,	HI
3C	ISODENDRION MACULATUM	VIOLACEAE	AUPAKA,	HI
1	* ISODENDRION MOLOKAIENSE	VIOLACEAE	AUPAKA,	HI
1	* ISODENDRION PYRIFOLIUM	VIOLACEAE	AUPAKA,	HI
3A	ISODENDRION REMYI	VIOLACEAE	AUPAKA,	HI
1	ISODENDRION SUBSESSILIFOLIUM	VIOLACEAE	AUPAKA,	HI
1	* ISODENDRION WAIANAEENSE	VIOLACEAE	AUPAKA,	HI
3C	ISOETES BOLANDERI VAR. PYGMAEA	ISOETACEAE		AZ CA
2	ISOETES EATONII	ISOETACEAE	QUILLWORT, EATON'S	CT MA NH NJ NY, Canada (Ont.)
3C	ISOETES FLACCIDA	ISOETACEAE		FL GA
3B	ISOETES FOVEOLATA	ISOETACEAE	QUILLWORT, PITTED	CT MA NH
3C	ISOETES LITHOPHYLLA	ISOETACEAE	QUILLWORT, ROCK	TX
2	ISOETES LOUISIANENSIS	ISOETACEAE	QUILLWORT, LOUISIANA	GA LA
2	ISOETES MELANOSPORA	ISOETACEAE		GA SC
3C	ISOETES ORCUTTII	ISOETACEAE		CA
2	ISOETES TEGETIFORMANS	ISOETACEAE		GA NC
2	ISOETES VIRGINICA	ISOETACEAE	QUILLWORT,	NC SC VA
LE	ISOTRIA MEDEOLOIDES	ORCHIDACEAE	WHORLED POGONIA, SMALL	CT IL ME MD MA MI MO NH NJ NY NC PA RI SC VT VA, Canada (Ont.)
3C	IVESIA ARGYROCOMA	ROSACEAE	IVESIA, SILVER-HAIRED	CA
2	IVESIA CALLIDA	ROSACEAE	IVESIA, TAHQUITZ	CA
S	IVESIA CORYMBOSA	*** SEE ***	IVESIA PANICULATA	
2	IVESIA CRYPTOCAULIS	ROSACEAE		NV
LT	IVESIA EREMICA	ROSACEAE	IVESIA, ASH MEADOWS	NV
S	IVESIA MULTIFOLIOLATA	*** SEE ***	POTENTILLA MULTIFOLIOLATA	
2	IVESIA PANICULATA	ROSACEAE	IVESIA, ASH CREEK	CA
3C	IVESIA PICKERINGII	ROSACEAE	IVESIA, PICKERING	CA
2	IVESIA RHYPARA	ROSACEAE	IVESIA, GRIMY	NV OR
2	JACQUEMONTIA CURTISSII	CONVOLVULACEAE	JACQUEMONTIA, PINELAND	FL
2	JACQUEMONTIA RECLINATA	CONVOLVULACEAE		FL
2	JAMESIANTHUS ALABAMENSIS	ASTERACEAE	JAMESIANTHUS, ALABAMA	AL
2	JAQUINIA UMBELLATA	THEOPHRASTACEAE		PR, Hispaniola
3C	JOINVILLEA ASCENDENS SSP. ASCENDENS	FLAGELLARIACEAE	'OHE	HI
2	JUGLANS HINDSII	JUGLANDACEAE	WALNUT, NORTHERN CALIFORNIA BLACK	CA
2	JUNCUS CAESARIENSIS	JUNCACEAE	RUSH, NEW JERSEY	MD NJ VA
3C	JUNCUS GYMNOCARPUS	JUNCACEAE	RUSH,	AL FL MS NC PA SC TN
1	JUNCUS LEIOSPERMUS VAR. AHARTII /INED.	JUNCACEAE	RUSH,	CA
2	JUNCUS LEIOSPERMUS VAR. LEIOSPERMUS /INED.	JUNCACEAE	RUSH, RED BLUFF	CA
3A	JUNCUS PERVETUS	JUNCACEAE	RUSH, BOG, BARNSTABLE	MA
3B	JUNCUS SLWOOKOORUM	JUNCACEAE		AK
2	JUNCUS TRIFIDUS SSP. CAROLINIANUS	JUNCACEAE		NC NY TN VA
1	JUSTICIA BORINQUENSIS	ACANTHACEAE		PR
1	JUSTICIA COOLEYI	ACANTHACEAE	WATER-WILLOW, COOLEY'S	FL
2	JUSTICIA CRASSIFOLIA	ACANTHACEAE	WATER-WILLOW, THICK-LEAVED	FL
1	JUSTICIA CULEBRITAE	ACANTHACEAE		PR, British V.I.
3B	JUSTICIA MORTUIFLUMINIS	ACANTHACEAE	WATER-WILLOW,	VA
2	JUSTICIA RUNYONII	ACANTHACEAE		TX, Mexico
3C	JUSTICIA WARNOCKII	ACANTHACEAE		TX
3C	JUSTICIA WRIGHTII	ACANTHACEAE		TX
2	KALLSTROEMIA PERENNANS	ZYGOPHYLLACEAE		TX
1	KALMIA CUNEATA	ERICACEAE	WHITE-WICKY	NC SC
3C	KALMIOPSIS LEACHIANA	ERICACEAE		OR
S	KOANOPHYLLON DROSEROLEPIS	*** SEE ***	EUPATORIUM DROSEROLEPIS	
LE	KOKIA COOKEI	MALVACEAE	KOKIO, COOKE'S	HI
LE	KOKIA DRYNARIOIDES	MALVACEAE	HAU-HELE'ULA (TREE COTTON, HAWAIIAN)	HI
2	KOKIA KAUAIENSIS	MALVACEAE	KOKI'O, KAUAI	HI
3A	KOKIA LANCEOLATA	MALVACEAE	KOKI'O,	HI
2	KOSTELETZKYA SMILACIFOLIA	MALVACEAE		AL FL
3A	LABORDIA BAILLONII	LOGANIACEAE		HI
2	LABORDIA CYRTANDRAE VAR. NAHIKUANA	LOGANIACEAE		HI
2	LABORDIA DECURRENS VAR. DECURRENS	LOGANIACEAE		HI
1	LABORDIA FAGRAEOIDEA VAR. FAGRAEOIDEA	LOGANIACEAE	KAMAKAHALA	HI
1	* LABORDIA FAGRAEOIDEA VAR. LONGISEPALA	LOGANIACEAE		HI
2	LABORDIA FAGRAEOIDEA VAR. SAINT-JOHNIANA	LOGANIACEAE		HI
1	LABORDIA FAGRAEOIDEA VAR. WAIANAEANA	LOGANIACEAE		HI
2	LABORDIA GLABRA	LOGANIACEAE		HI

STATUS		SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
1		LABORDIA HEDYOSMIFOLIA VAR. KILAUEANA	LOGANIACEAE		HI
1		LABORDIA HEDYOSMIFOLIA VAR. MAGNIFOLIA	LOGANIACEAE		HI
1		LABORDIA HEDYOSMIFOLIA VAR. ROBUSTA	LOGANIACEAE		HI
1		LABORDIA HEDYOSMIFOLIA VAR. ROCKII	LOGANIACEAE		HI
1		LABORDIA HEDYOSMIFOLIA VAR. SKOTTSBERGII	LOGANIACEAE		HI
1		LABORDIA HIRTELLA VAR. IMBRICATA	LOGANIACEAE		HI
1		LABORDIA HIRTELLA VAR. LAEVIS	LOGANIACEAE		HI
1		LABORDIA HIRTELLA VAR. LAEVISEPALA	LOGANIACEAE		HI
1	*	LABORDIA HIRTELLA VAR. MICROCALYX	LOGANIACEAE		HI
1	*	LABORDIA HIRTELLA VAR. MICROPHYLLA	LOGANIACEAE		HI
1	*	LABORDIA KAALAE VAR. BRACHYPODA	LOGANIACEAE		HI
1		LABORDIA KAALAE VAR. FOSBERGII	LOGANIACEAE		HI
1	*	LABORDIA KAALAE VAR. KAUAIENSIS	LOGANIACEAE		HI
1		LABORDIA KAALAE VAR. MENDAX	LOGANIACEAE		HI
2		LABORDIA LYDGATEI	LOGANIACEAE		HI
1		LABORDIA MEMBRANACEA	LOGANIACEAE	KAMAKAHALA	HI
1	*	LABORDIA MOLOKAIANA VAR. MOLOKAIANA	LOGANIACEAE		HI
1		LABORDIA MOLOKAIANA VAR. MUNROI	LOGANIACEAE		HI
1		LABORDIA MOLOKAIANA VAR. SETOSA	LOGANIACEAE		HI
2		LABORDIA NELSONII	LOGANIACEAE		HI
1	*	LABORDIA OLYMPIANA	LOGANIACEAE		HI
1	*	LABORDIA PALLIDA	LOGANIACEAE		HI
1	*	LABORDIA PEDUNCULATA	LOGANIACEAE		HI
2		LABORDIA TINIFOLIA VAR. EUPHORBIOIDEA	LOGANIACEAE		HI
1	*	LABORDIA TINIFOLIA VAR. FORBESII	LOGANIACEAE		HI
1	*	LABORDIA TINIFOLIA VAR. HONOLULENSIS	LOGANIACEAE		HI
2		LABORDIA TINIFOLIA VAR. LANAIENSIS	LOGANIACEAE		HI
1		LABORDIA TINIFOLIA VAR. MICROGYNA	LOGANIACEAE		HI
1	*	LABORDIA TINIFOLIA VAR. PARVIFOLIA	LOGANIACEAE		HI
1		LABORDIA TINIFOLIA VAR. TENUIFOLIA	LOGANIACEAE		HI
2		LABORDIA TRIFLORA	LOGANIACEAE		HI
2		LABORDIA WAWRANA	LOGANIACEAE		HI
3C		LACHNOCAULON BEYRICHIANUM	ERIOCAULACEAE		FL GA NC SC
2		LACHNOCAULON DIGYNUM	ERIOCAULACEAE		AL FL LA MS
5		LAPHAMIA CERNUA	*** SEE ***	PERITYLE CERNUA	
2		LAPLACEA PORTORICENSIS	THEACEAE	NINO DE COTA (MARICAO VERDE)	PR, Hispaniola
2		LASTHENIA BURKEI	ASTERACEAE	GOLDFIELDS, BURKE'S	CA
2		LASTHENIA CONJUGENS	ASTERACEAE	GOLDFIELDS, CONTRA COSTA	CA
2		LASTHENIA LEPTALEA	ASTERACEAE	BAERIA, SALINAS VALLEY	CA
2		LASTHENIA MACRANTHA SSP. PRISCA	ASTERACEAE		OR
3C		LASTHENIA MINOR SSP. MARITIMA	ASTERACEAE	GOLDFIELDS, SEASIDE	CA OR WA, Canada (B.C.)
2		LATHYRUS BIFLORUS	FABACEAE		CA
3C		LATHYRUS HITCHCOCKIANUS	FABACEAE	WILD PEA, BULLFROG MOUNTAIN	CA NV
2		LATHYRUS HOLOCHLORUS	FABACEAE		OR
2		LATHYRUS JEPSONII SSP. JEPSONII	FABACEAE	TULE-PEA, DELTA	CA
5		LAVATERA ASSURGENTIFLORA	*** SEE ***	LAVATERA ASSURGENTIFLORA SSP. ASSURGENTIFLORA	
3C		LAVATERA ASSURGENTIFLORA SSP. ASSURGENTIFLORA	MALVACEAE	MALVA ROSA	CA
2		LAYIA DISCOIDEA	ASTERACEAE	TIDYTIPS, RAYLESS	CA
2		LAYIA JONESII	ASTERACEAE	LAYIA, JONES	CA
2		LAYIA LEUCOPAPPA	ASTERACEAE	LAYIA, COMANCHE	CA
1		LAYIA ZIEGLERI	ASTERACEAE		CA
2		LEAVENWORTHIA ALABAMICA VAR. ALABAMICA	BRASSICACEAE		AL
2		LEAVENWORTHIA ALABAMICA VAR. BRACHYSTYLA	BRASSICACEAE	GLADE CRESS,	AL
3C		LEAVENWORTHIA AUREA	BRASSICACEAE	GLADE CRESS, GOLDEN	OK TX
2		LEAVENWORTHIA CRASSA VAR. CRASSA	BRASSICACEAE	GLADE CRESS,	AL
2		LEAVENWORTHIA CRASSA VAR. ELONGATA	BRASSICACEAE	GLADE CRESS,	AL
2		LEAVENWORTHIA EXIGUA VAR. EXIGUA	BRASSICACEAE		AL GA TN
1		LEAVENWORTHIA EXIGUA VAR. LACINIATA	BRASSICACEAE	GLADE CRESS,	KY
2		LEAVENWORTHIA EXIGUA VAR. LUTEA	BRASSICACEAE	GLADE CRESS,	AL TN
3C		LEAVENWORTHIA STYLOSA	BRASSICACEAE		AL TN
3C		LEAVENWORTHIA TORULOSA	BRASSICACEAE	GLADECRESS, NECKLACE	AL KY TN
2		LECHEA CERNUA	CISTACEAE		FL
2		LECHEA DIVARICATA	CISTACEAE		FL
2		LECHEA LAKELAE	CISTACEAE		FL
3C		LECHEA MARITIMA VAR. VIRGINICA	CISTACEAE	PINWEED, BEACH, VIRGINIAN	VA
2		LECHEA MENSALIS	CISTACEAE	PINWEED,	TX MEXICO
2		LEGENERE LIMOSA	CAMPANULACEAE	LEGENERE	CA
5		LEIBERGIA OROGENIOIDES	*** SEE ***	TAUSCHIA TENUISSIMA	
3C		LEITNERIA FLORIDANA	LEITNERIACEAE		AR FL GA MO TX
5		LENOPHYLLUM TEXANUM	*** SEE ***	SEDUM TEXANUM	
3C		LEPANTHES DODIANA	ORCHIDACEAE		PR
1		LEPANTHES ELTOROENSIS	ORCHIDACEAE		PR
2		LEPANTHOPSIS MELANANTHA	ORCHIDACEAE	ORCHID, TINY	FL, Cuba, Dominican Republic, Haiti, Jamaica
2		LEPECHINIA CARDIOPHYLLA	LAMIACEAE		CA
2		LEPECHINIA GANDERI	LAMIACEAE		CA
1	*	LEPIDIUM ARBUSCULUM	BRASSICACEAE		HI
1		LEPIDIUM BARNEBYANUM	BRASSICACEAE	PEPPER CRESS, BARNEBY	UT
3C		LEPIDIUM BIDENTATUM VAR. O-WAIHIENSE	BRASSICACEAE	ANAUNAU,	HI
1	*	LEPIDIUM BIDENTATUM VAR. REMYI	BRASSICACEAE	ANAUNAU, REMY'S	HI
2		LEPIDIUM DAVISII	BRASSICACEAE	PEPPER CRESS, DAVIS'	ID OR

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
2	LEPIDIUM FLAVUM VAR. FELIPENSE	BRASSICACEAE	PEPPER-GRASS, BORREGO VALLEY	CA
2	LEPIDIUM MONTANUM VAR. NEESEAE	BRASSICACEAE		UT
1	LEPIDIUM MONTANUM VAR. STELLAE	BRASSICACEAE		UT
3C	LEPIDIUM NANUM	BRASSICACEAE		NV
2	LEPIDIUM OSTLERI	BRASSICACEAE		UT
1	LEPIDIUM SERRA	BRASSICACEAE	ANAUNAU	HI
2	LEPIDOSPARTUM BURGESSII	ASTERACEAE		NM TX
5	LEPTOCEREUS QUADRICOSTATUS	*** SEE ***	CEREUS QUADRICOSTATUS	
3C	LEPTODACTYLON HAZELAE	POLEMONIACEAE		OR
2	LEPTODACTYLON JAEGERI	POLEMONIACEAE		CA
5	LEPTOGRAMMA PILOSA VAR. ALABAMENSIS	*** SEE ***	THELYPTERIS PILOSA VAR. ALABAMENSIS	
1	LESPEDEZA LEPTOSTACHYA	FABACEAE	BUSH-CLOVER, PRAIRIE	IL IA MN WI
3C	LESQUERELLA ANGUSTIFOLIA	BRASSICACEAE	BLADDERPOD, THREAD-LEAVED	OK TX
3B	LESQUERELLA ARCTICA VAR. SCAMMANAE	BRASSICACEAE		AK
3C	LESQUERELLA AUREA	BRASSICACEAE	BLADDERPOD, GOLDEN	NM
3C	LESQUERELLA CARINATA	BRASSICACEAE	BLADDERPOD, KEELED	ID MT WY
2	LESQUERELLA CONDENSATA	BRASSICACEAE		CO
3C	LESQUERELLA DENSIPILA	BRASSICACEAE	BLADDERPOD, DUCK RIVER	AL TN
1	LESQUERELLA FILIFORMIS	BRASSICACEAE	BLADDERPOD,	MO
3C	LESQUERELLA FREMONTII	BRASSICACEAE	BLADDERPOD, FREMONT'S	WY
3C	LESQUERELLA GARRETTII	BRASSICACEAE	BLADDERPOD, GARRETT	UT
2	LESQUERELLA GLOBOSA	BRASSICACEAE	BLADDERPOD, SHORT'S	IN KY TN
3B	LESQUERELLA GOODINGII	BRASSICACEAE	BLADDERPOD,	AZ NM
3C	LESQUERELLA HITCHCOCKII	BRASSICACEAE	BLADDERPOD,	NV
2	LESQUERELLA KAIBABENSIS	BRASSICACEAE		AZ
1	LESQUERELLA KINGII SSP. BERNARDINA	BRASSICACEAE	BLADDERPOD,	CA
3C	LESQUERELLA KINGII SSP. DIVERSIFOLIA	BRASSICACEAE	BLADDERPOD,	OR
3B	LESQUERELLA LATA	BRASSICACEAE	BLADDERPOD,	NM
3C	LESQUERELLA LESCURII	BRASSICACEAE		AL TN
2	LESQUERELLA LYRATA	BRASSICACEAE	BLADDERPOD, LYRATE	AL
3C	LESQUERELLA MACROCARPA	BRASSICACEAE	BLADDERPOD, LARGE-FRUITED	WY
3C	LESQUERELLA MCVAUGHIANA	BRASSICACEAE	BLADDERPOD,	TX
2	LESQUERELLA PALLIDA	BRASSICACEAE		TX
2	LESQUERELLA PARVIFLORA	BRASSICACEAE		CO
1	LESQUERELLA PERFORATA	BRASSICACEAE	BLADDERPOD, SPRING CREEK	TN
2	LESQUERELLA PRUINOSA	BRASSICACEAE	BLADDERPOD,	CO
3C	LESQUERELLA RUBICUNDULA	BRASSICACEAE	BLADDERPOD, BRYCE	UT
1	LESQUERELLA STONENSIS	BRASSICACEAE	BLADDERPOD, STONES RIVER	TN
2	LESQUERELLA THAMNOPHILA	BRASSICACEAE	BLADDERPOD,	TX
2	LESQUERELLA TUMULOSA	BRASSICACEAE	BLADDERPOD,	UT
3C	LESQUERELLA VALIDA	BRASSICACEAE	BLADDERPOD,STRONG	NM TX
1	LESSINGIA GERMANORUM VAR. GERMANORUM	ASTERACEAE	LESSINGIA, SAN FRANCISCO	CA
2	LESSINGIA GLANDULIFERA VAR. TOMENTOSA	ASTERACEAE	LESSINGIA, WARNER SPRINGS	CA
3C	LEWISIA CANTELOWII	PORTULACACEAE	LEWISIA, CANTELOW'S	CA
3C	LEWISIA COLUMBIANA VAR. WALLOWENSIS	PORTULACACEAE		ID MT OR
3C	LEWISIA CONGDONII	PORTULACACEAE		CA
2	LEWISIA COTYLEDON SSP. /SSP. NOV. INED.	PORTULACACEAE	LEWISIA, FRINGED	CA
2	LEWISIA COTYLEDON VAR. HECKNERI	PORTULACACEAE	LEWISIA, HECKNER'S	CA
2	LEWISIA COTYLEDON VAR. HOWELLII	PORTULACACEAE	LEWISIA, HOWELL'S	CA OR
2	LEWISIA COTYLEDON VAR. PURDYI	PORTULACACEAE		OR
3C	LEWISIA DISEPALA	PORTULACACEAE	BITTERROOT, YOSEMITE	CA
2	LEWISIA MAGUIREI	PORTULACACEAE		NV
3C	LEWISIA OPPOSITIFOLIA	PORTULACACEAE		CA OR
2	LEWISIA PYGMAEA SSP. LONGIPETALA	PORTULACACEAE	LEWISIA, LONG-PETALED	CA
2	LEWISIA SERRATA	PORTULACACEAE	LEWISIA, SAW-TOOTHED	CA
2	LEWISIA STEBBINSII	PORTULACACEAE	LEWISIA, STEBBINS	CA
3C	LEWISIA TWEEDYI	PORTULACACEAE		WA, Canada (B.C.)
3C	LIATRIS CYMOSA	ASTERACEAE		TX
2	LIATRIS HELLERI	ASTERACEAE		NC
1	LIATRIS OHLINGERAE	ASTERACEAE	BLAZINGSTAR,	FL
2	LIATRIS PROVINCIALIS	ASTERACEAE	BLAZINGSTAR, GODFREY'S	FL
3C	LIATRIS TENUIS	ASTERACEAE		TX
3B	LIGUSTICUM PORTERI VAR. BREVILOBUM	APIACEAE		UT
2	LILAEOPSIS CAROLINENSIS	APIACEAE		AL FL GA LA MS NC SC VA
2	LILAEOPSIS MASONII	APIACEAE		CA
2	LILAEOPSIS RECURVA	APIACEAE		AZ
3C	LILIUM BOLANDERI	LILIACEAE		CA OR
2	LILIUM FAIRCHILDII	LILIACEAE		CA
2	LILIUM GRAYI	LILIACEAE	LILY, GRAY'S	NC TN VA
2	LILIUM IRIDOLLAE	LILIACEAE	LILY, PANHANDLE	AL FL
2	LILIUM OCCIDENTALE	LILIACEAE	LILY, WESTERN	CA OR
2	LILIUM PARRYI	LILIACEAE		AZ CA
1	LILIUM PITKINENSE	LILIACEAE	LILY, PITKIN MARSH	CA
3C	LILIUM VOLLMERI	LILIACEAE	LILY, VOLLMER	CA OR
3B	LILIUM WASHINGTONIANUM VAR. MINUS	LILIACEAE		CA
3C	LILIUM WIGGINSII	LILIACEAE	LILY, WIGGINS	CA OR
2	LIMNANTHES BAKERI	LIMNANTHACEAE	MEADOWFOAM, BAKER'S	CA
2	LIMNANTHES DOUGLASII VAR. SULPHUREA	LIMNANTHACEAE	MEADOWFOAM, PT. REYES	CA
2	LIMNANTHES FLOCCOSA SSP. BELLINGERANA	LIMNANTHACEAE	MEADOWFOAM, BELLINGER'S	CA OR
1	LIMNANTHES FLOCCOSA SSP. CALIFORNICA	LIMNANTHACEAE		CA
2	LIMNANTHES FLOCCOSA SSP. GRANDIFLORA	LIMNANTHACEAE	MEADOWFOAM, WOOLY, LARGE-FLOWERED	OR
1	LIMNANTHES FLOCCOSA SSP. PUMILA	LIMNANTHACEAE	MEADOWFOAM, WOOLY, DWARF	OR
2	LIMNANTHES GRACILIS VAR. GRACILIS	LIMNANTHACEAE		OR

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2		LIMNANTHES GRACILIS VAR. PARISHII	LIMNANTHACEAE	MEADOWFOAM, PARISH'S	CA
2		LIMNANTHES VINCULANS	LIMNANTHACEAE	MEADOWFOAM, SEBASTOPOL	CA
3B		LIMONIUM CAROLINIANUM VAR. ANGUSTATUM	PLUMBAGINACEAE	SEA-LAVENDER,	FL
3C		LIMONIUM LIMBATUM	PLUMBAGINACEAE		AZ NM TX
2		LIMOSELLA PUBIFLORA	SCROPHULARIACEAE	MUDWORT,	AZ
3C		LINANTHUS ARENICOLA	POLEMONIACEAE		CA NV, Mexico (Baja California)
3C		LINANTHUS BELLUS	POLEMONIACEAE	DESERT BEAUTY	CA, Mexico
2		LINANTHUS HARKNESSII SSP. CONDENSATUS	POLEMONIACEAE		CA
2		LINANTHUS KILLIPII	POLEMONIACEAE	LINANTHUS, BALDWIN LAKE	CA
2		LINANTHUS MACULATUS	POLEMONIACEAE	LINANTHUS, SAN BERNARDINO MT., LITTLE	CA
2		LINANTHUS ORCUTTII	POLEMONIACEAE	LINANTHUS, ORCUTT	CA
S		LINANTHUS ORCUTTII SSP. PACIFICUS	*** SEE ***	LINANTHUS ORCUTTII	
PE		LINDERA MELISSIFOLIA	LAURACEAE		AL AR FL GA LA MS MO NC SC
2		LINDERA SUBCORIACEA	LAURACEAE		LA MS NC
1	*	LINDERNIA SAXICOLA	SCROPHULARIACEAE	FALSE PIMPERNEL,	GA NC
2		LINUM ARENICOLA	LINACEAE	FLAX, SAND	FL
1		LINUM CARTERI VAR. CARTERI	LINACEAE	FLAX,	FL
1		LINUM CARTERI VAR. SMALLII	LINACEAE	FLAX,	FL
2		LINUM MACROCARPUM	LINACEAE	FLAX,	AL
2		LINUM SULCATUM VAR. HARPERI	LINACEAE		AL FL GA
2		LINUM WESTII	LINACEAE	FLAX, WEST'S	FL GA
3B		LIPOCHAETA ALATA VAR. ALATA	ASTERACEAE	NEHE,	HI
1	*	LIPOCHAETA BRYANII	ASTERACEAE	NEHE, BRYAN'S	HI
1	*	LIPOCHAETA DEGENERI	ASTERACEAE	NEHE, SMALL-LEAVED	HI
S		LIPOCHAETA DELTOIDEA	*** SEE ***	LIPOCHAETA SUBCORDATA	HI
3B		LIPOCHAETA DUBIA	ASTERACEAE		HI
3B		LIPOCHAETA EXIGUA	ASTERACEAE	NEHE, LESSER	HI
1	*	LIPOCHAETA FAURIEI	ASTERACEAE	NEHE, FAURIE	HI
3B		LIPOCHAETA FLEXUOSA	ASTERACEAE	NEHE, FLEXUOUS	HI
3B		LIPOCHAETA FORBESII VAR. FORBESII	ASTERACEAE	NEHE, FORBES	HI
3C		LIPOCHAETA HETEROPHYLLA VAR. HETEROPHYLLA	ASTERACEAE		HI
3B		LIPOCHAETA HETEROPHYLLA VAR. MALVACEA	ASTERACEAE		HI
3B		LIPOCHAETA HETEROPHYLLA VAR. MOLOKAIENSIS	ASTERACEAE		HI
3B		LIPOCHAETA INTEGRIFOLIA VAR. ARGENTEA	ASTERACEAE		HI
3B		LIPOCHAETA INTEGRIFOLIA VAR. GRACILIS	ASTERACEAE	NEHE,	HI
3B		LIPOCHAETA INTEGRIFOLIA VAR. MAJOR	ASTERACEAE	NEHE,	HI
3B		LIPOCHAETA INTEGRIFOLIA VAR. MEGACEPHALA	ASTERACEAE	NEHE,	HI
3B		LIPOCHAETA INTERMEDIA	ASTERACEAE		HI
3B		LIPOCHAETA KAMOOLAWENSIS	ASTERACEAE	NEHE,	HI
1		LIPOCHAETA KAMOLENSIS	ASTERACEAE		HI
3B		LIPOCHAETA LAVARUM VAR. CONFERTA	ASTERACEAE		HI
3B		LIPOCHAETA LAVARUM VAR. HILLEBRANDIANA	ASTERACEAE		HI
3B		LIPOCHAETA LAVARUM VAR. LONGIFOLIA	ASTERACEAE	NEHE,	HI
3B		LIPOCHAETA LAVARUM VAR. MANELEANA	ASTERACEAE		HI
3B		LIPOCHAETA LAVARUM VAR. OVATA	ASTERACEAE		HI
3B		LIPOCHAETA LAVARUM VAR. SALICIFOLIA	ASTERACEAE	NEHE,	HI
3B		LIPOCHAETA LAVARUM VAR. SKOTTSBERGII	ASTERACEAE	NEHE,	HI
3B		LIPOCHAETA LAVARUM VAR. STEARNSII	ASTERACEAE		HI
3B		LIPOCHAETA LOBATA VAR. ALBESCENS	ASTERACEAE	NEHE,	HI
3B		LIPOCHAETA LOBATA VAR. APREVALLIANA	ASTERACEAE	NEHE,	HI
3B		LIPOCHAETA LOBATA VAR. GROSSEDENTATA	ASTERACEAE		HI
3B		LIPOCHAETA LOBATA VAR. HASTULATA	ASTERACEAE	NEHE,	HI
2		LIPOCHAETA LOBATA VAR. HASTULATOIDES	ASTERACEAE		HI
1		LIPOCHAETA LOBATA VAR. LEPTOPHYLLA	ASTERACEAE	NEHE,	HI
3C		LIPOCHAETA LOBATA VAR. LOBATA	ASTERACEAE	NEHE, LOBED,	HI
3B		LIPOCHAETA LOBATA VAR. MAKENENSIS	ASTERACEAE		HI
3B		LIPOCHAETA LOBATA VAR. MAUNALOENSIS	ASTERACEAE	NEHE, LOBED, MAUNA LOA	HI
1		LIPOCHAETA MICRANTHA	ASTERACEAE		HI
3B		LIPOCHAETA MINUSCULA	ASTERACEAE		HI
1	*	LIPOCHAETA OVATA	ASTERACEAE		HI
1	*	LIPOCHAETA PERDITA	ASTERACEAE	NEHE,	HI
3B		LIPOCHAETA POPULIFOLIA	ASTERACEAE	NEHE,	HI
3B		LIPOCHAETA POROPHILA	ASTERACEAE		HI
3B		LIPOCHAETA PROFUSA VAR. PROFUSA	ASTERACEAE	NEHE, MANY-FLOWERED	HI
3B		LIPOCHAETA PROFUSA VAR. ROBUSTIOR	ASTERACEAE	NEHE,	HI
3C		LIPOCHAETA REMYI	ASTERACEAE	NEHE, REMY'S	HI
3C		LIPOCHAETA ROCKII	ASTERACEAE	NEHE, ROCK'S	HI
3B		LIPOCHAETA ROCKII VAR. DISSECTA	ASTERACEAE	NEHE,	HI
3B		LIPOCHAETA ROCKII VAR. SUBOVATA	ASTERACEAE	NEHE,	HI
3B		LIPOCHAETA SCABRA	ASTERACEAE		HI
3B		LIPOCHAETA SUBCORDATA VAR. MEMBRANACEA	ASTERACEAE	NEHE,	HI
S		LIPOCHAETA SUBCORDATA VAR. POPULIFOLIA	*** SEE ***	LIPOCHAETA POPULIFOLIA	
3B		LIPOCHAETA SUCCULENTA VAR. ANGUSTATA	ASTERACEAE	NEHE,	HI
3C		LIPOCHAETA SUCCULENTA VAR. SUCCULENTA	ASTERACEAE	NEHE,	HI
3B		LIPOCHAETA SUCCULENTA VAR. TRIFIDA	ASTERACEAE	NEHE,	HI
1		LIPOCHAETA TENUIFOLIA	ASTERACEAE	NEHE, SLENDER-LEAVED	HI
2		LIPOCHAETA TENUIS	ASTERACEAE		HI
3B		LIPOCHAETA TRILOBATA	ASTERACEAE		HI
LE		LIPOCHAETA VENOSA	ASTERACEAE	NEHE,	HI

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
1	LIPOCHAETA WAIMEAENSIS	ASTERACEAE		HI
2	LISTERA AURICULATA	ORCHIDACEAE	TWAYBLADE, AURICLED	ME MI MN NH NY VT WI, Canada (Lab., N.B., Nfld., Ont., Que.)
1	LITHOPHRAGMA MAXIMUM	SAXIFRAGACEAE	WOODLAND STAR, SAN CLEMENTE ISLAND	CA
5	LITHOSPERMUM DRUMMONDII	*** SEE ***	MERTENSIA DRUMMONDII	
3C	LITSEA AESTIVALIS	LAURACEAE	PONDSPICE	FL GA NC SC TN VA
2	LOBELIA APPENDICULATA VAR. GATTINGERI	CAMPANULACEAE		TN
2	LOBELIA BOYKINII	CAMPANULACEAE	LOBELIA, BOYKIN'S	AL DE FL GA NC NJ SC
1	LOBELIA DUNBARIAE	CAMPANULACEAE		HI
5	LOBELIA GATTINGERI	*** SEE ***	LOBELIA APPENDICULATA VAR. GATTINGERI	
1	LOBELIA GAUDICHAUDII VAR. KOOLAUENSIS	CAMPANULACEAE		HI
1	LOBELIA HILLEBRANDII VAR. MONOSTACHYA	CAMPANULACEAE		HI
2	LOBELIA HYPOLEUCA VAR. ROCKII	CAMPANULACEAE		HI
1	LOBELIA NIIHAUENSIS	CAMPANULACEAE		HI
1	LOBELIA OAHUENSIS	CAMPANULACEAE		HI
3A	LOBELIA REMYI	CAMPANULACEAE		HI
1	LOBELIA TORTUOSA	CAMPANULACEAE		HI
3C	LOEFLINGIA SQUARROSA SSP. ARTEMISIARUM	CARYOPHYLLACEAE		CA
2	LOMATIUM ATTENUATUM	APIACEAE		WY
1	LOMATIUM BRADSHAWII	APIACEAE	DESERT-PARSLEY, BRADSHAW	OR
2	LOMATIUM CONCINNUM	APIACEAE		CO
1	LOMATIUM CONGDONII	APIACEAE	LOMATIUM, CONGDON'S	CA
3C	LOMATIUM CUSPIDATUM	APIACEAE		WA
2	LOMATIUM ERYTHROCARPUM	APIACEAE	DESERT-PARSELY, RED-FRUITED	OR
3C	LOMATIUM FOENICULACEUM SSP. INYOENSE	APIACEAE	LOMATIUM, INYO	CA ID
1	LOMATIUM GREENMANII	APIACEAE	DESERT-PARSLEY, GREENMAN'S	OR
3C	LOMATIUM HENDERSONII	APIACEAE		OR WA
3C	LOMATIUM HOWELLII	APIACEAE	LOMATIUM, HOWELL'S	CA OR
2	LOMATIUM LAEVIGATUM	APIACEAE		OR WA
2	LOMATIUM LATILOBUM	APIACEAE		CO UT
3C	LOMATIUM MINIMUM	APIACEAE		UT
3C	LOMATIUM MINUS	APIACEAE	DESERT-PARSLEY, DAY VALLEY	OR
2	LOMATIUM NELSONIANUM	APIACEAE		OR
2	LOMATIUM OREGANUM	APIACEAE		OR
5	LOMATIUM OROGENIOIDES	*** SEE ***	TAUSCHIA TENUISSIMA	
2	LOMATIUM PECKIANUM	APIACEAE	LOMATIUM, PECK'S	CA OR
3C	LOMATIUM QUINTUPLEX	APIACEAE		WA
3C	LOMATIUM RAVENII	APIACEAE	DESERT-PARSLEY, LASSEN	CA ID NV OR UT
3C	LOMATIUM RIGIDUM	APIACEAE	LOMATIUM, BIG PINE	CA
2	LOMATIUM ROLLINSII	APIACEAE		ID OR WA
3C	LOMATIUM SERPENTINUM	APIACEAE		ID OR WA
2	LOMATIUM STEBBINSII	APIACEAE		CA
2	LOMATIUM SUKSDORFII	APIACEAE	DESERT-PARSLEY, SUKSDORF'S	OR WA
3C	LOMATIUM THOMPSONII	APIACEAE		WA
2	LOMATIUM TUBEROSUM	APIACEAE	DESERT-PARSLEY, HOOVER'S	WA
2	LOTUS ARGOPHYLLUS SSP. ADSURGENS	FABACEAE	HOSACKIA, SILVER, SAN CLEMENTE IS.	CA
2	LOTUS ARGOPHYLLUS SSP. NIVEUS	FABACEAE	HOSACKIA, SILVER, SANTA CRUZ ISLAND	CA, Mexico
LE	LOTUS DENDROIDEUS SSP. TRASKIAE	FABACEAE	BROOM, SAN CLEMENTE ISLAND	CA
3C	LOTUS NUTTALLIANUS	FABACEAE	HOSACKIA, PROSTRATE	CA, Mexico
5	LOTUS SCOPARIUS SSP. TRASKIAE	*** SEE ***	LOTUS DENDROIDEUS SSP. TRASKIAE	
1	LUINA SERPENTINA	ASTERACEAE		OR
5	LUPINUS ABORTIVUS	*** SEE ***	LUPINUS CUSICKII SSP. ABORTIVUS	
2	LUPINUS ANTONINUS	FABACEAE		CA
2	LUPINUS ARBOREUS VAR. EXIMIUS	FABACEAE	TREE LUPINE, SAN MATEO	CA
1	LUPINUS ARIDORUM	FABACEAE		FL
2	LUPINUS ARIDUS SSP. ASHLANDENSIS	FABACEAE		OR
5	LUPINUS ARIDUS VAR. ABORTIVUS	*** SEE ***	LUPINUS CUSICKII SSP. ABORTIVUS	
2	LUPINUS BIDDLEI	FABACEAE		OR
3C	LUPINUS BURKEI SSP. CAERULEOMONTANUS	FABACEAE		OR
3C	LUPINUS CERVINUS	FABACEAE	LUPINE, SANTA LUCIA	CA
2	LUPINUS CITRINUS	FABACEAE		CA
5	LUPINUS CITRINUS VAR. DEFLEXUS	*** SEE ***	LUPINUS DEFLEXUS	
2	LUPINUS CONSTANCEI	FABACEAE	LUPINE, LASSICS	CA
2	LUPINUS CRASSUS	FABACEAE		CO
2	LUPINUS CULBERTSONII SSP. CULBERTSONII	FABACEAE		CA
2	LUPINUS CUSICKII	FABACEAE		OR
3C	LUPINUS CUTLERI	FABACEAE		AZ
2	LUPINUS DALESIAE	FABACEAE		CA
5	LUPINUS DEDECKERAE	*** SEE ***	LUPINUS PADRE-CROWLEYI	
2	LUPINUS DEFLEXUS	FABACEAE	LUPINE, MARIPOSA	CA
2	LUPINUS DURANII	FABACEAE		CA
2	LUPINUS EXCUBITUS VAR. MEDIUS	FABACEAE		CA
5	LUPINUS EXIMIUS	*** SEE ***	LUPINUS ARBOREUS VAR. EXIMIUS	
2	LUPINUS GUADALUPENSIS	FABACEAE	LUPINE, GUADALUPE ISLAND	CA, Mexico
3C	LUPINUS HOLMGRENIANUS	FABACEAE	LUPINE, HOLMGREN	CA NV
5	LUPINUS HUMBOLDTIENSIS /SP. NOV. INED.	*** SEE ***	LUPINUS CONSTANCEI	
3C	LUPINUS INYOENSIS	FABACEAE		CA
3C	LUPINUS JONESII	FABACEAE		UT
2	LUPINUS LUDOVICIANUS	FABACEAE	LUPINE, SAN LUIS	CA
2	LUPINUS MAGNIFICUS VAR. MAGNIFICUS	FABACEAE	LUPINE, PANAMINT MOUNTAINS	CA
3C	LUPINUS MALACOPHYLLUS	FABACEAE		NV
5	LUPINUS MARIANUS	*** SEE ***	LUPINUS SERVICEUS SSP. MARIANUS	

STATUS		SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
2		LUPINUS MILO-BAKERI	FABACEAE	LUPINE, MILO BAKER	CA
3C		LUPINUS MONTIGENUS VAR. MONTIGENUS	FABACEAE		CA NV
3B		LUPINUS MUCRONULATUS	FABACEAE		OR
2		LUPINUS NIPOMENSIS	FABACEAE	LUPINE, NIPOMO MESA	CA
2		LUPINUS PADRE-CROWLEYI	FABACEAE	LUPINE, DEDECKER'S	CA
3C		LUPINUS PEIRSONII	FABACEAE		CA
3C		LUPINUS SABINII	FABACEAE		OR WA
3C		LUPINUS SERICATUS	FABACEAE	LUPINE, COBB MOUNTAIN	CA
3C		LUPINUS SERVICEUS SSP. MARIANUS	FABACEAE		UT
2		LUPINUS SPECTABILIS	FABACEAE	LUPINE, SHAGGY HAIR	CA
2		LUPINUS TIDESTROMII VAR. LAYNEAE	FABACEAE	LUPINE, POINT REYES	CA
1		LUPINUS TIDESTROMII VAR. TIDESTROMII	FABACEAE	LUPINE, TIDESTROM	CA
3C		LUPINUS TRACYI	FABACEAE	LUPINE, TRACY'S	CA OR
3C		LUPINUS WESTIANUS	FABACEAE	LUPINE, GULFCOAST	FL
3C		LUZULA HAWAIIENSIS VAR. OAHUENSIS	JUNCACEAE		HI
2		LYCIUM BERBERIOIDES	SOLANACEAE		TX
1	*	LYCIUM HASSEI	SOLANACEAE	DESERT-THORN, SANTA CATALINA	CA
3C		LYCIUM TEXANUM	SOLANACEAE		TX
1	*	LYCIUM VERRUCOSUM	SOLANACEAE	DESERT-THORN, SAN NICOLAS	CA, Mexico
2		LYCOPODIUM HALEAKALAE	LYCOPODIACEAE		HI
1		LYCOPODIUM MANNII	LYCOPODIACEAE		HI
2		LYCOPODIUM NUTANS	LYCOPODIACEAE		HI
3B		LYCOPODIUM PORTORICENSE	LYCOPODIACEAE	CLUBMOSS,	PR
2		LYGODESMIA DOLORESENSIS	ASTERACEAE		CO
2		LYGODESMIA ENTRADA	ASTERACEAE	SKELETONPLANT, ENTRADA	CO UT
3C		LYGODESMIA GRANDIFLORA VAR. STRICTA	ASTERACEAE		UT
2		LYONOTHAMNUS FLORIBUNDUS SSP. ASPLENIFOLIUS	ROSACEAE	IRONWOOD, FERN-LEAVED	CA
2		LYONOTHAMNUS FLORIBUNDUS SSP. FLORIBUNDUS	ROSACEAE	IRONWOOD, CATALINA	CA
3C		LYSILOMA MICROPHYLLA VAR. THORNBERI	FABACEAE		AZ
1		LYSIMACHIA ASPERULAEFOLIA	PRIMULACEAE		NC SC
S		LYSIMACHIA CILIATA	*** SEE ***	STEIRONEMA LAEVIGATUM	
2		LYSIMACHIA FILIFOLIA	PRIMULACEAE		HI
1		LYSIMACHIA HILLEBRANDII VAR. HILLEBRANDII	PRIMULACEAE	PUA-HEKILI	HI
1		LYSIMACHIA KALALAUENSIS	PRIMULACEAE		HI
2		LYSIMACHIA OVATA	PRIMULACEAE		HI
2		LYSIMACHIA SP. /SP. NOV. INED.	PRIMULACEAE	(WAIHOI VALLEY, MAUI CO.)	HI
2		LYTHRUM CURTISSII	LYTHRACEAE		FL GA
2		LYTHRUM FLAGELLARE	LYTHRACEAE		FL
3C		LYTHRUM OVALIFOLIUM	LYTHRACEAE		TX
2		MACBRIDEA ALBA	LAMIACEAE	BIRDS-IN-A-NEST, WHITE	FL
3B		MACHAERANTHERA AMMOPHILA	ASTERACEAE		CA NV
3B		MACHAERANTHERA ARIZONICA	ASTERACEAE		AZ
3C		MACHAERANTHERA AUREA	ASTERACEAE	MACHAERANTHERA, HOUSTON	TX
3C		MACHAERANTHERA CANESCENS VAR. ZIEGLERI	ASTERACEAE		CA
S		MACHAERANTHERA COGNATA	*** SEE ***	XYLORHIZA COGNATA	
S		MACHAERANTHERA GLABRIUSCULA VAR. CONFERTIFOLIA	*** SEE ***	XYLORHIZA CONFERTIFOLIA	
3C		MACHAERANTHERA GRINDELIOIDES VAR. DEPRESSA	ASTERACEAE		AZ NV UT
3C		MACHAERANTHERA KINGII	ASTERACEAE		UT
2		MACHAERANTHERA LAGUNENSIS	ASTERACEAE	ASTER, LAGUNA MOUNTAINS	CA
3C		MACHAERANTHERA LEUCANTHEMIFOLIA	ASTERACEAE		CA NV
3C		MACHAERANTHERA MUCRONATA	ASTERACEAE		AZ
S		MACHAERANTHERA ORCUTTII	*** SEE ***	XYLORHIZA ORCUTTII	
2		MADIA HALLII	ASTERACEAE		CA
3C		MADIA STEBBINSII	ASTERACEAE		CA
3C		MAGNOLIA ASHEI	MAGNOLIACEAE	MAGNOLIA, ASHE'S	FL
2		MAHONIA NERVOSA VAR. MENDOCINENSIS	BERBERIDACEAE	BARBERRY, MENDOCINO	CA
1		MAHONIA NEVINII	BERBERIDACEAE	BARBERRY, NEVIN'S	CA
2		MAHONIA PINNATA SSP. INSULARIS	BERBERIDACEAE	BARBERRY, ISLAND	CA
LE		MAHONIA SONNEI	BERBERIDACEAE	BARBERRY, TRUCKEE	CA
1	*	MALACOTHAMNUS ABBOTTII	MALVACEAE	BUSH-MALLOW, ABBOTT'S	CA
LE		MALACOTHAMNUS CLEMENTINUS	MALVACEAE	BUSH-MALLOW, SAN CLEMENTE ISLAND	CA
2		MALACOTHAMNUS FASCICULATUS VAR. NESIOTICUS	MALVACEAE	BUSH-MALLOW, SANTA CRUZ ISLAND	CA
1	*	MALACOTHAMNUS MENDOCINENSIS	MALVACEAE	BUSH-MALLOW, MENDOCINO	CA
2		MALACOTHAMNUS PALMERI VAR. INVOLUCRATUS	MALVACEAE	BUSH-MALLOW, CARMEL VALLEY	CA
2		MALACOTHAMNUS PALMERI VAR. LUCIANUS	MALVACEAE	BUSH-MALLOW, ARROYO SECO	CA
3C		MALACOTHAMNUS PALMERI VAR. PALMERI	MALVACEAE	BUSH-MALLOW, PALMER	CA
2		MALACOTHAMNUS PARISHII	MALVACEAE	BUSH-MALLOW, PARISH'S	CA
2		MALACOTHRIX SAXATILIS VAR. ARACHNOIDEA	ASTERACEAE	MALACOTHRIX, CARMEL VALLEY	CA
1		MALPIGHIA INFESTISSIMA	MALPIGHIACEAE	STINGINGBUSH	VI
S		MALPIGHIA PALLENS	*** SEE ***	MALPIGHIA INFESTISSIMA	
S		MAMMILLARIA LEEI	*** SEE ***	CORYPHANTHA SNEEDII VAR. LEEI	
S		MAMMILLARIA NELLIEAE	*** SEE ***	CORYPHANTHA MINIMA	
3C		MAMMILLARIA ORESTERA	CACTACEAE		AZ
S		MAMMILLARIA SNEEDII	*** SEE ***	CORYPHANTHA SNEEDII VAR. SNEEDII	
PT		MAMMILLARIA THORNBERI	CACTACEAE		AZ, Mexico
S		MAMMILLARIA TOBUSCHII	*** SEE ***	ANCISTROCACTUS TOBUSCHII	
3C		MANIHOT DAVISIAE	EUPHORBIACEAE		AZ

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
1	MANIHOT WALKERAE	EUPHORBIACEAE		TX, Mexico
5	MANISURIS TUBERCULOSA	*** SEE ***	COELORACHIS TUBERCULOSA	
2	MARGARANTHUS LEMMONII	SOLANACEAE		AZ
2	MARINA ORCUTTII VAR. ORCUTTII	FABACEAE		CA
1	MARISCUS URBANII	CYPERACEAE		PR
1	MARLIEREA SINTENISII	MYRTACEAE	MURTA	PR
1	MARSDENIA ELLIPTICA	ASCLEPIADACEAE		PR
2	MARSHALLIA GRANDIFLORA	ASTERACEAE	BARBARA'S BUTTONS, LARGE-FLOWERED	KY MD NC PA TN WV
2	MARSHALLIA MOHRII	ASTERACEAE	BARBARA'S BUTTONS,	AL GA
3C	MARSHALLIA RAMOSA	ASTERACEAE		FL GA
1	MARSILEA VILLOSA	MARSILEACEAE		HI
2	MATELEA ALABAMENSIS	ASCLEPIADACEAE	ANGLEPOD,	AL FL GA
3C	MATELEA BREVICORONATA	ASCLEPIADACEAE		TX
3C	MATELEA EDWARDSENSIS	ASCLEPIADACEAE	MILKVINE, PLATEAU	TX
2	MATELEA FLORIDANA	ASCLEPIADACEAE		FL
3C	MATELEA PARVIFLORA	ASCLEPIADACEAE		TX
2	MATELEA RADIATA	ASCLEPIADACEAE	ANGLEPOD (MILKVINE), FALFURRIAS	TX
2	MATELEA TEXENSIS	ASCLEPIADACEAE		TX
2	MAURANDYA PETROPHILA	SCROPHULARIACEAE	ROCK LADY	CA
2	MAYTENUS CYMOSA	CELASTRACEAE		PR VI, British V.I.
2	MAYTENUS ELONGATA	CELASTRACEAE	CUERO DE SAPO	PR
1	MAYTENUS PONCEANA	CELASTRACEAE	CUERO DE SAPO	PR
2	MELANTHERA PARVIFOLIA	ASTERACEAE		FL
2	MENTZELIA ARGILLOSA	LOASACEAE	BLAZING STAR, CLAY	CO UT
2	MENTZELIA DENSA	LOASACEAE		CO
3C	MENTZELIA HIRSUTISSIMA VAR. STENOPHYLLA	LOASACEAE	STICKLEAF, HAIRY	CA
LT	MENTZELIA LEUCOPHYLLA	LOASACEAE	BLAZING STAR, ASH MEADOWS	NV
2	MENTZELIA MOLLIS	LOASACEAE	STICKLEAF, SMOOTH	ID NV OR
3A	MENTZELIA NITENS VAR. LEPTOCAULIS	LOASACEAE	STICKLEAF,	AZ
2	MENTZELIA PACKARDIAE	LOASACEAE	STICKLEAF, PACKARD'S	OR
2	MERTENSIA DRUMMONDII	BORAGINACEAE	BLUEBELL, DRUMMOND	AK, Canada (N.W.T., Yukon)
5	MERTENSIA LANCEOLATA VAR. DRUMMONDII	*** SEE ***	MERTENSIA DRUMMONDII	
3C	MERTENSIA TOYABENSIS	BORAGINACEAE	BLUEBELLS,	NV
3C	MERTENSIA VIRIDIS VAR. CANA	BORAGINACEAE	BLUEBELLS, CANESCENT	CO UT
3C	MERTENSIA VIRIDIS VAR. DILATATA	BORAGINACEAE	BLUEBELLS, SMOOTH-LEAF	CO UT WY
2	MESADENUS PORTORICENSIS	ORCHIDACEAE		PR
1	METROSIDEROS COLLINA VAR. NEWELLII	MYRTACEAE		HI
PE	MEZONEURON KAVAIENSE	FABACEAE	UHIUHI	HI
5	MICONIA OTTOSCHULZII	*** SEE ***	GRAFFENRIEDA OTTOSCHULZII	
1	* MICRANTHEMUM MICRANTHEMOIDES	SCROPHULARIACEAE	MICRANTHEMUM, NUTTALL'S	DE DC MD NJ NY PA VA
2	MICROSERIS DECIPIENS	ASTERACEAE	MICROSERIS, SANTA CRUZ	CA
3C	MICROSERIS DETLINGII	ASTERACEAE		OR
2	MICROSERIS HOWELLII	ASTERACEAE		OR
5	MICROSERIS LACINIATA SSP. DETLINGII	*** SEE ***	MICROSERIS DETLINGII /SP. NOV. INED.	
5	MICROSERIS LACINIATA SSP. SISKIYOUENSIS	*** SEE ***	MICROSERIS NUTANS SSP. SISKIYOUENSIS /INED.	
3C	MICROSERIS NUTANS SSP. SISKIYOUENSIS /INED.	ASTERACEAE		CA OR
1	MIKANIA STEVENSIANA	ASTERACEAE	GUACO	PR
5	MIMULUS ARIDUS	*** SEE ***	DIPLACUS ARIDUS	
1	* MIMULUS BRANDEGEI	SCROPHULARIACEAE	MONKEYFLOWER, SANTA CRUZ ISLAND	CA
2	MIMULUS EXIGUUS	SCROPHULARIACEAE	MONKEYFLOWER, MEAN	CA
2	MIMULUS GEMMIPARUS	SCROPHULARIACEAE		CO
2	MIMULUS GLABRATUS VAR. MICHIGANENSIS	SCROPHULARIACEAE	MONKEYFLOWER,	MI
3B	MIMULUS GUTTATUS SSP. ARENICOLA	SCROPHULARIACEAE	MONKEYFLOWER,	CA
3C	MIMULUS JUNGERMANNIOIDES	SCROPHULARIACEAE		OR WA
2	MIMULUS PATULUS	SCROPHULARIACEAE	MONKEY FLOWER, STALK-LEAVED	ID OR WA
3C	MIMULUS PICTUS	SCROPHULARIACEAE	MONKEYFLOWER, CALICO	CA
2	MIMULUS PURPUREUS VAR. FURPUREUS	SCROPHULARIACEAE	MONKEYFLOWER, PURFLE	CA
2	MIMULUS PYGMAEUS	SCROPHULARIACEAE	MONKEYFLOWER, PYGMY	CA OR
2	MIMULUS RINGENS VAR. COLPOPHILUS	SCROPHULARIACEAE	MONKEYFLOWER, SQUARE-STEMMED	ME, Canada.
2	MIMULUS RUPICOLA	SCROPHULARIACEAE	MONKEYFLOWER, DEATH VALLEY	CA
2	MIMULUS SP./SP. NOV. INED. (KERN CO.)	SCROPHULARIACEAE		CA
2	MIMULUS SP./SP. NOV. INED. (TULARE CO.)	SCROPHULARIACEAE		CA
1	* MIMULUS TRASKIAE	SCROPHULARIACEAE	MONKEYFLOWER, SANTA CATALINA	CA
3B	MIMULUS WASHOENSIS	SCROPHULARIACEAE		NV
1	* MIMULUS WHIPPLEI	SCROPHULARIACEAE	MONKEYFLOWER, WHIPPLE'S	CA
2	MINUARTIA DECUMBENS	CARYOPHYLLACEAE	SANDWORT, LASSICS	CA
2	MINUARTIA GODFREYI	CARYOPHYLLACEAE		AL AR FL NC SC
3C	MINUARTIA MARCESCENS	CARYOPHYLLACEAE		VT, Canada
2	MINUARTIA ROSEI	CARYOPHYLLACEAE	SANDWORT, PEANUT	CA
3C	MINUARTIA UNIFLORA	CARYOPHYLLACEAE		AL GA NC SC
LE	MIRABILIS MACFARLANEI	NYCTAGINACEAE	FOUR-O'CLOCK, MACFARLANE'S	ID OR
3C	MIRABILIS PUDICA	NYCTAGINACEAE		NV
2	MIRABILIS ROTUNDIFOLIA	NYCTAGINACEAE		CO
2	MITRACARPUS MAXWELLIAE	RUBIACEAE		PR
1	MITRACARPUS POLYCLADUS	RUBIACEAE		PR
3C	MONARDA STIPITATOGLANDULOSA	LAMIACEAE	HORSE-MINT,	AR OK
3C	MONARDELLA ANTONINA	LAMIACEAE		CA
3C	MONARDELLA BENITENSIS	LAMIACEAE	MONARDELLA, SAN BENITO	CA
2	MONARDELLA CRISPA	LAMIACEAE	MONARDELLA, CRISP	CA
2	MONARDELLA DIABOLI /SP. NOV. INED.	LAMIACEAE		CA

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
2	MONARDELLA DOUGLASSII VAR. VENOSA	LAMIACEAE		CA
2	MONARDELLA HYPOLEUCA SSP. LANATA	LAMIACEAE	MONARDELLA, FELT LEAF	CA
1	* MONARDELLA LEUCOCEPHALA	LAMIACEAE	MONARDELLA, MERCED	CA
2	MONARDELLA LINOIDES SSP. OBLONGA	LAMIACEAE		CA
2	MONARDELLA LINOIDES SSP. VIMINEA	LAMIACEAE	MONARDELLA, WILLOWY	CA
3C	MONARDELLA MACRANTHA SSP. HALLII	LAMIACEAE	MONARDELLA, HALL'S	CA
2	MONARDELLA NANA SSP. LEPTOSIPHON	LAMIACEAE	MONARDELLA, SAN FELIPE	CA
3C	MONARDELLA PALMERI	LAMIACEAE		CA
1	* MONARDELLA PRINGLEI	LAMIACEAE	MONARDELLA, PRINGLE	CA
3C	MONARDELLA PURPUREA	LAMIACEAE		CA OR
2	MONARDELLA ROBISONII	LAMIACEAE	MONARDELLA, ROBISON	CA
2	MONARDELLA SCELERATA /SP. NOV. INED.	LAMIACEAE	MONARDELLA, STONE CORRAL CANYON	CA
2	MONARDELLA STEBBINSII SP. NOV. /INED.	LAMIACEAE	MONARDELLA, STEBBINS'	CA
2	MONARDELLA UNDULATA VAR. FRUTESCENS	LAMIACEAE	MONARDELLA, CURLY-LEAVED, SAN LUIS O	CA
3C	MONARDELLA VIRIDIS SSP. SAXICOLA	LAMIACEAE		CA
2	MONOTROPA BRITTONII	ERICACEAE		FL
5	MONOTROPA CALIFORNICUS	*** SEE ***	PITYOPUS CALIFORNICUS	
2	MONOTROPSIS REYNOLDSIAE	ERICACEAE	PINESAP, SWEET	FL
2	MONTIA BOSTOCKII	PORTULACACEAE		AK, Canada (Yukon)
3C	MORINDA SANDWICENSIS	RUBIACEAE		HI
1	MORINDA TRIMERA	RUBIACEAE	NONI-KUAHIWI	HI
3B	MUHLENBERGIA CURTISETOSA	POACEAE	MUHLY,	IL MO OH PA
3B	MUHLENBERGIA PILOSA	POACEAE		TX
5	MUHLENBERGIA SCHREBERI VAR. CURTISETOSA	*** SEE ***	MUHLENBERGIA CURTISETOSA	
1	MUHLENBERGIA TORREYANA	POACEAE	MUHLY, TORREY'S	DE GA MD NJ NY TN
3B	MUHLENBERGIA VILLOSA	POACEAE	MUHLY, VILLOUS	NM TX
2	MUILLA CLEVELANDII	LILIACEAE	GOLDENSTAR, SAN DIEGO	CA, Mexico (Baja California)
3B	MUILLA CORONATA	LILIACEAE		CA
1	MUNROIDENDRON RACEMOSUM	ARALIACEAE		HI
2	MUNZOTHAMNUS BLAIRII	ASTERACEAE		CA
2	MUSINEON LINEARE	APIACEAE		UT
2	MYOPORUM SANDWICENSE VAR. STELLATUM	MYOPORACEAE	NAIO	HI
2	MYOSURUS MINIMUS SSP. APUS	RANUNCULACEAE		CA OR
1	MYRCIA PAGANII	MYRTACEAE	AUSU	PR
2	MYRCIANTHES FRAGRANS VAR. SIMPSONII	MYRTACEAE		FL
3C	MYRICA HARTWEGII	MYRICACEAE	SWEET-BAY, SIERRA	CA
2	MYRIOPHYLLUM LAXUM	HALORAGACEAE		AL FL GA NC SC
1	MYRSINE FERNSEEI	MYRSINACEAE	KOLEA	HI
1	MYRSINE LANAIENSIS VAR. OAHUENSIS	MYRSINACEAE		HI
1	MYRSINE LINEARIFOLIA VAR. LINEARIFOLIA	MYRSINACEAE		HI
1	MYRSINE MEZII	MYRSINACEAE		HI
2	MYRSINE PETIOLATA	MYRSINACEAE		HI
1	MYRSINE ST-JOHNII	MYRSINACEAE		HI
2	* NAJAS CAESPITOSA	NAJADACEAE	NAIAD, FISH LAKE	UT
5	NAJAS FLEXILIS SSP. CAESPITOSA	*** SEE ***	NAJAS CAESPITOSA	
3C	NAMA RETRORSUM	HYDROPHYLLACEAE		AZ UT
3C	NAMA XYLOPODUM	HYDROPHYLLACEAE		NM TX
2	NAPAEA DIOICA	MALVACEAE		IL IN OH MN WI
2	NARTHECIUM AMERICANUM	LILIACEAE		DE NJ NY NC SC
5	NARTHECIUM OSSIFRAGUM VAR. AMERICANUM	*** SEE ***	NARTHECIUM AMERICANUM	
2	NASTURTIUM GAMBELII	BRASSICACEAE	WATERCRESS, GAMBEL'S	CA
5	NAVAJOA PEEBLESIANUS	*** SEE ***	PEDIOCACTUS PEEBLESIANUS VAR. PEEBLESIANUS	
2	NAVARRETIA FOSSALIS	POLEMONIACEAE	NAVARRETIA, NO-NAMED	CA, Mexico (Baja California)
2	NAVARRETIA PAUCIFLORA	POLEMONIACEAE	NAVARRETIA, FEW-FLOWERED	CA
2	NAVARRETIA PLIEANTHA	POLEMONIACEAE	NAVARRETIA, MANY-FLOWERED	CA
3C	NAVARRETIA PROLIFERA SSP. LUTEA	POLEMONIACEAE	NAVARRETIA, YELLOW-BUR	CA
2	NAVARRETIA SETILOBA	POLEMONIACEAE	NAVARRETIA, COVILLE'S	CA
2	NEMACLADUS TWISSELMANNII	CAMPANULACEAE	NEMACLADUS, TWISSELMANN'S	CA
2	NEMASTYLIS FLORIDANA	IRIDACEAE	IXIA, FALL-FLOWERING	FL
5	NEMOPANTHUS COLLINUS	*** SEE ***	ILEX COLLINA	
1	NEOLLOYDIA ERECTOCENTRA VAR. ACUNENSIS	CACTACEAE		AZ, Mexico
2	NEOLLOYDIA ERECTOCENTRA VAR. ERECTOCENTRA	CACTACEAE		AZ
2	NEOLLOYDIA GAUTII	CACTACEAE		TX
LT	NEOLLOYDIA MARIPOSENSIS	CACTACEAE	CACTUS, MARIPOSA, LLOYD'S	TX, Mexico (Coahuila)
3C	NEOLLOYDIA WARNOCKII	CACTACEAE		TX
2	NEOPARRYA LITHOPHILA	APIACEAE		CO
2	NEOSTAPFIA COLUSANA	POACEAE	GRASS, COLUSA	CA
1	NEOWAWRAEA PHYLLANTHOIDES	EUPHORBIACEAE	MEHAMEHAME	HI
2	NEPHROPETALUM PRINGLEI	STERCULIACEAE	KIDNEYPETAL, PRINGLE'S	TX, Mexico
1	NERAUDIA ANGULATA	URTICACEAE		HI
3A	NERAUDIA COOKII	URTICACEAE		HI
3A	NERAUDIA KAHOOLAWENSIS	URTICACEAE		HI
1	NERAUDIA KAUAIENSIS	URTICACEAE		HI
2	NERAUDIA MELASTOMIFOLIA	URTICACEAE	MA'OLOA	HI
1	NERAUDIA OVATA	URTICACEAE	MA'OLOA, BIG ISLAND	HI
1	NERAUDIA SERICEA	URTICACEAE		HI
5	NESAEA LONGIPES	*** SEE ***	HEIMIA LONGIPES	
2	NESTRONIA UMBELLULA	SANTALACEAE		AL GA MS NC SC TN VA
2	NEVIUSIA ALABAMENSIS	ROSACEAE		AL AR MS MO TN

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
LE	NITROPHILA MOHAVENSIS	CHENOPODIACEAE	NITERWORT, AMARGOSA	CA
2	NOLINA ARENICOLA	LILIACEAE		TX
2	NOLINA ATOPOCARPA	LILIACEAE	BEAR-GRASS,	FL
2	NOLINA BRITTONIANA	LILIACEAE	BEAR-GRASS,	FL
1	NOLINA INTERRATA	LILIACEAE	BEAR-GRASS, DEHESA (SAN DIEGO)	CA, Mexico
1	NOTHOCESTRUM BREVIFLORUM	SOLANACEAE		HI
1	NOTHOCESTRUM LATIFOLIUM	SOLANACEAE	'AIEA,	HI
1	NOTHOCESTRUM LONGIFOLIUM VAR. RUFIPILOSUM	SOLANACEAE	'AIEA,	HI
1	NOTHOCESTRUM PELTATUM	SOLANACEAE	'AIEA,	HI
1	NOTHOCESTRUM SUBCORDATUM	SOLANACEAE	'AIEA,	HI
2	NOTHOLAENA LEMMONII	POLYPODIACEAE		AZ, Mexico
3C	NOTHOLAENA SCHAFFNERI VAR. NEALLEYI	POLYPODIACEAE		TX
1	NOTOTRICHIUM HUMILE	AMARANTHACEAE		HI
3B	NOTOTRICHIUM SANDWICENSE VAR. DECIPIENS	AMARANTHACEAE		HI
3B	NOTOTRICHIUM SANDWICENSE VAR. DUBIUM	AMARANTHACEAE		HI
3B	NOTOTRICHIUM SANDWICENSE VAR. FORBESII	AMARANTHACEAE		HI
3B	NOTOTRICHIUM SANDWICENSE VAR. HELLERI	AMARANTHACEAE		HI
3B	NOTOTRICHIUM SANDWICENSE VAR. KOLEKOLENSE	AMARANTHACEAE		HI
3B	NOTOTRICHIUM SANDWICENSE VAR. LANAIENSE	AMARANTHACEAE		HI
3B	NOTOTRICHIUM SANDWICENSE VAR. LANCEOLATUM	AMARANTHACEAE		HI
3B	NOTOTRICHIUM SANDWICENSE VAR. LATIFOLIUM	AMARANTHACEAE		HI
3B	NOTOTRICHIUM SANDWICENSE VAR. LEPTOPODUM	AMARANTHACEAE		HI
3B	NOTOTRICHIUM SANDWICENSE VAR. LONGESPICATUM	AMARANTHACEAE		HI
3B	NOTOTRICHIUM SANDWICENSE VAR. MACROPHYLLUM	AMARANTHACEAE		HI
3B	NOTOTRICHIUM SANDWICENSE VAR. MAUIENSE	AMARANTHACEAE		HI
3B	NOTOTRICHIUM SANDWICENSE VAR. NIIHAUENSE	AMARANTHACEAE		HI
3B	NOTOTRICHIUM SANDWICENSE VAR. OLOKELEANUM	AMARANTHACEAE		HI
3B	NOTOTRICHIUM SANDWICENSE VAR. PULCHELLOIDES	AMARANTHACEAE		HI
3B	NOTOTRICHIUM SANDWICENSE VAR. PULCHELLUM	AMARANTHACEAE		HI
3B	NOTOTRICHIUM SANDWICENSE VAR. SUBCORDATUM	AMARANTHACEAE		HI
3B	NOTOTRICHIUM SANDWICENSE VAR. SYRINGIFOLIUM	AMARANTHACEAE		HI
3B	NOTOTRICHIUM VIRIDE	AMARANTHACEAE		HI
2	NUPHAR LUTEUM SSP. ULVACEUM	NYMPHAEACEAE		FL
3C	OCHROSIA COMPTA	APOCYNACEAE		HI
2	OCHROSIA KILAUEAENSIS	APOCYNACEAE	HOLEI	HI
2	OENOTHERA ACUTISSIMA	ONAGRACEAE	EVENING-PRIMROSE,	CO UT
LE	OENOTHERA AVITA SSP. EUREKENSIS	ONAGRACEAE	EVENING-PRIMROSE, EUREKA VALLEY	CA
5	OENOTHERA CAESPITOSA VAR. PSAMMOPHILA	*** SEE ***	OENOTHERA PSAMMOPHILA	
LE	OENOTHERA DELTOIDES SSP. HOWELLII	ONAGRACEAE	EVENING-PRIMROSE, ANTIOCH DUNES	CA
5	OENOTHERA GOULDII	*** SEE ***	CAMISSONIA GOULDII	
5	OENOTHERA HOOKERI SSP. WOLFII	*** SEE ***	OENOTHERA WOLFII	
2	* OENOTHERA KLEINII	ONAGRACEAE	EVENING-PRIMROSE, KLEIN'S	CO
5	OENOTHERA MEGALANTHA	*** SEE ***	CAMISSONIA MEGALANTHA	
2	OENOTHERA ORGANENSIS	ONAGRACEAE		NM
1	OENOTHERA PILOSELLA SSP. SESSILIS	ONAGRACEAE	EVENING-PRIMROSE,	AR LA TX
2	OENOTHERA PSAMMOPHILA	ONAGRACEAE	EVENING-PRIMROSE,	ID
5	OENOTHERA SESSILIS	*** SEE ***	OENOTHERA PILOSELLA SSP. SESSILIS	
2	OENOTHERA WOLFII	ONAGRACEAE	EVENING-PRIMROSE, WOLF'S	CA OR
2	ONCIDIUM CARTHAGENENSE	ORCHIDACEAE	DANCING-LADY, COOT BAY	FL, Mexico, Central America, South America
3C	ONOSMODIUM HELLERI	BORAGINACEAE		TX
3C	ONOSMODIUM MOLLE SSP. MOLLE	BORAGINACEAE		AL IL KY TN
5	OONOPSIS MONOCEPHALA	*** SEE ***	HAPLOPAPPUS FREMONTII SSP. MONOCEPHALUS	
2	OPERCULINA TRIQUETRA	CONVOLVULACEAE		VI
5	OPHIOGLOSSUM CALIFORNICUM	*** SEE ***	OPHIOGLOSSUM LUSITANICUM VAR. CALIFORNICUM	
1	OPHIOGLOSSUM CONCINNUM	OPHIOGLOSSACEAE	ADDER'S-TONGUE,	HI
3C	OPHIOGLOSSUM DENDRONEURON	OPHIOGLOSSACEAE		FL, Cuba, Mexico, Philippines, Africa, South America
3C	OPHIOGLOSSUM LUSITANICUM VAR. CALIFORNICUM	OPHIOGLOSSACEAE	ADDER'S-TONGUE, CALIFORNIA	CA, Mexico
3C	OPHIOGLOSSUM PALMATUM	OPHIOGLOSSACEAE	FERN, HAND	FL, West Indies, Central America, South America
2	OPUNTIA ARENARIA	CACTACEAE		NM TX, Mexico
2	OPUNTIA BASILARIS VAR. BRACHYCLADA	CACTACEAE		CA
3B	OPUNTIA BASILARIS VAR. LONGIAREOLATA	CACTACEAE		AZ
1	OPUNTIA BASILARIS VAR. TRELEASEI	CACTACEAE	BEAVERTAIL CACTUS, KERN	CA
3B	OPUNTIA BASILARIS VAR. WOODBURYI	CACTACEAE		UT
2	OPUNTIA BIGELOVII VAR. HOFFMANNII	CACTACEAE		CA
3B	OPUNTIA BORINQUENSIS	CACTACEAE	OLAGA	PR
2	OPUNTIA IMBRICATA VAR. ARGENTEA	CACTACEAE		TX
2	** OPUNTIA LINDHEIMERI VAR. LINGUIFORMIS	CACTACEAE	PRICKLY PEAR, COW-TONGUE	TX
2	OPUNTIA MUNZII	CACTACEAE	CHOLLA, MUNZ	CA

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2	OPUNTIA PARRYI VAR. SERPENTINA	CACTACEAE	CHOLLA, SNAKE	CA, Mexico (Baja California)
3C	OPUNTIA PHAEACANTHA VAR. FLAVISPINA	CACTACEAE		AZ
3B	OPUNTIA PHAEACANTHA VAR. MOJAVENSIS	CACTACEAE		AZ CA
3B	OPUNTIA PHAEACANTHA VAR. SUPERBOSPINA	CACTACEAE		AZ
3C	OPUNTIA PULCHELLA	CACTACEAE	CHOLLA, SAND	AZ NV UT
2	OPUNTIA SPINOSISSIMA	CACTACEAE		FL PR VI, Jamaica, British V.I.
2	OPUNTIA STRIGIL VAR. FLEXOSPINA	CACTACEAE	PRICKLY-PEAR,	TX
2	OPUNTIA TRIACANTHA	CACTACEAE		FL PR VI, Guadeloupe, Lesser Antilles
2	OPUNTIA WHIPPLEI VAR. MULTIGENICULATA	CACTACEAE		AZ NV
2	OPUNTIA WIGGINSII	CACTACEAE	CHOLLA, WIGGINS	AZ CA
5	ORCHIS FLAVA	*** SEE ***	PLATANTHERA FLAVA	
5	ORCHIS INTEGRA	*** SEE ***	PLATANTHERA INTEGRA	
5	ORCHIS LEUCOPHAEA	*** SEE ***	PLATANTHERA LEUCOPHAEA	
1	ORCUTTIA CALIFORNICA	POACEAE	ORCUTT GRASS, CALIFORNIA	CA, Mexico
5	ORCUTTIA CALIFORNICA VAR. INEQUALIS	*** SEE ***	ORCUTTIA INAEQUALIS	
5	ORCUTTIA CALIFORNICA VAR. VISCIDA	*** SEE ***	ORCUTTIA VISCIDA	
5	ORCUTTIA GREENEI	*** SEE ***	TUCTORIA GREENEI	
1	ORCUTTIA INEQUALIS	POACEAE	ORCUTT GRASS, SAN JOAQUIN	CA
LE	ORCUTTIA MUCRONATA	POACEAE	GRASS, SOLANO (CRAMPTON'S ORCUTT)	CA
1	ORCUTTIA PILOSA	POACEAE	ORCUTT GRASS, PILOSE	CA
1	ORCUTTIA TENUIS	POACEAE	ORCUTT GRASS, SLENDER	CA
1	ORCUTTIA VISCIDA	POACEAE	ORCUTT GRASS, SACRAMENTO	CA
2	OREONANA PURPURASCENS	APIACEAE		CA
2	OROBANCHE PARISHII SSP. BRACHYLOBA	OROBANCHACEAE	BROOMRAPE, SHORT-LOBED	CA
2	OROBANCHE VALIDA SSP. VALIDA	OROBANCHACEAE	BROOMRAPE, ROCK CREEK	CA
2	ORTHOCARPUS CAMPESTRIS VAR. SUCCULENTUS	SCROPHULARIACEAE	OWL'S-CLOVER, SUCCULENT	CA
2	ORTHOCARPUS CASTILLEJOIDES VAR. HUMBOLDTIENSIS	SCROPHULARIACEAE	OWL'S-CLOVER, HUMBOLDT	CA
2	ORTHOCARPUS FLORIBUNDUS	SCROPHULARIACEAE	OWL'S-CLOVER, SAN FRANCISCO	CA
2	ORTHOCARPUS LASIORHYNCHUS	SCROPHULARIACEAE	ORTHOCARPUS, SAN BERNARDINO	CA
1	* ORTHOCARPUS PACHYSTACHYUS	SCROPHULARIACEAE	OWL-CLOVER, SHASTA	CA
5	ORTHOCARPUS SUCCULENTUS	*** SEE ***	ORTHOCARPUS CAMPESTRIS VAR. SUCCULENTUS	
2	ORYCTES NEVADENSIS	SOLANACEAE		CA NV
3C	ORYZOPSIS HYMENOIDES VAR. CONTRACTA	POACEAE		CO WY
2	ORYZOPSIS SWALLENII	POACEAE		WY
5	OSMIA BORINQUENSIS	*** SEE ***	EUPATORIUM BORINQUENSE	
2	OSMORHIZA MEXICANA SSP. BIPATRIATA	APIACEAE		TX, Mexico
2	OSTRYA CHISOSENSIS	BETULACEAE		TX
1	OTTOSCHULZIA RHODOXYLON	ICACINACEAE	PALO DE ROSA	PR, Hispaniola
5	OXYBAPHUS ROTUNDIFOLIUS	*** SEE ***	MIRABILIS ROTUNDIFOLIA	
PE	OXYPOLIS CANBYI	APIACEAE	DROPWORT, CANBY'S	DE GA MD NC SC
2	OXYPOLIS GREENMANII	APIACEAE	WATER-DROPWORT, GIANT (GREENMAN'S)	FL
3C	OXYTHECA WATSONII	POLYGONACEAE		NV
3C	OXYTROPIS BESSEYI VAR. OBNAPIFORMIS	FABACEAE		CO UT WY
1	OXYTROPIS CAMPESTRIS VAR. CHARTACEA	FABACEAE		WI
2	OXYTROPIS CAMPESTRIS VAR. JOHANNENSIS	FABACEAE		ME, Canada
2	OXYTROPIS GLABERRIMA	FABACEAE		AK
5	OXYTROPIS JOHANNENSIS	*** SEE ***	OXYTROPIS CAMPESTRIS VAR. JOHANNENSIS	
3C	OXYTROPIS JONESII	FABACEAE		UT
3C	OXYTROPIS KOBUKENSIS	FABACEAE	LOCOWEED, KOBUK	AK
2	OXYTROPIS KOKRINENSIS	FABACEAE	OXYTROPE, KOKRINES	AK
5	OXYTROPIS OBNAPIFORMIS	*** SEE ***	OXYTROPIS BESSEYI VAR. OBNAPIFORMIS	
2	OXYTROPIS SORDIDA SSP. BARNEBYANA	FABACEAE		AK
5	PACHISTIMA CANBYI	*** SEE ***	PAXISTIMA CANBYI	
5	PACHYSTIMA CANBYI	*** SEE ***	PAXISTIMA CANBYI	
2	PALAFOXIA ARIDA VAR. GIGANTEA	ASTERACEAE	SPANISHNEEDLE, GIANT	CA
5	PALAFOXIA LINEARIS VAR. GIGANTEA	*** SEE ***	PALAFOXIA ARIDA VAR. GIGANTEA	
3C	PANAX QUINQUEFOLIUS	ARALIACEAE	GINSENG, AMERICAN	AL AR CT DE DC FL IL IN IA KY LA ME MD MA MI MN MS MO NE NH NJ NY NC OH OK PA RI SC TN VT VA WV WI, Canada
3B	PANICUM ACULEATUM	POACEAE	PANIC GRASS,	DC NY RI VA
1	PANICUM ALAKAIENSE	POACEAE	PANIC GRASS,	HI
LE	PANICUM CARTERI	POACEAE	PANICGRASS, CARTER'S	HI
2	PANICUM FAURIEI	POACEAE	PANIC GRASS, FAURIE'S	HI
2	PANICUM HIRSTII	POACEAE	PANIC GRASS, HIRST'S	GA NJ
3B	PANICUM LAMIATILE	POACEAE	PANIC GRASS,	HI
3B	PANICUM LITHOPHILUM	POACEAE		GA SC
3B	PANICUM LUSTRIALE	POACEAE	PANIC GRASS,	HI
3B	PANICUM MUNDUM	POACEAE	PANIC GRASS, FERNALD'S	VA
2	PANICUM NIIHAUENSE	POACEAE	PANIC GRASS, NIIHAU	HI
2	PANICUM NUDICAULE	POACEAE		AL FL MS
2	PANICUM PINETORUM	POACEAE		FL
3B	PANICUM SHASTENSE	POACEAE	PANIC GRASS, SHASTA	CA
2	PANICUM STEVENSIANUM	POACEAE		PR, Cuba
5	PANICUM THERMALE	*** SEE ***	DICHANTHELIUM LANUGINOSUM VAR. THERMALE	
3C	PAPAVER ALBOROSEUM	PAPAVERACEAE	POPPY, PALE	AK, Canada (B.C., Yukon), U.S.S.R.
3C	PAPAVER WALPOLEI	PAPAVERACEAE	POPPY, WALPOLE	AK, Canada (Yukon), U.S.S.R.

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
2	PARNASSIA CAROLINIANA	SAXIFRAGACEAE		AL FL MS NC SC
3B	PARNASSIA KOTZEBUEI VAR. PUMILA	SAXIFRAGACEAE	GRASS-OF-PARNASSUS, KOTZEBUE'S,	WA
3B	PARONYCHIA ARGYROCOMA VAR. ALBIMONTANA	CARYOPHYLLACEAE	SILVERLING	MA ME NH
2	PARONYCHIA CHARTACEA	CARYOPHYLLACEAE	WHITLOW-WORT,	FL
3C	PARONYCHIA CHORIZANTHOIDES	CARYOPHYLLACEAE		TX
1	PARONYCHIA CONGESTA	CARYOPHYLLACEAE	WHITLOW-WORT,	TX
3C	PARONYCHIA DRUMMONDII SSP. PARVIFLORA	CARYOPHYLLACEAE		TX
2	PARONYCHIA MACCARTII	CARYOPHYLLACEAE	WHITLOW-WORT, MCCART'S	TX
3C	PARONYCHIA MONTICOLA	CARYOPHYLLACEAE		TX
3B	PARONYCHIA NUDATA	CARYOPHYLLACEAE		TX
3B	PARONYCHIA RUGELII VAR. INTERIOR	CARYOPHYLLACEAE	WHITLOW-WORT,	FL GA
2	PARONYCHIA VIRGINICA VAR. PARKSII	CARYOPHYLLACEAE		TX
2	PARONYCHIA VIRGINICA VAR. VIRGINICA	CARYOPHYLLACEAE	NAILWORT, SILVERY	DC MD VA WV
2	PARONYCHIA WILKINSONII	CARYOPHYLLACEAE		TX, Mexico
3C	PARRYA NUDICAULIS	BRASSICACEAE		AK MT, Canada, U.S.S.R.
3C	PARRYA RYDBERGII	BRASSICACEAE	PARRYA, RYDBERG	UT
3C	PARTHENIUM ALPINUM	ASTERACEAE		CO WY
3C	PARTHENIUM LIGULATUM	ASTERACEAE	FEVERFEW,	CO UT WY
2	PARTHENIUM TETRANEURIS	ASTERACEAE		CO
2	PARVISEDUM LEIOCARPUM	CRASSULACEAE	STONECROP, LAKE COUNTY	CA
2	PASSIFLORA BILOBATA	PASSIFLORACEAE		PR, Hispaniola
2	PASSIFLORA MURUCUJA	PASSIFLORACEAE		PR, Hispaniola
2	PAXISTIMA CANBYI	CELASTRACEAE		KY OH PA TN VA WV
2	PECTIS IMBERBIS	ASTERACEAE		AZ
3C	PECTIS RUSBYI	ASTERACEAE	FETID-MARIGOLD, RUSBY'S	AZ
3C	PEDICULARIS DUDLEYI	SCROPHULARIACEAE	LOUSEWORT, DUDLEY'S	CA
LE	PEDICULARIS FURBISHIAE	SCROPHULARIACEAE	LOUSEWORT, FURBISH	ME, Canada (N.B.)
3C	PEDICULARIS HOWELLII	SCROPHULARIACEAE	LOUSEWORT, HOWELL	CA OR
3C	PEDICULARIS RAINIERENSIS	SCROPHULARIACEAE		WA
LE	PEDIOCACTUS BRADYI	CACTACEAE	PINCUSHION CACTUS, BRADY	AZ
1	PEDIOCACTUS DESPAINII	CACTACEAE		UT
5	PEDIOCACTUS GLAUCUS	*** SEE ***	SCLEROCACTUS GLAUCUS	
LE	PEDIOCACTUS KNOWLTONII	CACTACEAE	CACTUS, KNOWLTON	CO NM
5	PEDIOCACTUS MESAE-VERDAE	*** SEE ***	SCLEROCACTUS MESAE-VERDAE	
2	PEDIOCACTUS PAPYRACANTHUS	CACTACEAE		AZ NM
1	PEDIOCACTUS PARADINEI	CACTACEAE		AZ
1	PEDIOCACTUS PEEBLESIANUS VAR. FICKEISENIAE	CACTACEAE		AZ
LE	PEDIOCACTUS PEEBLESIANUS VAR. PEEBLESIANUS	CACTACEAE	CACTUS, PEEBLES NAVAJO	AZ
LE	PEDIOCACTUS SILERI	CACTACEAE	PINCUSHION CACTUS, SILER	AZ UT
1	PEDIOCACTUS WINKLERI	CACTACEAE		UT
5	PEDIOCACTUS WRIGHTIAE	*** SEE ***	SCLEROCACTUS WRIGHTIAE	
5	PEDIOMELUM SUBACAULIS	*** SEE ***	PSORALEA SUBACAULIS	
2	PELEA ANISATA VAR. HAUPUANA	RUTACEAE		HI
5	PELEA APODA	*** SEE ***	PELEA PARVIFOLIA VAR. APODA	
1	PELEA BALLOUI	RUTACEAE		HI
1	PELEA CHRISTOPHERSENII	RUTACEAE		HI
1	PELEA CINEREA	RUTACEAE	MANENA	HI
1	PELEA CINEREOPS	RUTACEAE		HI
1	PELEA CLUSIAEFOLIA VAR. PICKERINGII	RUTACEAE		HI
1	PELEA DEGENERI	RUTACEAE		HI
1	PELEA DESCENDENS	RUTACEAE		HI
3A	PELEA ELLIPTICA VAR. MAUIENSIS	RUTACEAE		HI
1	PELEA GLABRA	RUTACEAE		HI
1	PELEA GRANDIFOLIA VAR. LIANOIDES	RUTACEAE		HI
1	PELEA GRANDIFOLIA VAR. MONTANA	RUTACEAE		HI
2	PELEA GRANDIFOLIA VAR. OVALIFOLIA	RUTACEAE		HI
5	PELEA GRANDIFOLIA VAR. TERMINALIS	*** SEE ***	PELEA GRANDIFOLIA VAR. OVALIFOLIA	
1	PELEA HAUPUENSIS	RUTACEAE		HI
1	PELEA HAWAIENSIS	RUTACEAE	MANENA	HI
2	PELEA HIIAKAE	RUTACEAE		HI
1	PELEA HOSAKAE	RUTACEAE		HI
2	PELEA KAUAENSIS	RUTACEAE		HI
1	PELEA KAVAIENSIS	RUTACEAE		HI
1	* PELEA KNUDSENII	RUTACEAE		HI
1	PELEA LAKAE	RUTACEAE		HI
1	* PELEA LANCEOLATA	RUTACEAE		HI
1	PELEA LEVEILLEI	RUTACEAE		HI
1	PELEA LYDGATEI	RUTACEAE		HI
1	* PELEA MACROPUS	RUTACEAE		HI
1	PELEA MAKAHAE	RUTACEAE		HI
1	* PELEA MUCRONULATA	RUTACEAE	ALANI	HI
1	PELEA MULTIFLORA	RUTACEAE		HI
1	* PELEA MUNROI	RUTACEAE		HI
1	PELEA NEALIAE	RUTACEAE	PELEA, NEAL	HI
5	PELEA NIUENSIS	*** SEE ***	PELEA PEDUNCULARIS VAR. NIUENSIS	
1	PELEA OBLONGIFOLIA	RUTACEAE		HI
3A	PELEA OBOVATA	RUTACEAE		HI
1	PELEA OLOWALUENSIS	RUTACEAE		HI
1	PELEA ORBICULARIS	RUTACEAE		HI
1	* PELEA OVALIS	RUTACEAE		HI
1	PELEA OVATA	RUTACEAE		HI

STATUS		SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
1		PELEA PALLIDA	RUTACEAE	ALANI	HI
1		PELEA PANICULATA	RUTACEAE	ALANI	HI
1	*	PELEA PARVIFOLIA VAR. APODA	RUTACEAE	APODA	HI
1		PELEA PARVIFOLIA VAR. SESSILIS	RUTACEAE		HI
1		PELEA PEDUNCULARIS VAR. CORDATA	RUTACEAE		HI
1		PELEA PEDUNCULARIS VAR. NIUENSIS	RUTACEAE		HI
1		PELEA PEDUNCULARIS VAR. NUMMULARIA	RUTACEAE		HI
S		PELEA PICKERINGII	*** SEE ***	PELEA CLUSIAEFOLIA VAR. PICKERINGII	
1		PELEA PLUVIALIS	RUTACEAE		HI
1		PELEA PUAULUENSIS	RUTACEAE		HI
1		PELEA QUADRANGULARIS	RUTACEAE		HI
1		PELEA RECURVATA	RUTACEAE	ALANI	HI
1		PELEA REFLEXA	RUTACEAE		HI
1		PELEA SAINT-JOHNII	RUTACEAE	PELEA, ST. JOHN'S	HI
1	*	PELEA SANDWICENSIS	RUTACEAE		HI
3A		PELEA STOREYANA	RUTACEAE		HI
1		PELEA TOMENTOSA	RUTACEAE		HI
1		PELEA VOLCANICA VAR. KOHALAE	RUTACEAE		HI
1		PELEA WAHIAWAENSIS	RUTACEAE		HI
2		PELEA WAIMEAENSIS	RUTACEAE		HI
1		PELEA ZAHLBRUCKNERI	RUTACEAE		HI
S		PENIOCEREUS GREGII	*** SEE ***	CEREUS GREGGII	
3C		PENSTEMON ABIETINUS	SCROPHULARIACEAE	BEARDTONGUE,	NV UT
2		PENSTEMON ABSORKENSIS	SCROPHULARIACEAE		WY
3C		PENSTEMON ACAULIS	SCROPHULARIACEAE	BEARDTONGUE, STEMLESS	UT WY
2		PENSTEMON ALAMOSENSIS	SCROPHULARIACEAE		NM TX
1		PENSTEMON ALBIFLUVIS	SCROPHULARIACEAE	BEARDTONGUE,	CO UT
2		PENSTEMON AMMOPHILUM	SCROPHULARIACEAE		UT
3C		PENSTEMON ANGUSTIFOLIUS VAR. VERNALENSIS	SCROPHULARIACEAE		UT
2		PENSTEMON ARENARIUS	SCROPHULARIACEAE		NV
2		PENSTEMON ATWOODII	SCROPHULARIACEAE		UT
3C		PENSTEMON BACCHARIFOLIUS	SCROPHULARIACEAE		TX, Mexico
2		PENSTEMON BARRETTIAE	SCROPHULARIACEAE		OR WA
2		PENSTEMON BICOLOR SSP. BICOLOR	SCROPHULARIACEAE		NV
2		PENSTEMON BICOLOR SSP. ROSEUS	SCROPHULARIACEAE		AZ NV
2		PENSTEMON BRACTEATUS	SCROPHULARIACEAE	BEARDTONGUE, RED CANYON	UT
3B		PENSTEMON CAESPITOSUS VAR. SUFFRUTICOSUS	SCROPHULARIACEAE	BEARDTONGUE,	UT
3C		PENSTEMON CALCAREUS	SCROPHULARIACEAE	PENSTEMON, LIMESTONE	CA NV
3C		PENSTEMON CALIFORNICUS	SCROPHULARIACEAE	PENSTEMON, CALIFORNIA	CA, Mexico
3C		PENSTEMON CARYI	SCROPHULARIACEAE	BEARDTONGUE, CARY	MT WY
3C		PENSTEMON CINICOLA	SCROPHULARIACEAE	PENSTEMON, ASH	CA OR
3C		PENSTEMON CLUTEI	SCROPHULARIACEAE	BEARDTONGUE,	AZ
3C		PENSTEMON COBAEA VAR. PURPUREUS	SCROPHULARIACEAE		AR MO
2		PENSTEMON COMPACTUS	SCROPHULARIACEAE	BEARDTONGUE, CACHE	UT
2		PENSTEMON CONCINNUS	SCROPHULARIACEAE	BEARDTONGUE, TUNNEL SPRINGS	NV UT
3B		PENSTEMON DECURVUS	SCROPHULARIACEAE	BEARDTONGUE,	NV UT
2		PENSTEMON DEGENERI	SCROPHULARIACEAE	BEARDTONGUE, DEGENER	CO
1		PENSTEMON DISCOLOR	SCROPHULARIACEAE	BEARDTONGUE,	AZ
3C		PENSTEMON DISSECTUS	SCROPHULARIACEAE		GA
2		PENSTEMON DISTANS	SCROPHULARIACEAE		AZ
3C		PENSTEMON ELEGANTULUS	SCROPHULARIACEAE		ID OR
3C		PENSTEMON FILIFORMIS	SCROPHULARIACEAE	PENSTEMON, THREAD-LEAVED	CA
2		PENSTEMON FLOWERSII	SCROPHULARIACEAE		UT
3C		PENSTEMON FRANCISCI-PENNELLII	SCROPHULARIACEAE		NV
2		PENSTEMON FRUTICIFORMIS SSP. AMARGOSAE	SCROPHULARIACEAE	PENSTEMON, AMARGOSA	CA NV
3B		PENSTEMON GARRETTII	SCROPHULARIACEAE	BEARDTONGUE, GARRETT'S	UT
2		PENSTEMON GIBBENSII	SCROPHULARIACEAE		CO WY
2		PENSTEMON GLAUCINUS	SCROPHULARIACEAE	BEARDTONGUE,	OR
2		PENSTEMON GOODRICHII	SCROPHULARIACEAE		UT
1		PENSTEMON GRAHAMII	SCROPHULARIACEAE	BEARDTONGUE, GRAHAM	CO UT
2		PENSTEMON HARRINGTONII	SCROPHULARIACEAE	BEARDTONGUE, HARRINGTON	CO
1		PENSTEMON HAYDENII	SCROPHULARIACEAE		NE
3C		PENSTEMON HUMILIS VAR. BREVIFOLIUS	SCROPHULARIACEAE		UT
3C		PENSTEMON HUMILIS VAR. OBTUSIFOLIUS	SCROPHULARIACEAE		UT
3C		PENSTEMON KECKII	SCROPHULARIACEAE	BEARDTONGUE,	NV
3C		PENSTEMON LEIOPHYLLUS	SCROPHULARIACEAE	BEARDTONGUE,	NV UT
2		PENSTEMON LEMHIENSIS	SCROPHULARIACEAE	BEARDTONGUE, LEMHI	ID MT
2		PENSTEMON LEPTANTHUS	SCROPHULARIACEAE		UT
S		PENSTEMON MODESTUS	*** SEE ***	PENSTEMON PROCERUS VAR. MODESTUS	
3C		PENSTEMON MORIAHENSIS	SCROPHULARIACEAE		NV
3B		PENSTEMON MULTICAULIS	SCROPHULARIACEAE		AR
3C		PENSTEMON NANUS	SCROPHULARIACEAE	BEARDTONGUE, LOW	UT
2		PENSTEMON NAVAJOA	SCROPHULARIACEAE		UT
3B		PENSTEMON NYEENSIS	SCROPHULARIACEAE	BEARDTONGUE,	NV
1		PENSTEMON PAHUTENSIS	SCROPHULARIACEAE	BEARDTONGUE,	NV
3C		PENSTEMON PAPILLATUS	SCROPHULARIACEAE	PENSTEMON, INYO	CA
2	*	PENSTEMON PARVIFLORUS	SCROPHULARIACEAE		CO
3C		PENSTEMON PARVUS	SCROPHULARIACEAE	BEARDTONGUE, SMALL	UT
3C		PENSTEMON PATRICUS	SCROPHULARIACEAE		NV UT
3C		PENSTEMON PAYSONIORUM	SCROPHULARIACEAE	BEARDTONGUE, PAYSON	WY
2		PENSTEMON PECKII	SCROPHULARIACEAE		OR
2		PENSTEMON PERSONATUS	SCROPHULARIACEAE	BEARDTONGUE, CLOSED-LIP	CA
3C		PENSTEMON PROCERUS VAR. MODESTUS	SCROPHULARIACEAE		NV

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2	PENSTEMON PUDICUS	SCROPHULARIACEAE		NV
1	PENSTEMON RETRORSUS	SCROPHULARIACEAE	BEARDTONGUE,	CO
3C	PENSTEMON RUBICUNDUS	SCROPHULARIACEAE	BEARDTONGUE,	NV
3C	PENSTEMON SP. /SP. NOV. INED.	SCROPHULARIACEAE	BEARDTONGUE (RANDLETT, UINTAH CO.)	UT
2	PENSTEMON SPATULATUS	SCROPHULARIACEAE	BEARDTONGUE, WALLOWA	OR
2	PENSTEMON STEPHENSII	SCROPHULARIACEAE	PENSTEMON, STEPHENS	CA
3C	PENSTEMON THOMPSONIAE SSP. JAEGERI	SCROPHULARIACEAE		NV
3B	PENSTEMON THURBERI VAR. ANESTIUS	SCROPHULARIACEAE		NV
2	PENSTEMON TIDESTROMII	SCROPHULARIACEAE	BEARDTONGUE, TIDESTROM	UT
2	PENSTEMON TRACYI	SCROPHULARIACEAE	PENSTEMON, TRACY	CA
3C	PENSTEMON UINTAHENSIS	SCROPHULARIACEAE	BEARDTONGUE, UINTAH	UT
3C	PENSTEMON VIRGATUS SSP. PSEUDOPUTUS	SCROPHULARIACEAE		AZ
2	PENSTEMON WARDII	SCROPHULARIACEAE	BEARDTONGUE, WARD	UT
3C	PENSTEMON WASHINGTONENSIS	SCROPHULARIACEAE		WA
3C	PENSTEMON YAMPAENSIS	SCROPHULARIACEAE		CO
2	PENTACHAETA BELLIDIFLORA	ASTERACEAE	PENTACHAETA, WHITE-RAYED	CA
2	PENTACHAETA EXILIS SSP. AEOLICA	ASTERACEAE	PENTACHAETA, SLENDER	CA
2	PENTACHAETA LYONII	ASTERACEAE	PENTACHAETA, LYON'S	CA
2	PEPEROMIA COOKIANA VAR. MINUTILIMBA	PIPERACEAE		HI
1	* PEPEROMIA CORNIFOLIA	PIPERACEAE		HI
1	PEPEROMIA DEGENERI	PIPERACEAE		HI
2	PEPEROMIA EXPALLESCENS VAR. BREVIPILOSA	PIPERACEAE		HI
2	PEPEROMIA FAURIEI	PIPERACEAE		HI
2	PEPEROMIA FLORIDANA	PIPERACEAE		FL
2	PEPEROMIA FORBESII	PIPERACEAE		HI
2	PEPEROMIA HAUPUENSIS	PIPERACEAE		HI
2	PEPEROMIA HELLERI VAR. KNUDSENII	PIPERACEAE		HI
1	PEPEROMIA KULENSIS	PIPERACEAE		HI
2	PEPEROMIA LILIFOLIA VAR. OBTUSATA	PIPERACEAE		HI
2	PEPEROMIA MAUNAKEANA	PIPERACEAE		HI
1	PEPEROMIA OAHUENSIS VAR. ST-JOHNII	PIPERACEAE		HI
3A	PEPEROMIA PLINERVATA	PIPERACEAE		HI
2	PEPEROMIA RIGIDILIMBA	PIPERACEAE		HI
2	PEPEROMIA SUBPETIOLATA	PIPERACEAE		HI
1	PEPEROMIA TRELEASEI	PIPERACEAE		HI
2	PEPEROMIA WAIKAMOIANA	PIPERACEAE		HI
1	PEPEROMIA WHEELERI	PIPERACEAE		PR
3C	PERIDERIDIA BACIGALUPII	APIACEAE	YAMPAH, MOTHER LODE	CA
2	PERIDERIDIA ERYTHRORHIZA	APIACEAE		OR
2	PERIDERIDIA GAIRDNERI SSP. GAIRDNERI	APIACEAE	YAMPAH, GAIRDNER'S	CA
3C	PERIDERIDIA LEPTOCARPA	APIACEAE	YAMPAH, NARROW-SEEDED	CA
3C	PERIDERIDIA PRINGLEI	APIACEAE	YAMPAH, ADOBE	CA
2	PERITYLE AJOENSIS	ASTERACEAE	ROCK-DAISY, AJO	AZ
2	PERITYLE BISETOSA VAR. BISETOSA	ASTERACEAE	ROCK-DAISY, TWO-SPIKE,	TX
2	PERITYLE BISETOSA VAR. SCALARIS	ASTERACEAE	ROCK-DAISY, TWO-SPIKE,	TX
2	PERITYLE CERNUA	ASTERACEAE		NM
3C	PERITYLE CINEREA	ASTERACEAE	ROCK-DAISY, GRAY	TX
2	PERITYLE COCHISENSIS	ASTERACEAE		AZ
3C	PERITYLE GILENSIS VAR. SALENSIS	ASTERACEAE	ROCK-DAISY,	AZ
2	PERITYLE INYOENSIS	ASTERACEAE	LAPHAMIA, INYO	CA
3C	PERITYLE LEMMONII	ASTERACEAE		AZ NM
3C	PERITYLE LINDHEIMERI	ASTERACEAE	ROCK-DAISY,	TX
3B	PERITYLE LINDHEIMERI VAR. HALIMIFOLIA	ASTERACEAE	ROCK-DAISY,	TX
3C	PERITYLE MEGALOCEPHALA VAR. INTRICATA	ASTERACEAE		CA NV
3C	PERITYLE PARRYI	ASTERACEAE		TX
5	PERITYLE ROTUNDATA	*** SEE ***	PERITYLE LINDHEIMERI VAR. LINDHEIMERI	
2	PERITYLE SAXICOLA	ASTERACEAE		AZ
3C	PERITYLE STAUROPHYLLA	ASTERACEAE		NM
2	PERITYLE VILLOSA	ASTERACEAE	LAPHAMIA, HANAUPAH	CA
2	PERITYLE VITREOMONTANA	ASTERACEAE	ROCK-DAISY, GLASS MOUNTAIN	TX
2	PERITYLE WARNOCKII	ASTERACEAE		TX
3C	PERSEA BORBONIA VAR. HUMILIS	LAURACEAE		FL GA
3B	PERSICARIA PALUDICOLA	POLYGONACEAE		FL
2	PETALONYX THURBERI SSP. GILMANII	LOASACEAE	SANDPAPERPLANT, DEATH VALLEY	CA
5	PETALOSTEMUM FOLIOSUM	*** SEE ***	DALEA FOLIOSA	
5	PETALOSTEMUM GATTINGERI	*** SEE ***	DALEA GATTINGERI	
5	PETALOSTEMUM REVERCHONII	*** SEE ***	DALEA REVERCHONII	
5	PETALOSTEMUM SABINALE	*** SEE ***	DALEA SABINALIS	
5	PETALOSTEMUM SCARIOSUM	*** SEE ***	DALEA SCARIOSA	
3C	PETERIA THOMPSONAE	FABACEAE		AZ ID NV UT
2	PETROPHYTUM CINERASCENS	ROSACEAE	ROCKMAT, CHELAN	WA
3C	PETROPHYTUM HENDERSONII	ROSACEAE		WA
2	PEUCEDANUM KAUAIENSE	APIACEAE		HI
2	PEUCEDANUM SANDWICENSE VAR. SANDWICENSE	APIACEAE	MAKOU	HI
2	PHACELIA AMABILIS	HYDROPHYLLACEAE	PHACELIA, SALINE VALLEY	CA
3C	PHACELIA ANELSONII	HYDROPHYLLACEAE	PHACELIA, MACBRIDE	CA NV UT
2	PHACELIA ARGENTEA	HYDROPHYLLACEAE		CA OR
LE	PHACELIA ARGILLACEA	HYDROPHYLLACEAE	PHACELIA, CLAY	UT
2	PHACELIA BEATLEYAE	HYDROPHYLLACEAE	PHACELIA, BEATLEY	NV
2	PHACELIA CAPITATA	HYDROPHYLLACEAE	PHACELIA,	OR
2	PHACELIA CEPHALOTES	HYDROPHYLLACEAE	PHACELIA, VIRGIN	AZ UT
2	PHACELIA CILIATA VAR. OPACA	HYDROPHYLLACEAE		CA
1	* PHACELIA CINEREA	HYDROPHYLLACEAE	PHACELIA, ASHY	CA

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3C	PHACELIA CONSTANCEI	HYDROPHYLLACEAE		AZ UT
2	PHACELIA COOKEI	HYDROPHYLLACEAE	PHACELIA, COOKE'S	CA
2	PHACELIA DALESIANA	HYDROPHYLLACEAE	PHACELIA, TRINITY	CA
3C	PHACELIA DEMISSA VAR. HETEROTRICHA	HYDROPHYLLACEAE	PHACELIA,	UT
S	PHACELIA DIVARICATA VAR. INSULARIS	*** SEE ***	PHACELIA INSULARIS VAR. INSULARIS	
3C	PHACELIA DUBIA VAR. GEORGIANA	HYDROPHYLLACEAE		AL GA
3B	PHACELIA FILIFORMIS	HYDROPHYLLACEAE	PHACELIA,	AZ
2	PHACELIA FLORIBUNDA	HYDROPHYLLACEAE		CA
LE	PHACELIA FORMOSULA	HYDROPHYLLACEAE	PHACELIA, NORTH PARK	CO
3C	PHACELIA GLABERRIMA	HYDROPHYLLACEAE		NV
2	PHACELIA GREENEI	HYDROPHYLLACEAE	PHACELIA, SCOTT VALLEY	CA
3C	PHACELIA HOWELLIANA	HYDROPHYLLACEAE	PHACELIA, HOWELL	AZ UT
2	PHACELIA INCONSPICUA	HYDROPHYLLACEAE		ID NV
3B	PHACELIA INDECORA	HYDROPHYLLACEAE	PHACELIA, DRAB	UT
2	PHACELIA INSULARIS VAR. CONTINENTIS	HYDROPHYLLACEAE		CA
2	PHACELIA INSULARIS VAR. INSULARIS	HYDROPHYLLACEAE	PHACELIA, ISLAND	CA
3C	PHACELIA INTEGRIFOLIA VAR. TEXANA	HYDROPHYLLACEAE		NM TX
2	PHACELIA LENTA	HYDROPHYLLACEAE	PHACELIA, STICKY	WA
3C	PHACELIA MAMMILLARENSIS	HYDROPHYLLACEAE	PHACELIA, NIPPLE BENCH	UT
2	PHACELIA MONOENSIS	HYDROPHYLLACEAE	PHACELIA, MONO	CA NV
3C	PHACELIA MUSTELINA	HYDROPHYLLACEAE	PHACELIA, ROUND LEAF, DEATH VALLEY	CA NV
2	* PHACELIA NEVADENSIS	HYDROPHYLLACEAE		NV
2	PHACELIA NOVENMILLENSIS	HYDROPHYLLACEAE	PHACELIA, NINE MILE CANYON	CA
3C	PHACELIA OROGENES	HYDROPHYLLACEAE	PHACELIA, MOUNTAIN	CA
2	PHACELIA PALLIDA	HYDROPHYLLACEAE	PHACELIA, PALE	TX MEXICO
3C	PHACELIA PARISHII	HYDROPHYLLACEAE		CA NV
3C	PHACELIA PECKII	HYDROPHYLLACEAE		OR
2	PHACELIA PHACELIOIDES	HYDROPHYLLACEAE	PHACELIA, MT. DIABLO	CA
3C	PHACELIA RAFAELENSIS	HYDROPHYLLACEAE	PHACELIA,	AZ UT
3C	PHACELIA SERRATA	HYDROPHYLLACEAE		AZ
2	PHACELIA STEBBINSII	HYDROPHYLLACEAE	PHACELIA, STEBBINS	CA
2	PHACELIA SUAVEOLENS SSP. KECKII	HYDROPHYLLACEAE		CA
2	PHACELIA SUBMUTICA	HYDROPHYLLACEAE	PHACELIA,	CO
3C	PHACELIA UTAHENSIS	HYDROPHYLLACEAE	PHACELIA, UTAH	UT
2	PHACELIA VERNA	HYDROPHYLLACEAE		OR
2	PHACELIA WELSHII	HYDROPHYLLACEAE	PHACELIA,	AZ
3C	PHASEOLUS SUPINUS	FABACEAE	BEAN, SUPINE	AZ UT
3C	PHILADELPHUS ERNESTII	SAXIFRAGACEAE		TX
3C	PHILADELPHUS MEARNSII	SAXIFRAGACEAE		NM
3C	PHILADELPHUS TEXENSIS VAR. TEXENSIS	SAXIFRAGACEAE		TX
3C	PHIPPSIA ALGIDA	POACEAE	ICE GRASS	AK CO WY, Canada, U.S.S.R.
2	PHLOX BIFIDA SSP. STELLARIA	POLEMONIACEAE	PHLOX, CLEFT,	AR IL IN KY MO TN
3C	PHLOX BUCKLEYI	POLEMONIACEAE		VA WV
2	PHLOX CARYOPHYLLA	POLEMONIACEAE	PHLOX,	CO NM
3C	PHLOX CLUTEANA	POLEMONIACEAE	PHLOX, NAVAJO MOUNTAIN	AZ UT
2	PHLOX DOLICHANTHA	POLEMONIACEAE	PHLOX, BEAR VALLEY	CA
3C	PHLOX GLADIFORMIS	POLEMONIACEAE	PHLOX, RED CANYON	NV UT
3B	PHLOX GRAHAMII	POLEMONIACEAE	PHLOX,	UT
2	PHLOX HIRSUTA	POLEMONIACEAE	PHLOX, YREKA	CA
1	PHLOX IDAHONIS	POLEMONIACEAE	PHLOX, CLEARWATER	ID
3B	PHLOX JONESII	POLEMONIACEAE	PHLOX,	UT
S	PHLOX LONGIPILOSA	*** SEE ***	PHLOX PILOSA VAR. LONGIPILOSA	OK
3C	PHLOX MISSOULENSIS	POLEMONIACEAE	PHLOX,	MT
3B	PHLOX MOLLIS	POLEMONIACEAE	PHLOX,	ID OR WA
1	PHLOX NIVALIS SSP. TEXENSIS	POLEMONIACEAE	PHLOX, TRAILING, TEXAS	TX
3C	PHLOX OKLAHOMENSIS	POLEMONIACEAE	PHLOX,	KS OK TX
3B	PHLOX PECKII	POLEMONIACEAE		OR
3C	PHLOX PILOSA VAR. LONGIPILOSA	POLEMONIACEAE	PHLOX, LONG-HAIRED	OK
3C	PHLOX PULCHRA	POLEMONIACEAE	PHLOX,	AL
2	PHLOX SP. NOV. /INED.	POLEMONIACEAE		WY
2	PHOLISMA ARENARIUM	LENNOACEAE	PHOLISMA	AZ CA, MEXICO (Baja California)
2	PHOLISMA SONORAE	LENNOACEAE		AZ
3C	PHYLLANTHUS ERICOIDES	EUPHORBIACEAE	LEAF-FLOWER,	TX, Mexico
3C	PHYLLANTHUS LIEBMANNIANUS SSP. PLATYLEPIS	EUPHORBIACEAE		FL
2	PHYLLANTHUS PENTAPHYLLUS SSP. FLORIDANUS	EUPHORBIACEAE		FL
2	PHYLLANTHUS SANDWICENSIS VAR. DEGENERI	EUPHORBIACEAE		HI
2	PHYLLITIS SCOLOPENDRIUM VAR. AMERICANUM	POLYPODIACEAE	FERN, AMERICAN	AL MI NY TN, Canada
1	* PHYLLOSTEGIA BREVIDENS VAR. AMBIGUA	LAMIACEAE		HI
2	PHYLLOSTEGIA BREVIDENS VAR. DEGENERI	LAMIACEAE		HI
1	PHYLLOSTEGIA BREVIDENS VAR. HETERODOXA	LAMIACEAE		HI
1	* PHYLLOSTEGIA BREVIDENS VAR. HIRSUTULA	LAMIACEAE		HI
1	* PHYLLOSTEGIA BREVIDENS VAR. LONGIPES	LAMIACEAE		HI
1	* PHYLLOSTEGIA BREVIDENS VAR. PUBESCENS	LAMIACEAE		HI
1	PHYLLOSTEGIA FLORIBUNDA VAR. FORBESII	LAMIACEAE		HI
1	PHYLLOSTEGIA GLABRA VAR. LANAIENSIS	LAMIACEAE		HI
1	PHYLLOSTEGIA HELLERI VAR. IMMINUTA	LAMIACEAE		HI
2	PHYLLOSTEGIA HILLEBRANDII	LAMIACEAE		HI
2	PHYLLOSTEGIA HIRSUTA	LAMIACEAE		HI
1	PHYLLOSTEGIA KNUDSENII	LAMIACEAE		HI
3A	PHYLLOSTEGIA LEDYARDII	LAMIACEAE		HI

STATUS		SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
3A		PHYLLOSTEGIA LONGIMONTIS	LAMIACEAE		HI
1		PHYLLOSTEGIA MACROPHYLLA VAR. VELUTINA	LAMIACEAE		HI
1		PHYLLOSTEGIA MOLLIS VAR. FAGERLINDII	LAMIACEAE		HI
1		PHYLLOSTEGIA MOLLIS VAR. HOCHREUTINERI	LAMIACEAE	NEHE KUHIWA	HI
1	*	PHYLLOSTEGIA MOLLIS VAR. LYDGATEI	LAMIACEAE		HI
1		PHYLLOSTEGIA MOLLIS VAR. MICRANTHA	LAMIACEAE		HI
1	*	PHYLLOSTEGIA PARVIFLORA VAR. CANESCENS	LAMIACEAE		HI
1	*	PHYLLOSTEGIA PARVIFLORA VAR. GLABRIUSCULA	LAMIACEAE		HI
1	*	PHYLLOSTEGIA PARVIFLORA VAR. HONOLULENSIS	LAMIACEAE		HI
1	*	PHYLLOSTEGIA VARIABILIS	LAMIACEAE		HI
2		PHYLLOSTEGIA YAMAGUCHII	LAMIACEAE	PHYLLOSTEGIA, YAMAGUCHI	HI
3C		PHYSALIS VISCOSA VAR. ELLIOTII	SOLANACEAE		FL
2		PHYSARIA ACUTIFOLIA VAR. PURPUREA	BRASSICACEAE		UT
5		PHYSARIA ALPESTRIS VAR. LYRATA	*** SEE ***	PHYSARIA DIDYMOCARPA VAR. LYRATA	
5		PHYSARIA ALPESTRIS VAR. PURPUREA	*** SEE ***	PHYSARIA GEYERI VAR. PURPUREA	
2		PHYSARIA BELLII	BRASSICACEAE		CO
3C		PHYSARIA CONDENSATA	BRASSICACEAE	TWINPOD, DENSE	WY
2		PHYSARIA DIDYMOCARPA VAR. LYRATA	BRASSICACEAE		ID
2		PHYSARIA DORNII	BRASSICACEAE		WY
3C		PHYSARIA GEYERI VAR. PURPUREA	BRASSICACEAE		ID
3C		PHYSARIA GRAHAMII	BRASSICACEAE	TWINPOD, GRAHAM'S	UT
2		PHYSARIA OBCORDATA	BRASSICACEAE		CO
2		PHYSOSTEGIA CORRELLII	LAMIACEAE	FALSE DRAGON-HEAD, CORRELL'S	TX
2		PHYSOSTEGIA LEPTOPHYLLA	LAMIACEAE		FL GA NC SC VA
2		PHYSOSTEGIA LONGISEPALA	LAMIACEAE		LA TX
3B		PHYSOSTEGIA MICRANTHA	LAMIACEAE		OK TX
3B		PHYSOSTEGIA VERONICIFORMIS	LAMIACEAE	DRAGONHEAD, FALSE	FL GA
3C		PIERIS PHILLYREAEFOLIA	ERICACEAE		AL FL GA MS SC
1		PILEA LEPTOPHYLLA	URTICACEAE		PR
1		PILEA MULTICAULIS	URTICACEAE		PR
2		PILEA RICHARDII	URTICACEAE		PR VI
2		PILEA YUNQUENSIS	URTICACEAE		PR
3C		PILOSTYLES THURBERI	RAFFLESIACEAE	PILOSTYLES, THURBER'S	AZ CA NV TX, Mexico (Baja California)
3C		PINCKNEYA PUBENS	RUBIACEAE		FL GA SC
2		PINGUICULA IONANTHA	LENTIBULARIACEAE	BUTTERWORT,	FL
2		PINGUICULA PLANIFOLIA	LENTIBULARIACEAE	BUTTERWORT, CHAPMAN'S	AL FL MS
2		PINUS TORREYANA	PINACEAE	PINE, TORREY	CA
3A		PISONIA FLORIDANA	NYCTAGINACEAE		FL
3B		PITTOSPORUM ACUMINATUM VAR. LEPTOPODUM	PITTOSPORACEAE		HI
3B		PITTOSPORUM ACUMINATUM VAR. MAGNIFOLIUM	PITTOSPORACEAE		HI
3B		PITTOSPORUM ACUMINATUM VAR. WAIMEANUM	PITTOSPORACEAE		HI
3B		PITTOSPORUM AMPLECTENS	PITTOSPORACEAE		HI
3B		PITTOSPORUM ARGENTIFOLIUM VAR. ARGENTIFOLIUM	PITTOSPORACEAE		HI
3B		PITTOSPORUM ARGENTIFOLIUM VAR. SESSILE	PITTOSPORACEAE		HI
3C		PITTOSPORUM CAULIFLORUM VAR. CAULIFLORUM	PITTOSPORACEAE		HI
3B		PITTOSPORUM CAULIFLORUM VAR. CLADANTHOIDES	PITTOSPORACEAE		HI
2		PITTOSPORUM CAULIFLORUM VAR. PEDICELLATUM	PITTOSPORACEAE		HI
3B		PITTOSPORUM CLADANTHUM VAR. GRACILIPES	PITTOSPORACEAE		HI
2		PITTOSPORUM CONFERTIFLORUM VAR. LONGIPES	PITTOSPORACEAE		HI
3B		PITTOSPORUM CONFERTIFLORUM VAR. MICROPHYLLUM	PITTOSPORACEAE		HI
3B		PITTOSPORUM GLABRUM VAR. GLOMERATUM	PITTOSPORACEAE		HI
3B		PITTOSPORUM GLABRUM VAR. INTERMEDIUM	PITTOSPORACEAE		HI
3B		PITTOSPORUM GLABRUM VAR. TINIFOLIUM	PITTOSPORACEAE		HI
3B		PITTOSPORUM HALOPHILOIDES	PITTOSPORACEAE		HI
3B		PITTOSPORUM HALOPHILUM	PITTOSPORACEAE		HI
1		PITTOSPORUM HAWAIIENSE	PITTOSPORACEAE		HI
3B		PITTOSPORUM HELLERI	PITTOSPORACEAE		HI
3B		PITTOSPORUM HOSMERI VAR. HOSMERI	PITTOSPORACEAE		HI
3B		PITTOSPORUM HOSMERI VAR. SAINT-JOHNII	PITTOSPORACEAE		HI
3B		PITTOSPORUM INSIGNE VAR. MICRANTHUM	PITTOSPORACEAE		HI
3B		PITTOSPORUM KAHANANUM	PITTOSPORACEAE		HI
3B		PITTOSPORUM KAUAIENSE VAR. REPENS	PITTOSPORACEAE		HI
3B		PITTOSPORUM TERMINALIOIDES VAR. LANAIENSE	PITTOSPORACEAE		HI
3B		PITTOSPORUM TERMINALIOIDES VAR. MACROPUS	PITTOSPORACEAE		HI
3B		PITTOSPORUM TERMINALIOIDES VAR. MAUIENSE	PITTOSPORACEAE		HI
2		PITYOPSIS FLEXUOSA	ASTERACEAE		FL
LE		PITYOPSIS RUTHII	ASTERACEAE	GOLDEN-ASTER, RUTH'S	TN
3C		PITYOPUS CALIFORNICUS	ERICACEAE		CA OR
5		PITYOPUS OREGONA	*** SEE ***	PITYOPUS CALIFORNICUS	
5		PITYOTHAMNUS TETRAMERUS	*** SEE ***	ASIMINA TETRAMERA	
2	*	PLAGIOBOTHRYS DIFFUSUS	BORAGINACEAE	POPCORNFLOWER, SAN FRANCISCO	CA
3C		PLAGIOBOTHRYS DISTANTIFLORUS	BORAGINACEAE		CA
2		PLAGIOBOTHRYS GLABER	BORAGINACEAE	ALLOCARYA, GLABROUS	CA
2		PLAGIOBOTHRYS GLYPTOCARPUS VAR. MODESTUS	BORAGINACEAE	ALLOCARYA, CEDAR VALLEY	CA
2	*	PLAGIOBOTHRYS HIRTUS VAR. CORALLICARPUS	BORAGINACEAE		OR

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
2	PLAGIOBOTHRYS HIRTUS VAR. HIRTUS	BORAGINACEAE	POPCORNFLOWER,	OR
2	PLAGIOBOTHRYS HYSTRICULUS	BORAGINACEAE	ALLOCARYA, BEARDED	CA
2	• PLAGIOBOTHRYS LAMPROCARPUS	BORAGINACEAE	POPCORNFLOWER,	OR
2	PLAGIOBOTHRYS MOLLIS VAR. VESTITUS	BORAGINACEAE	ALLOCARYA, PETALUMA	CA
2	PLAGIOBOTHRYS SCRIPTUS	BORAGINACEAE	ALLOCARYA, SCRIBE	CA
2	PLAGIOBOTHRYS STRICTUS	BORAGINACEAE	ALLOCARYA, CALISTOGA	CA
S	PLANCHONELLA AUAHIENSIS	*** SEE ***	POUTERIA AUAHIENSIS	
S	PLANCHONELLA RHYNCHOSPERMA	*** SEE ***	POUTERIA RHYNCHOSPERMA	
2	PLANTAGO CORDATA	PLANTAGINACEAE	PLANTAIN, HEART-LEAVED	AL AR DC FL GA IL IN KY LA MD MI MO NY NC OH VA WI, Canada (Ont.)
2	PLANTAGO PRINCEPS VAR. ACAULIS	PLANTAGINACEAE		HI
1	PLANTAGO PRINCEPS VAR. DENTICULATA	PLANTAGINACEAE		HI
1	PLANTAGO PRINCEPS VAR. ELATA	PLANTAGINACEAE		HI
1	PLANTAGO PRINCEPS VAR. LAXIFOLIA	PLANTAGINACEAE		HI
1	PLANTAGO PRINCEPS VAR. PRINCEPS	PLANTAGINACEAE	ALE	HI
2	PLANTAGO PRINCEPS VAR. QUELENIANA	PLANTAGINACEAE		HI
3C	PLATANTHERA FLAVA	ORCHIDACEAE	REIN ORCHID, SOUTHERN	AR DE DC FL GA IL IN IA KY LA ME MD MA MI MN MO NH NJ NY NC OH OK PA RI SC SD TN TX VT VA WV WI
1	PLATANTHERA HOLOCHILA	ORCHIDACEAE		HI
3C	PLATANTHERA INTEGRA	ORCHIDACEAE		AL FL GA LA MS NJ NC SC TN TX
2	PLATANTHERA INTEGRILABIA	ORCHIDACEAE		AL KY MS NC SC TN
2	PLATANTHERA LEUCOPHAEA	ORCHIDACEAE	ORCHID, WHITE-FRINGED, PRAIRIE	AR IL IN IA KS LA ME MI MN MO NE NY ND OH OK PA SD VA WI, Canada, Ont.)
3C	PLATANTHERA PERAMOENA	ORCHIDACEAE	ORCHID, PURPLE, FRINGELESS	AL AR DE IL IN KY MD MS MO NJ NY NC OH PA SC TN VA WV
3B	PLATANTHERA UNALASCENSIS SSP. MARITIMA	ORCHIDACEAE	REIN ORCHID, ALASKA	CA OR WA, Canada (B.C.)
1	PLATYDESMA REMYI	RUTACEAE	PILOKEA, REMY	HI
2	PLATYSTEMON CALIFORNICUS VAR. CILIATUS	PAPAVERACEAE		CA
1	PLEODENDRON MACRANTHUM	CANELLACEAE	CHUPAGALLO (CHUPACALLOS)	PR
S	PLEOMELE AUREA	*** SEE ***	DRACAENA AUREA	
S	PLEOMELE FORBESII	*** SEE ***	DRACAENA FORBESII	
2	PLEUROPOGON HOOVERANUS	POACEAE	SEMAPHORE GRASS, HOOVER'S	CA
2	PLEUROPOGON OREGONUS	POACEAE	SEMAPHORE GRASS, OREGON	OR
3C	PLUMMERA AMBIGENS	ASTERACEAE		AZ
3B	PLUMMERA FLORIBUNDA	ASTERACEAE		AZ
1	POA ATROPURPUREA	POACEAE	BLUE GRASS, SAN BERNADINO	CA
3C	POA CURTIFOLIA	POACEAE		WA
2	POA EYERDAMII	POACEAE		AK
2	POA FIBRATA	POACEAE	BLUE GRASS, LASSEN COUNTY	CA
2	POA INVOLUTA	POACEAE	BLUE GRASS, BIG BEND	TX
3C	POA LAXIFLORA	POACEAE		AK OR WA, Canada (B.C.)
1	POA MANNII	POACEAE	BLUE GRASS, MANN'S	HI
3C	POA MARCIDA	POACEAE		OR WA, Canada (B.C.)
2	POA MERRILLIANA	POACEAE		AK
2	POA NAPENSIS	POACEAE	BLUE GRASS, NAPA	CA
2	POA NORBERGII	POACEAE		AK
S	POA PACHYPHOLIS	*** SEE ***	POA UNILATERALIS	
2	POA PALUDIGENA	POACEAE	BLUE GRASS,	IL IN MI MN NY OH PA WI
3C	POA PIPERI	POACEAE		CA OR
2	POA RHIZOMATA	POACEAE		CA
1	POA SANDVICENSIS	POACEAE	BLUE GRASS, HAWAIIAN	HI
1	POA SIPHONOGLOSSA	POACEAE		HI
1	POA UNILATERALIS	POACEAE	BLUE GRASS, SEA CLIFF	WA
2	PODISTERA YUKONENSIS	APIACEAE		AK, Canada (Yukon)
LE	POGOGYNE ABRAMSII	LAMIACEAE	MESA MINT, SAN DIEGO	CA
2	POGOGYNE CLAREANA	LAMIACEAE	POGOGYNE, SANTA LUCIA	CA
2	POGOGYNE DOUGLASII SSP. PARVIFLORA	LAMIACEAE		CA
1	POGOGYNE NUDIUSCULA	LAMIACEAE	POGOGYNE, LOMA ALTA	CA, Mexico
3C	POLEMONIUM CHARTACEUM	POLEMONIACEAE		CA
3B	POLEMONIUM NEVADENSE	POLEMONIACEAE		NV
2	• POLEMONIUM OCCIDENTALE VAR. LACUSTRE	POLEMONIACEAE	JACOB'S LADDER,	MN
2	POLEMONIUM PAUCIFLORUM SSP. HINCKLEYI	POLEMONIACEAE	JACOB'S LADDER,	AZ TX
2	POLEMONIUM PECTINATUM	POLEMONIACEAE		WA
2	POLEMONIUM REPTANS VAR. VILLOSUM	POLEMONIACEAE	JACOB'S LADDER,	KY OH
2	POLEMONIUM VANBRUNTIAE	POLEMONIACEAE	JACOB'S LADDER,	CT MD NJ NY PA VT WV, Canada (N.B., Que.)
3C	POLIANTHES MACULOSA	LILIACEAE		TX
2	POLIANTHES RUNYONII	LILIACEAE	HUACO, RUNYON	TX
3C	POLIOMINTHA GLABRESCENS	LAMIACEAE	ROSEMARY-MINT	TX, Mexico
2	POLYCTENIUM WILLIAMSIAE	BRASSICACEAE	COMBLEAF	NV
S	POLYGALA ARENICOLA	*** SEE ***	POLYGALA SMALLII	
2	POLYGALA BOYKINII VAR. SPARSIFOLIA	POLYGALACEAE		FL
2	POLYGALA COWELLII	POLYGALACEAE	PALO DE VIOLETA (VIOLET TREE)	PR
1	POLYGALA LEWTONII	POLYGALACEAE	POLYGALA, LEWTON'S	FL
3C	POLYGALA MARAVILLASENSIS	POLYGALACEAE	MILKWORT, MARAVILLAS	TX
3B	POLYGALA PILIOPHORA	POLYGALACEAE		AZ
3C	POLYGALA RIMULICOLA	POLYGALACEAE		NM TX

STATUS		SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
LE		POLYGALA SMALLII	POLYGALACEAE	POLYGALA, TINY	FL
3C		POLYGALA SUBSPINOSA VAR. HETERORHYNCA	POLYGALACEAE		CA NV
1		POLYGONELLA CILIATA VAR. BASIRAMIA	POLYGONACEAE	JOINTWEED,	FL
1		POLYGONELLA MACROPHYLLA	POLYGONACEAE	JOINTWEED, LARGE-LEAVED	AL FL
3C		POLYGONELLA MYRIOPHYLLA	POLYGONACEAE	JOINTWEED,	FL
3C		POLYGONELLA PARKSII	POLYGONACEAE	JOINTWEED, PARKS'	TX
3C		POLYGONUM BIDWELLIAE	POLYGONACEAE	KNOTWEED, BIDWELL	CA
3C		POLYGONUM CASCADENSE	POLYGONACEAE		OR
2		POLYGONUM FUSIFORME	POLYGONACEAE		AZ CA
2		POLYGONUM MARINENSE	POLYGONACEAE	KNOTWEED, MARIN	CA
1	*	POLYGONUM MONTEREYENSE	POLYGONACEAE	KNOTWEED, MONTEREY	CA
2		POLYGONUM PENSYLVANICUM VAR. EGLANDULOSUM	POLYGONACEAE	PINKWEED, LAKE ERIE	MO OH, Canada (Ont.)
3C		POLYGONUM STRIATULUM	POLYGONACEAE		TX
3C		POLYGONUM TEXENSE	POLYGONACEAE	KNOTWEED,	TX
3B	-	POLYGONUM UTAHENSE	POLYGONACEAE		UT
2		POLYMNIA LAEVIGATA	ASTERACEAE		AL FL GA KY MO TN
2		POLYSTICHUM ALEUTICUM	POLYPODIACEAE	SHIELD FERN (HOLLY FERN), ALEUTIAN	AK
3C		POLYSTICHUM DUDLEYI	POLYPODIACEAE	SWORD FERN, DUDLEY'S	CA
3C		POLYSTICHUM KRUCKEBERGII	POLYPODIACEAE		CA ID MT OR WA, Canada (B.C.)
3B		POPULUS HINCKLEYANA	SALICACEAE	COTTONWOOD, GOAT CANYON	TX
3C		POROPHYLLUM GREGGII	ASTERACEAE		TX
2		PORTULACA CAULERPOIDES	PORTULACACEAE		PR
1		PORTULACA HAWAIIENSIS	PORTULACACEAE	PORTULACA, HAWAII	HI
1		PORTULACA SCLEROCARPA	PORTULACACEAE	IHI-MAKOLE	HI
3C		PORTULACA SMALLII	PORTULACACEAE		GA NC VA
1		POTAMOGETON CLYSTOCARPUS	POTAMOGETONACEAE	PONDWEED,	TX
2		POTAMOGETON FLORIDANUS	POTAMOGETONACEAE		FL
3C		POTAMOGETON HILLII	POTAMOGETONACEAE	PONDWEED, HILL'S	CT MA MI NY OH PA VT, Canada (Ont.)
3B		POTAMOGETON LATERALIS	POTAMOGETONACEAE		CT MA MI MN NH NY VT
3B		POTAMOGETON PORTERI	POTAMOGETONACEAE		PA
2		POTENTILLA EFFUSA VAR. RUPINCOLA	ROSACEAE		CO
2		POTENTILLA HICKMANII VAR. HICKMANII	ROSACEAE	CINQUEFOIL, HICKMAN'S	CA
2		POTENTILLA HICKMANII VAR. ULIGINOSA /INED.	ROSACEAE	CINQUEFOIL, CUNNINGHAM MARSH	CA
3C		POTENTILLA MULTIFOLIOLATA	ROSACEAE		AZ
1	*	POTENTILLA MULTIJUGA	ROSACEAE	CINQUEFOIL, BALLONA	CA
2		POTENTILLA PATELLIFERA	ROSACEAE	CINQUEFOIL, KINGSTON MOUNTAINS	CA
LE		POTENTILLA ROBBINSIANA	ROSACEAE	CINQUEFOIL, ROBBINS'	NH VT
S		POTENTILLA RUFINCOLA	*** SEE ***	POTENTILLA EFFUSA VAR. RUPINCOLA	
3C		POTENTILLA SIERRA-BLANCAE	ROSACEAE		NM
S		POTENTILLA ULIGINOSA	*** SEE ***	POTENTILLA HICKMANII VAR. ULIGINOSA VAR. NOV. /INED.	
1		POUTERIA AUAHIENSIS	SAPOTACEAE	ALA'A	HI
1		POUTERIA RHYNCHOSPERMA	SAPOTACEAE	ALA'A	HI
2		PRENANTHES BOOTTII	ASTERACEAE	RATTLESNAKE ROOT, BOOTT'S	ME NH NY VT
3C		PRENANTHES ROANENSIS	ASTERACEAE	RATTLESNAKE ROOT, MOUNTAIN	NC TN VA
2		PRIMULA CAPILLARIS	PRIMULACEAE	PRIMROSE,	NV
3C		PRIMULA CUSICKIANA	PRIMULACEAE	PRIMROSE, WALLOWA	ID OR
2		PRIMULA HUNNEWELLII	PRIMULACEAE		AZ
LT		PRIMULA MAGUIREI	PRIMULACEAE	PRIMROSE, MAGUIRE	UT
3C		PRIMULA MISTASSINICA	PRIMULACEAE	PRIMROSE, BIRD'S-EYE	IL IA ME MI MN NY VT WI, Canada
2		PRIMULA NEVADENSIS	PRIMULACEAE	PRIMROSE,	NV
3C		PRIMULA SPECUICOLA	PRIMULACEAE	PRIMROSE,	AZ UT
2		PRIMULA WILCOXIANA /SP. NOV. INED.	PRIMULACEAE		ID
1		PRITCHARDIA AYLMER-ROBINSONII	ARECACEAE	HAWANE (LO'ULU)	HI
2		PRITCHARDIA ELLIPTICA	ARECACEAE	LO'ULU	HI
1		PRITCHARDIA ERIOPHORA	ARECACEAE	LO'ULU	HI
2		PRITCHARDIA GAUDICHAUDII	ARECACEAE	LO'ULU	HI
1		PRITCHARDIA GLABRATA	ARECACEAE		HI
1		PRITCHARDIA HILLEBRANDII	ARECACEAE	LO'ULU-LELO	HI
3C		PRITCHARDIA KAALAE VAR. KAALAE	ARECACEAE	LO'ULU	HI
3C		PRITCHARDIA KAALAE VAR. MINIMA	ARECACEAE	LO'ULU	HI
2		PRITCHARDIA KAHANAE	ARECACEAE	LO'ULU	HI
1		PRITCHARDIA LANAIENSIS	ARECACEAE	LO'ULU	HI
1		PRITCHARDIA MONTIS-KEA	ARECACEAE		HI
1		PRITCHARDIA MUNROII	ARECACEAE		HI
1		PRITCHARDIA REMOTA	ARECACEAE		HI
2		PRITCHARDIA SCHATTAUERI	ARECACEAE		HI
3B		PRIVA PORTORICENSIS	VERBENACEAE		PR
2		PROBOSCIDEA SABULOSA	PEDALIACEAE		NM TX
2		PROBOSCIDEA SPICATA	PEDALIACEAE		TX
3C		PRUNUS ALLEGHANIENSIS	ROSACEAE	PLUM, ALLEGHANY	CT MD MI NJ PA VA WV
1		PRUNUS GENICULATA	ROSACEAE	PLUM, SCRUB	FL
S		PRUNUS GRAVESII	*** SEE ***	PRUNUS MARITIMA VAR. GRAVESII	
3C		PRUNUS HAVARDII	ROSACEAE		TX
2		PRUNUS MARITIMA VAR. GRAVESII	ROSACEAE	PLUM, BEACH, GRAVE'S	CT
3C		PRUNUS MINUTIFLORA	ROSACEAE		TX
3C		PRUNUS MURRAYANA	ROSACEAE		TX
3C		PRUNUS TEXANA	ROSACEAE		TX

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
2	PSEUDOBAHIA BAHIAEFOLIA	ASTERACEAE	PSEUDOBAHIA, HARTWEG'S	CA
2	PSEUDOBAHIA PEIRSONII	ASTERACEAE	PSEUDOBAHIA, TULARE	CA
5	PSEUDOTAENIDIA MONTANA	*** SEE ***	TAENIDIA MONTANA	
1	PSIDIUM SINTENISII	MYRTACEAE	HOJA MENUDA	PR
2	PSORALEA EPIPSILA	FABACEAE	SCURF-PEA,	AZ UT
1	* PSORALEA MACROPHYLLA	FABACEAE	SCURF-PEA,	NC
2	PSORALEA PARIENSIS	FABACEAE		UT
2	PSORALEA RYDBERGII	FABACEAE		TX MEXICO
1	* PSORALEA STIPULATA	FABACEAE	SCURF-PEA,	IN KY
3C	PSORALEA SUBACAULIS	FABACEAE		AL GA TN
2	PSORALEA TRINERVATA	FABACEAE		NM TX MEXICO
3C	PSOROTHAMNUS ARBORESCENS	FABACEAE		CA
3C	PSOROTHAMNUS KINGII	FABACEAE		NV
2	PSOROTHAMNUS POLYADENIUS VAR. JONESII	FABACEAE		UT
3C	PSOROTHAMNUS THOMPSONAE VAR. THOMPSONAE	FABACEAE		AZ UT
2	PSOROTHAMNUS THOMPSONAE VAR. WHITINGII	FABACEAE		AZ UT
1	PSYCHOTRIA GRANDIFLORA	RUBIACEAE		HI
1	* PSYCHOTRIA INSULARUM VAR. PARADISII	RUBIACEAE		HI
3B	PTERALYXIA CAUMIANA	APOCYNACEAE	KAULU	HI
1	PTERALYXIA KAUAIENSIS	APOCYNACEAE	PTERALYXIA, KAUAI	HI
1	PTERIS LIDGATEI	POLYPODIACEAE		HI
2	PTEROGLOSSASPIS ECRISTATA	ORCHIDACEAE		FL GA LA MS NC SC, Cuba
2	PTILAGROSTIS MONGHOLICA SSP. PORTERI	POACEAE	NEEDLE GRASS, PORTER'S	CO
5	PTILAGROSTIS PORTERI	*** SEE ***	PTILAGROSTIS MONGHOLICA SSP. PORTERI	
2	PTILIMNIUM FLUVIATILE	APIACEAE	HARPERELLA,	AL MD NC WV
2	PTILIMNIUM NODOSUM	APIACEAE		GA SC
2	PUCCINELLIA PARISHII	POACEAE	ALKALI GRASS, PARISH'S	AZ CA NM
2	PUCCINELLIA TRIFLORA	POACEAE		AK
3B	PYCNANTHEMUM CURVIPES	LAMIACEAE	MOUNTAIN-MINT,	AL GA TN
3C	PYCNANTHEMUM FLORIDANUM	LAMIACEAE		FL
3A	PYCNANTHEMUM MONOTRICHUM	LAMIACEAE	MOUNTAIN-MINT,	VA
5	PYRROCOMA ACUMINATA	*** SEE ***	HAPLOPAPPUS CONTRACTUS	
5	PYRROCOMA LIATRIFORMIS	*** SEE ***	HAPLOPAPPUS LIATRIFORMIS	
5	PYRROCOMA RADIATUS	*** SEE ***	HAPLOPAPPUS RADIATUS	
5	PYRROCOMA UNIFLORA VAR. GOSSYPINA	*** SEE ***	HAPLOPAPPUS UNIFLORUS SSP. GOSSYPINUS	
1	PYXIDANTHERA BARBULATA VAR. BREVIFOLIA	DIAPENSIACEAE	PIXIE-MOSS, WELL'S (SANDHILL)	NC SC
5	PYXIDANTHERA BREVIFOLIA	*** SEE ***	PYXIDANTHERA BARBULATA VAR. BREVIFOLIA	
3C	QUERCUS ARKANSANA	FAGACEAE		AL AR
3C	QUERCUS GEORGIANA	FAGACEAE		AL GA SC
3C	QUERCUS GRACILIFORMIS	FAGACEAE	OAK, SLENDER	TX
1	QUERCUS HINCKLEYI	FAGACEAE	OAK, HINCKLEY'S	TX
2	QUERCUS OGLETHORPENSIS	FAGACEAE		GA LA SC
3C	QUERCUS PARVULA	FAGACEAE	OAK, SANTA CRUZ ISLAND	CA
2	QUERCUS SHUMARDII VAR. ACERIFOLIA	FAGACEAE		AR
2	QUERCUS TARDIFOLIA	FAGACEAE	OAK, CHIZOS MOUNTAINS	TX
3C	QUERCUS TOMENTELLA	FAGACEAE	OAK, ISLAND	CA, MEXICO
3C	RAILLARDELLA MUIRII	ASTERACEAE	RAILLARDELLA, MUIR'S	CA
3C	RAILLARDELLA PRINGLEI	ASTERACEAE	RAILLARDELLA, SHOWY	CA
3C	RAILLARDELLA SCABRIDA	ASTERACEAE		CA
5	RAILLIARDIA ARBOREA	*** SEE ***	DUBAUTIA ARBOREA	
5	RAILLIARDIA HILLEBRANDII	*** SEE ***	DUBAUTIA HILLEBRANDII	
5	RAILLIARDIA LONCHOPHYLLA	*** SEE ***	DUBAUTIA LONCHOPHYLLA	
5	RAILLIARDIA RETICULATA	*** SEE ***	DUBAUTIA RETICULATA	
5	RAILLIARDIA SHERFFIANA	*** SEE ***	DUBAUTIA SHERFFIANA	
2	RANDIA PORTORICENSIS	RUBIACEAE		PR
1	RANUNCULUS ACRIFORMIS VAR. AESTIVALIS	RANUNCULACEAE	BUTTERCUP, SHARP, AUTUMN	UT
2	RANUNCULUS AUSTRO-OREGANUS	RANUNCULACEAE		OR
3B	RANUNCULUS FASCICULARIS VAR. CUNEIFORMIS	RANUNCULACEAE	CROWFOOT, KERR	TX
3C	RANUNCULUS INAMOENUS VAR. SUBAFFINIS	RANUNCULACEAE	BUTTERCUP,	AZ
3C	RANUNCULUS OCCIDENTALIS SSP. NELSONII	RANUNCULACEAE		AK
2	RANUNCULUS RECONDITUS	RANUNCULACEAE		OR WA
3B	RANUNCULUS SUBCORDATUS	RANUNCULACEAE	BUTTERCUP, BLADEN	NC
3B	RAUVOLFIA HELLERI	APOCYNACEAE		HI
3B	RAUVOLFIA MAUIENSIS	APOCYNACEAE		HI
3B	RAUVOLFIA MOLOKAIENSIS VAR. PARVIFOLIA	APOCYNACEAE		HI
3B	RAUVOLFIA REMOTIFLORA	APOCYNACEAE	WAHAULA HEIAU	HI
3B	RAUVOLFIA SANDWICENSIS VAR. SANDWICENSIS	APOCYNACEAE	HAO	HI
3B	RAUVOLFIA SANDWICENSIS VAR. SUBACUMINATA	APOCYNACEAE		HI
2	RAVENIA URBANII	RUTACEAE	TORTUGO ARIETO	PR
1	REMYA KAUAIENSIS	ASTERACEAE		HI
1	REMYA MAUIENSIS	ASTERACEAE	REMYA, MAUI	HI
5	REYNOLDSIA DEGENERI	*** SEE ***	REYNOLDSIA SANDWICENSIS	
5	REYNOLDSIA HILLEBRANDII	*** SEE ***	REYNOLDSIA SANDWICENSIS	
5	REYNOLDSIA HUEHUENSIS	*** SEE ***	REYNOLDSIA SANDWICENSIS	
5	REYNOLDSIA MAUIENSIS	*** SEE ***	REYNOLDSIA SANDWICENSIS	
3C	REYNOLDSIA SANDWICENSIS	ARALIACEAE	'OHE	HI
5	REYNOLDSIA VENUSTA	*** SEE ***	REYNOLDSIA SANDWICENSIS	
3C	RHAPIDOPHYLLUM HYSTRIX	ARECACEAE	PALM, NEEDLE	AL FL GA MS SC
2	RHEXIA ARISTOSA	MELASTOMATACEAE	MEADOWBEAUTY, AWNED	AL DE GA NC NJ SC VA
2	RHEXIA PARVIFLORA	MELASTOMATACEAE	MEADOWBEAUTY,	AL FL GA
2	RHEXIA SALICIFOLIA	MELASTOMATACEAE	MEADOWBEAUTY, PANHANDLE	AL FL
3C	RHINANTHUS ARCTICUS	SCROPHULARIACEAE		AK
3C	RHIPSALIS BACCIFERA	CACTACEAE		FL, Mexico, Sri Lanka, Africa, Central America, South America, West Indies

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
S	RHIPSALIS CASSUTHA	*** SEE ***	RHIPSALIS BACCIFERA	
3C	RHODODENDRON AUSTRINUM	ERICACEAE		AL FL GA MS
3C	RHODODENDRON BAKERI	ERICACEAE	RHODODENDRON, BAKER'S	AL GA KY NC TN VA
LE	RHODODENDRON CHAPMANII	ERICACEAE	RHODODENDRON, CHAPMAN	FL
S	RHODODENDRON MINUS VAR. CHAPMANII	*** SEE ***	RHODODENDRON CHAPMANII	
2	RHODODENDRON PRUNIFOLIUM	ERICACEAE	AZALEA, PLUMLEAF	AL GA
3C	RHODODENDRON VASEYI	ERICACEAE	AZALEA, PINK-SHELL	NC
3C	RHODODON CILIATUS	LAMIACEAE		TX
S	RHUS AROMATICA VAR. ARENARIA	*** SEE ***	RHUS TRILOBATA VAR. ARENARIA	
3C	RHUS KEARNEYI	ANACARDIACEAE	SUMAC, KEARNEY'S	AZ
1	RHUS MICHAUXII	ANACARDIACEAE		GA NC SC
2	RHUS TRILOBATA VAR. ARENARIA	ANACARDIACEAE	SUMAC,	IL IN OH
S	RHYNCHOPHORUM FLORIDANUM	*** SEE ***	PEPEROMIA FLORIDANA	
2	RHYNCHOSIA CINEREA	FABACEAE		FL
2	RHYNCHOSPORA CALIFORNICA	CYPERACEAE	BEAKED-RUSH, CALIFORNIA	CA
2	RHYNCHOSPORA CRINIPES	CYPERACEAE	BEAKED-RUSH,	AL
3C	RHYNCHOSPORA CULIXA	CYPERACEAE		FL GA
2	RHYNCHOSPORA GLOBULARIS VAR. SAXICOLA	CYPERACEAE		GA
1	RHYNCHOSPORA KNIESKERNII	CYPERACEAE	BEAKED-RUSH, KNIESKERN'S	DE NJ
2	RHYNCHOSPORA PUNCTATA	CYPERACEAE		FL GA
3C	RHYSOPTERUS PLURIJUGUS	APIACEAE		OR
2	RIBES CANTHARIFORME	SAXIFRAGACEAE	CURRANT, MORENO	CA
LT	RIBES ECHINELLUM	SAXIFRAGACEAE	GOOSEBERRY, FLORIDA (MICCOSUKEE)	FL SC
1	ROLLANDIA ANGUSTIFOLIA VAR. OCHREATA	CAMPANULACEAE		HI
2	ROLLANDIA CALYCINA	CAMPANULACEAE		HI
1	ROLLANDIA CRISPA VAR. CRISPA	CAMPANULACEAE		HI
1	ROLLANDIA DEGENERANA	CAMPANULACEAE		HI
1	ROLLANDIA HUMBOLDTIANA	CAMPANULACEAE		HI
1	ROLLANDIA LANCEOLATA	CAMPANULACEAE	ROLLANDIA, LANCEOLATE-LEAVED	HI
1	* ROLLANDIA PARVIFOLIA	CAMPANULACEAE		HI
1	ROLLANDIA PINNATIFIDA	CAMPANULACEAE		HI
2	ROLLANDIA PURPURELLIFOLIA	CAMPANULACEAE		HI
1	ROLLANDIA SESSILIFOLIA	CAMPANULACEAE		HI
1	ROLLANDIA ST-JOHNII	CAMPANULACEAE		HI
1	ROLLANDIA WAIANAEENSIS	CAMPANULACEAE		WI
3C	ROMANZOFFIA THOMPSONII /SP. NOV. (NED.	HYDROPHYLLACEAE		OR
2	RORIPPA CALYCINA	BRASSICACEAE		MT ND WY, Canada (N.W.T.)
S	RORIPPA CALYCINA VAR. COLUMBIAE	*** SEE ***	RORIPPA COLUMBIAE	
2	* RORIPPA COLORADENSIS	BRASSICACEAE	WATER CRESS,	CO
2	RORIPPA COLUMBIAE	BRASSICACEAE		CA OR WA
1	RORIPPA SUBUMBELLATA	BRASSICACEAE		CA NV
2	ROSA STELLATA	ROSACEAE		AZ NM TX
1	ROYSTONEA ELATA	ARECACEAE	PALM, ROYAL, FLORIDA	FL
3C	RUBUS BARTONIANUS	ROSACEAE		ID OR
3C	RUBUS DUPLARIS	ROSACEAE	DEWBERRY,	TX
2	RUBUS GLAUCIFOLIUS VAR. GANDERI	ROSACEAE		CA
3C	RUBUS MISSOURICUS	ROSACEAE		MO
1	RUBUS NIGERRIMUS	ROSACEAE		WA
2	RUBUS WHARTONIAE	ROSACEAE		KY TN
3C	RUDBECKIA AURICULATA	ASTERACEAE		AL
2	RUDBECKIA HELIOPSIDIS	ASTERACEAE		AL GA NC SC VA
2	RUDBECKIA NITIDA VAR. NITIDA	ASTERACEAE		FL GA
2	RUDBECKIA TRILOBA VAR. PINNATILOBA	ASTERACEAE		FL NC
3C	RUELLIA DRUMMONDIANA	ACANTHACEAE		TX
1	RUMEX ORTHONEURUS	POLYGONACEAE	DOCK,	AZ
3C	RUMEX SPIRALIS	POLYGONACEAE		TX
3B	RUPPIA ANOMALA	RUPPIACEAE	YERBA DE ZANJA	PR
3C	SAGERETIA MINUTIFLORA	RHAMNACEAE		AL FL GA MS SC
LE	SAGITTARIA FASCICULATA	ALISMATACEAE	ARROWHEAD, BUNCHED	NC SC
2	SAGITTARIA SANFORDII	ALISMATACEAE		CA
2	SAGITTARIA SECUNDIFLORA	ALISMATACEAE		AL
2	SALIX ARIZONICA	SALICACEAE		AZ
2	SALIX FLORIDANA	SALICACEAE	WILLOW, FLORIDA	FL GA
3C	SALIX FLUVIATILIS	SALICACEAE		OR WA
3B	SALIX INTERIOR VAR. EXTERIOR	SALICACEAE	WILLOW, SANDBAR, INSIDE-OUTSIDE	ME PA
2	SALIX OVALIFOLIA VAR. GLACIALIS	SALICACEAE	WILLOW, ROUND-LEAF	AK
3B	SALVIA BLODGETTII	LAMIACEAE	SAGE, BLODGETT'S	FL
2	SALVIA BRANDEGEI	LAMIACEAE	SAGE, BRANDEGEE'S	CA
3B	SALVIA COLUMBARIAE VAR. ZIEGLERI	LAMIACEAE	CHIA, ZIEGLER'S	CA
3C	SALVIA EREMOSTACHYA	LAMIACEAE		CA
3C	SALVIA FUNEREA	LAMIACEAE		CA NV
2	SALVIA GREATAE	LAMIACEAE		CA
2	SALVIA PENSTEMONOIDES	LAMIACEAE		TX
2	SANICULA HOFFMANNII	APIACEAE	SANICLE, HOFFMAN	CA
2	SANICULA MARITIMA	APIACEAE	SANICLE, ADOBE	CA
3C	SANICULA PECKIANA	APIACEAE	SANICLE, PECK'S	CA OR
3C	SANICULA PURPUREA	APIACEAE	SANICLE, PURPLE-FLOWERED	HI
2	SANICULA SANDWICENSIS	APIACEAE		HI
2	SANICULA SAXATILIS	APIACEAE	SANICLE, ROCK	CA
2	SANICULA TRACYI	APIACEAE	SANICLE, TRACY'S	CA
S	SANIDOPHYLLUM CUMULICOLA	*** SEE ***	HYPERICUM CUMULICOLA	
3B	SANTALUM ELLIPTICUM VAR. LITTORALE	SANTALACEAE		HI

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
PE	SANTALUM FREYCINETIANUM VAR. LANAIENSE	SANTALACEAE	SANDALWOOD, LANAI	HI
S	SANTALUM LANAIENSE	*** SEE ***	SANTALUM FREYCINETIANUM VAR. LANAIENSE	
3B	SANTALUM SALICIFOLIUM	SANTALACEAE		HI
S	SARRACENIA ALABAMENSIS SSP. ALABAMENSIS	*** SEE ***	SARRACENIA RUBRA SSP. ALABAMENSIS	
S	SARRACENIA ALABAMENSIS SSP. WHERRYI	*** SEE ***	SARRACENIA RUBRA SSP. WHERRYI	
S	SARRACENIA JONESII	*** SEE ***	SARRACENIA RUBRA SSP. JONESII	
LE	SARRACENIA OREOPHILA	SARRACENIACEAE	PITCHERPLANT, GREEN	AL GA TN
3C	SARRACENIA PSITTACINA	SARRACENIACEAE	PITCHERPLANT, PARROT	AL FL GA LA MS
1	SARRACENIA RUBRA SSP. ALABAMENSIS	SARRACENIACEAE	PITCHERPLANT, ALABAMA CANEBREAK	AL
1	SARRACENIA RUBRA SSP. JONESII	SARRACENIACEAE	PITCHERPLANT, SWEET,	NC SC
3C	SARRACENIA RUBRA SSP. RUBRA	SARRACENIACEAE	PITCHERPLANT, SWEET, RED-FLOWERED	AL FL GA MS NC SC
2	SARRACENIA RUBRA SSP. WHERRYI	SARRACENIACEAE		AL MS
2	SATUREJA CHANDLERI	LAMIACEAE	SAVORY, SAN MIGUEL	CA, Mexico
3C	SAUSSUREA WEBERI	ASTERACEAE		CO MT
2	SAXIFRAGA ALEUTICA	SAXIFRAGACEAE	SAXIFRAGE, ALEUTIAN	AK
2	SAXIFRAGA CAREYANA	SAXIFRAGACEAE	SAXIFRAGE,	GA NC SC TN VA
2	SAXIFRAGA CAROLINIANA	SAXIFRAGACEAE	SAXIFRAGE, GRAY'S	GA NC TN VA WV
3C	SAXIFRAGA FORBESII	SAXIFRAGACEAE	SAXIFRAGE, FORBES	IL IN IA MN MO WI
2	SAXIFRAGA HITCHCOCKIANA	SAXIFRAGACEAE	SAXIFRAGE, SADDLE MOUNTAIN	OR
S	SAXIFRAGA OCCIDENTALIS VAR. LATIPETIOLATA	*** SEE ***	SAXIFRAGA HITCHCOCKIANA	
PE	SCAEVOLA CORIACEA	GOODENIACEAE	NAUPAKA, DWARF	HI
3C	SCAEVOLA GAUDICHAUDI	GOODENIACEAE	NAUPAKA, MOUNTAIN	HI
1	SCAEVOLA KILAUEAE	GOODENIACEAE	NAUPAKA, KILAUEA	HI
1	SCAEVOLA SKOTTSBERGII	GOODENIACEAE		HI
LE	SCHIEDEA ADAMANTIS	CARYOPHYLLACEAE	SCHIEDEA, DIAMOND HEAD	HI
1	* SCHIEDEA AMPLEXICAULIS	CARYOPHYLLACEAE	MA'OLI'OLI	HI
1	SCHIEDEA APOKREMNOS	MA'OLI'OLI	CARYOPHYLLACEAE	HI
3B	SCHIEDEA GLOBOSA VAR. FOLIOSIOR	CARYOPHYLLACEAE		HI
3C	SCHIEDEA GLOBOSA VAR. GLOBOSA	CARYOPHYLLACEAE	MA'OLI'OLI	HI
3C	SCHIEDEA GLOBOSA VAR. GRAMINIFOLIA	CARYOPHYLLACEAE	MA'OLI'OLI	HI
1	SCHIEDEA HALEAKALENSIS	CARYOPHYLLACEAE	MA'OLI'OLI	HI
3B	SCHIEDEA HAWAIIENSIS	CARYOPHYLLACEAE	MA'OLI'OLI	HI
1	* SCHIEDEA HELLERI	CARYOPHYLLACEAE	MA'OLI'OLI	HI
2	SCHIEDEA HOOKERI	CARYOPHYLLACEAE	MA'OLI'OLI	HI
1	* SCHIEDEA IMPLEXA	CARYOPHYLLACEAE	MA'OLI'OLI	HI
1	SCHIEDEA KAALAE	CARYOPHYLLACEAE	MA'OLI'OLI	HI
3C	SCHIEDEA KEALIAE	CARYOPHYLLACEAE	MA'OLI'OLI	HI
1	SCHIEDEA LIGUSTRINA VAR. NEMATOPODA	CARYOPHYLLACEAE		HI
1	SCHIEDEA LYDGATEI	CARYOPHYLLACEAE	MA'OLI'OLI	HI
3C	SCHIEDEA MANNII	CARYOPHYLLACEAE		HI
2	SCHIEDEA MEMBRANACEA	CARYOPHYLLACEAE		HI
2	SCHIEDEA MENZIESII VAR. MENZIESII	CARYOPHYLLACEAE		HI
2	SCHIEDEA MENZIESII VAR. SPERGULACEA	CARYOPHYLLACEAE	MA'OLI'OLI	HI
S	SCHIEDEA PUBESCENS VAR. LANAIENSIS	*** SEE ***	SCHIEDEA PUBESCENS VAR. PUBESCENS	
2	SCHIEDEA PUBESCENS VAR. PUBESCENS	CARYOPHYLLACEAE	MA'OLI'OLI	HI
2	SCHIEDEA SALICARIA	CARYOPHYLLACEAE	MA'OLI'OLI	HI
1	SCHIEDEA SPERGULINA	CARYOPHYLLACEAE	MA'OLI'OLI	HI
1	* SCHIEDEA STELLARIOIDES	CARYOPHYLLACEAE	MA'OLI'OLI	HI
1	SCHIEDEA VERTICILLATA	CARYOPHYLLACEAE		HI
3C	SCHISANDRA GLABRA	SCHISANDRACEAE		AL AR FL GA LA MS NC SC TN
2	SCHIZACHYRIUM NIVEUM	POACEAE		FL GA
3B	SCHIZACHYRIUM RHIZOMATUM	POACEAE		FL
2	SCHIZAEA GERMANII	SCHIZAEACEAE	FERN, CURLY-GRASS	FL, Belize, Cuba, Guadeloupe
2	SCHIZAEA PUSILLA	SCHIZAEACEAE	FERN, CURLY-GRASS	NJ NY, Canada (Nfld., N.S., Ont.), St. Pierre & Miquelon
S	SCHIZOSTEGE LIDGATEI	*** SEE ***	PTERIS LIDGATEI	
S	SCHMALTZIA KEARNEYI	*** SEE ***	RHUS KEARNEYI	
1	SCHOENOCRAMBE BARNEBYI	BRASSICACEAE		UT
S	SCHOENOLIRION BRACTEOSUM	*** SEE ***	HASTINGSIA BRACTEOSA	
S	SCHOENOLIRION TEXANUM	*** SEE ***	SCHOENOLIRION WRIGHTII	
3C	SCHOENOLIRION WRIGHTII	LILIACEAE	SUNNYBELL, TEXAS	AL AR TX
2	SCHOEPFIA ARENARIA	OLACACEAE	ARANA	PR
1	SCHRANKIA PORTORICENSIS	FABACEAE	ZARZARILLA	PR
2	SCHWALBEA AMERICANA	SCROPHULARIACEAE	CHAFFSEED	CT DE FL GA KY LA MA MD MS NC NJ NY SC TN VA
2	SCIRPUS ANCISTROCHAETUS	CYPERACEAE	BULRUSH, NORTHEASTERN	MA NY PA VA VT
3C	SCIRPUS FLACCIDIFOLIUS	CYPERACEAE		NC VA
2	SCIRPUS LONGII	CYPERACEAE	BULRUSH, LONG'S	CT ME MA NJ NY, Canada (N.S.)
1	SCLERIA DORADOENSIS	CYPERACEAE		PR
S	SCLEROCACTUS FRANKLINII	*** SEE ***	SCLEROCACTUS GLAUCUS	
LT	SCLEROCACTUS GLAUCUS	CACTACEAE	CACTUS, HOOKLESS, UINTA BASIN	CO UT
LT	SCLEROCACTUS MESAE-VERDAE	CACTACEAE	CACTUS, MESA VERDE	CO NM
3C	SCLEROCACTUS POLYANCISTRUS	CACTACEAE	FISHHOOK CACTUS, MOHAVE	CA NV
3C	SCLEROCACTUS PUBISPINUS	CACTACEAE	FISHHOOK CACTUS, GREAT BASIN	NV UT
3C	SCLEROCACTUS SP. /SP. NOV. INED.	CACTACEAE	CACTUS (DUCHESNE & UINTAH COS.)	UT
3C	SCLEROCACTUS SPINOSIOR	CACTACEAE		UT
2	SCLEROCACTUS WHIPPLEI VAR. HEILII	CACTACEAE		NM
3C	SCLEROCACTUS WHIPPLEI VAR. REEVESII	CACTACEAE		NM

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
LE	SCLEROCACTUS WRIGHTIAE	CACTACEAE	FISHHOOK CACTUS, WRIGHT	UT
2	SCROPHULARIA ATRATA	SCROPHULARIACEAE	FIGWORT, BLACK-FLOWERED	CA
1	SCROPHULARIA COCCINEA	SCROPHULARIACEAE	FIGWORT,	NM
5	SCROPHULARIA MACRANTHA	*** SEE ***	SCROPHULARIA COCCINEA	
2	SCROPHULARIA VILLOSA	SCROPHULARIACEAE	FIGWORT, SANTA CATALINA	CA
2	SCUTELLARIA FLORIDANA	LAMIACEAE		FL
2	SCUTELLARIA HOLMGRENIORUM	LAMIACEAE	SKULLCAP, RAVENDALE	CA
2	SCUTELLARIA LAEVIS	LAMIACEAE		TX
1	SCUTELLARIA MONTANA	LAMIACEAE	SKULLCAP, LARGE-FLOWERED	GA TN
1	* SCUTELLARIA OCMULGEE	LAMIACEAE	SKULLCAP,	GA
2	SCUTELLARIA OVATA SSP. PSEUDOARGUTA	LAMIACEAE	SKULLCAP, HEART-LEAVED,	VA WV
5	- SCUTELLARIA SERRATA VAR. MONTANA	*** SEE ***	SCUTELLARIA MONTANA	
2	SCUTELLARIA THIERETII	LAMIACEAE		LA
2	SEDUM ALBOMARGINATUM	CRASSULACEAE	STONECROP, FEATHER RIVER	CA
1	SEDUM LAXUM SSP. EASTWOODIAE	CRASSULACEAE		CA
2	SEDUM LAXUM SSP. FLAVIDUM	CRASSULACEAE	STONECROP, PALE YELLOW	CA
3C	SEDUM LAXUM SSP. HECKNERI	CRASSULACEAE		CA OR
2	SEDUM MORANII	CRASSULACEAE	STONECROP,	OR
2	SEDUM NEVII	CRASSULACEAE	STONECROP,	AL GA TN
3C	SEDUM NIVEUM	CRASSULACEAE		CA
2	SEDUM OBLANCEOLATUM	CRASSULACEAE		OR
2	SEDUM OBTUSATUM SSP. PARADISUM	CRASSULACEAE	STONECROP, CANYON CREEK	CA
2	* SEDUM PINETORUM	CRASSULACEAE	STONECROP, PINE CITY	CA
2	SEDUM PUSILLUM	CRASSULACEAE		GA NC SC
3B	SEDUM RADIATUM SSP. DEPAUPERATUM	CRASSULACEAE		CA OR
2	SEDUM ROBERTSIANUM	CRASSULACEAE		TX
2	SEDUM ROSEA VAR. ROANENSIS	CRASSULACEAE		NC TN
3C	SEDUM TEXANUM	CRASSULACEAE	STONECROP, TEXAS	TX
5	SEDUM TEXANUM	*** SEE ***	LENOPHYLLUM TEXANUM	
2	SELAGINELLA UTAHENSIS	SELAGINELLACEAE		NV UT
3C	SELENIA JONESII	BRASSICACEAE	SELENIA, JONES'	TX
3C	SENECIO ANTENNARIIFOLIUS	ASTERACEAE	RAGWORT, PUSSYTOES	MD PA VA WV
2	SENECIO BERNARDINUS	ASTERACEAE	BUTTERWEED, SAN BERNARDINO	CA
3C	SENECIO CARDAMINE	ASTERACEAE		AZ NM
3B	SENECIO CLEVELANDII VAR. HETEROPHYLLUS	ASTERACEAE		CA
2	SENECIO DIMORPHOPHYLLUS VAR. INTERMEDIUS	ASTERACEAE	GROUNDSEL, INTERMEDIATE	CO UT
1	SENECIO ERTTERAE	ASTERACEAE	RAGWORT, ERTTER'S	OR
LT	SENECIO FRANCISCANUS	ASTERACEAE	GROUNDSEL, SAN FRANCISCO PEAKS	AZ
2	SENECIO GANDERI	ASTERACEAE	BUTTERWEED, GANDER	CA
3B	SENECIO HALLII VAR. DISCOIDEA	ASTERACEAE	GROUNDSEL,	CO
2	SENECIO HESPERIUS	ASTERACEAE		OR
1	SENECIO HUACHUCANUS	ASTERACEAE	GROUNDSEL, HUACHUCA	AZ, Mexico
2	SENECIO LAYNEAE	ASTERACEAE	BUTTERWEED, LAYNE'S	CA
3B	SENECIO LYNCEUS VAR. LEUCOREUS	ASTERACEAE		NV
3C	SENECIO MILLEFOLIUM	ASTERACEAE		GA NC SC
3C	SENECIO NEOWEBSTERI	ASTERACEAE		WA
3C	SENECIO PORTERI	ASTERACEAE	GROUNDSEL, PORTER'S	CO OR
2	SENECIO QUAERENS	ASTERACEAE		NM
3B	SENECIO SANDVICENSIS	ASTERACEAE		HI
3B	SENECIO WARNOCKII	ASTERACEAE		TX
1	SERIANTHES NELSONII	FABACEAE	HAYUN LAGU	GU, Rota
1	SESBANIA ARBOREA	FABACEAE	'OHAI	HI
1	SESBANIA HAWAIIENSIS	FABACEAE	'OHAI	HI
1	* SESBANIA HOBDYI	FABACEAE	'OHAI	HI
1	SESBANIA MOLOKAIENSIS	FABACEAE	'OHAI	HI
1	SESBANIA TOMENTOSA	FABACEAE	'OHAI	HI
5	SESBANIA TOMENTOSA VAR. MOLOKAIENSIS	*** SEE ***	SESBANIA MOLOKAIENSIS	
1	* SESUVIUM TRIANTHEMOIDES	AIZOACEAE	SEA-PURSLANE, TEXAS	TX
3B	- SEYMERIA HAVARDII	SCROPHULARIACEAE		TX
2	SHORTIA GALACIFOLIA	DIAPENSIACEAE	OCONEE-BELLS	GA NC SC
3B	SHORTIA GALACIFOLIA VAR. BREVISTYLA	DIAPENSIACEAE	OCONEE-BELLS, SHORT-STYLED	GA NC SC
2	SHOSHONEA PULVINATA	APIACEAE		WY
1	* SIBARA FILIFOLIA	BRASSICACEAE	ROCK CRESS, ISLAND	CA
2	SIBARA GRISEA	BRASSICACEAE		NM
3C	SIBARA ROSULATA	BRASSICACEAE		CA
5	SICYOS ATOLLENSIS	*** SEE ***	CLADOCARPA ATOLLENSIS	
5	SICYOS CAUMII	*** SEE ***	CLADOCARPA CAUMII	
5	SICYOS LAMOUREUXII	*** SEE ***	CLADOCARPA LAMOUREUXII	
1	SICYOS LAYSANENSIS	CUCURBITACEAE		HI
5	SICYOS MAXIMOWICZII	*** SEE ***	CLADOCARPA MAXIMOWICZII	
1	SICYOS NIHOAENSIS	CUCURBITACEAE		HI
5	SICYOS NIIHAUENSIS	*** SEE ***	CLADOCARPA NIIHAUENSIS	
5	SICYOS SEMITONSUS	*** SEE ***	CLADOCARPA SEMITONSUS	
5	SIDA EGGERSII	*** SEE ***	ABUTILON VIRGINIANUM	
3C	SIDA HERMAPHRODITA	MALVACEAE	MALLOW, VIRGINIA	DC IN KY MD MI OH PA TN VA WV
2	SIDA INFLEXA	MALVACEAE		VA
2	SIDA LEDYARDII	MALVACEAE		HI
2	SIDA NELSONII	MALVACEAE		HI
2	SIDA RUBROMARGINATA	MALVACEAE		FL
2	SIDALCEA CAMPESTRIS	MALVACEAE	CHECKER-MALLOW, MEADOW	OR
3C	SIDALCEA CANDIDA	MALVACEAE		CO NV NM UT WY
2	SIDALCEA COVILLEI	MALVACEAE	CHECKER-MALLOW, OWENS VALLEY	CA

STATUS		SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
3C		SIDALCEA CUSICKII	MALVACEAE		OR
2		SIDALCEA HICKMANII SSP. ANOMALA	MALVACEAE	SIDALCEA, CUESTA PASS	CA
2		SIDALCEA HICKMANII SSP. HICKMANII	MALVACEAE	SIDALCEA, HICKMAN	CA
2		SIDALCEA HICKMANII SSP. PARISHII	MALVACEAE	SIDALCEA, PRAISH	CA
2		SIDALCEA HICKMANII SSP. VIRIDIS	MALVACEAE	MALLOW, MARIN	CA
1	*	SIDALCEA KECKII	MALVACEAE	SIDALCEA, KECK	CA
3C		SIDALCEA MALVAEFLORA SSP. ELEGANS	MALVACEAE		CA OR
2		SIDALCEA NELSONIANA	MALVACEAE	CHECKER-MALLOW, NELSON'S	OR
3B		SIDALCEA OREGANA SSP. HYDROPHILA	MALVACEAE	CHECKER-MALLOW, WATER-LOVING	CA
2		SIDALCEA OREGANA SSP. VALIDA	MALVACEAE	CHECKER-MALLOW, KENWOOD MARSH	CA
2		SIDALCEA OREGANA VAR. CALVA	MALVACEAE		WA
LE		SIDALCEA PEDATA	MALVACEAE	CHECKER-MALLOW, PEDATE	CA
2		SIDALCEA ROBUSTA	MALVACEAE	SIDALCEA, BUTTE COUNTY	CA
3C		SIDALCEA SETOSA	MALVACEAE		CA OR
1		SIDALCEA STIPULARIS	MALVACEAE		CA
1		SILENE ALEXANDRI	CARYOPHYLLACEAE		HI
3C		SILENE APERTA	CARYOPHYLLACEAE		CA
1		SILENE CAMPANULATA SSP. CAMPANULATA	CARYOPHYLLACEAE		CA
2		SILENE CLOKEYI	CARYOPHYLLACEAE		NV
1	*	SILENE CRYPTOPETALA	CARYOPHYLLACEAE		HI
1	*	SILENE DEGENERI	CARYOPHYLLACEAE		HI
2		SILENE DOUGLASII VAR. ORARIA	CARYOPHYLLACEAE	CATCHFLY, CASCADE HEAD	OR
1		SILENE HAWAIIENSIS	CARYOPHYLLACEAE		HI
3C		SILENE INVISA	CARYOPHYLLACEAE	CATCHFLY, RED FIR	CA
1		SILENE LANCEOLATA	CARYOPHYLLACEAE		HI
1		SILENE MARMORENSIS	CARYOPHYLLACEAE	CATCHFLY, MARBLE MOUNTAINS	CA
2		SILENE OCCIDENTALIS SSP. LONGISTIPITATA	CARYOPHYLLACEAE	CAMPION, BUTTE COUNTY	CA
1		SILENE PETERSONII VAR. MINOR	CARYOPHYLLACEAE	CATCHFLY, RED CANYON	UT
2		SILENE PETERSONII VAR. PETERSONII	CARYOPHYLLACEAE	CATCHFLY, PLATEAU	UT
3C		SILENE PLANKII	CARYOPHYLLACEAE		NM TX
1		SILENE POLYPETALA	CARYOPHYLLACEAE		FL GA
2		SILENE RECTIRAMEA	CARYOPHYLLACEAE		AZ
2		SILENE REGIA	CARYOPHYLLACEAE		AL AR GA IL IN KS KY MO OH OK
3C		SILENE SCAPOSA VAR. LOBATA	CARYOPHYLLACEAE		ID NV OR
2		SILENE SCAPOSA VAR. SCAPOSA	CARYOPHYLLACEAE		OR
2		SILENE SEELYI	CARYOPHYLLACEAE		WA
2		SILENE SPALDINGII	CARYOPHYLLACEAE		ID MT OR WA
2		SILENE VERECUNDA SSP. VERECUNDA	CARYOPHYLLACEAE	CAMPION, DOLORES	CA
3C		SILENE WRIGHTII	CARYOPHYLLACEAE		NM
2		SILPHIUM BRACHIATUM	ASTERACEAE	ROSINWEED,	AL TN
2		SILPHIUM CONFERTIFOLIUM	ASTERACEAE		AL MS
3B		SILPHIUM INTEGRIFOLIUM VAR. GATTINGERI	ASTERACEAE	ROSINWEED,	TN
3B		SISYMBRIUM KEARNEYI	BRASSICACEAE		AZ
2		SISYRINCHIUM SARMENTOSUM	IRIDACEAE		WA
2		SIUM FLORIDANUM	APIACEAE	WATER-PARSNIP, FLORIDA	AL FL
2		SMELOWSKIA BOREALIS VAR. VILLOSA	BRASSICACEAE		AK
3C		SMELOWSKIA HOLMGRENII	BRASSICACEAE		NV
2		SMELOWSKIA OVALIS SSP. CONGESTA	BRASSICACEAE		CA
2		SMELOWSKIA PYRIFORMIS	BRASSICACEAE		AK
1	*	SMILAX LEPTANTHERA	LILIACEAE		GA
2		SMILAX MELASTOMIFOLIA VAR. MELASTOMIFOLIA	LILIACEAE		HI
3A		SOLANUM BAHAMENSE VAR. RUGELII	SOLANACEAE	NIGHTSHADE,	FL
2		SOLANUM CAROLINENSE VAR. FLORIDANUM	SOLANACEAE		FL
1	*	SOLANUM CAROLINENSE VAR. HIRSUTUM	SOLANACEAE	HORSE-NETTLE,	GA
1	*	SOLANUM CONOCARPUM	SOLANACEAE		VI
1		SOLANUM DRYMOPHILUM	SOLANACEAE	ERUBIA	PR
S		SOLANUM GODFREYI	*** SEE ***	SOLANUM CAROLINENSE VAR. FLORIDANUM	
1	*	SOLANUM HALEAKALAENSE	SOLANACEAE		HI
1	*	SOLANUM HILLEBRANDII	SOLANACEAE		HI
1		SOLANUM INCOMPLETUM	SOLANACEAE	POPOLO, THORNY	HI
1		SOLANUM KAUAIENSE	SOLANACEAE	POPOLO-'AI-A-KE-AKUA	HI
2		SOLANUM MUCRONATUM	SOLANACEAE		PR VI
3C		SOLANUM NELSONII VAR. NELSONII	SOLANACEAE	NIGHTSHADE, NELSON	HI
1		SOLANUM NELSONII VAR. THOMASIAEFOLIUM	SOLANACEAE		HI
1		SOLANUM SANDWICENSE	SOLANACEAE		HI
2		SOLANUM TENUILOBATUM	SOLANACEAE	NIGHTSHADE, NARROW-LEAVED	CA, Mexico
2		SOLANUM WOODBURYI	SOLANACEAE		PR
1		SOLIDAGO ALBOPILOSA	ASTERACEAE	GOLDENROD,	KY
3C		SOLIDAGO ARGUTA VAR. HARRISSII	ASTERACEAE		MD PA VA WV
S		SOLIDAGO HARRISSII	*** SEE ***	SOLIDAGO ARGUTA VAR. HARRISSII	
1		SOLIDAGO HOUGHTONII	ASTERACEAE	GOLDENROD, HOUGHTON'S	MI, Canada (Ont.)
3C		SOLIDAGO LINDHEIMERIANA	ASTERACEAE		TX
3C		SOLIDAGO MOLLIS VAR. ANGUSTATA	ASTERACEAE		TX
2	*	SOLIDAGO PORTERI	ASTERACEAE	GOLDENROD, PORTER'S	GA
1		SOLIDAGO PULCHRA	ASTERACEAE		NC
LE		SOLIDAGO SHORTII	ASTERACEAE	GOLDENROD, SHORT'S	KY
LT		SOLIDAGO SPITHAMAEA	ASTERACEAE		NC TN
2		SOLIDAGO VERNA	ASTERACEAE		NC SC
3C		SOPHORA ARIZONICA	FABACEAE		AZ
2		SOPHORA CHRYSOPHYLLA VAR. CIRCULARIS	FABACEAE		HI
2		SOPHORA CHRYSOPHYLLA VAR. ELLIPTICA	FABACEAE		HI

STATUS		SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
2		SOPHORA CHRYSOPHYLLA VAR. GLABRATA	FABACEAE		HI
1		SOPHORA CHRYSOPHYLLA VAR. GRISEA	FABACEAE		HI
2		SOPHORA CHRYSOPHYLLA VAR. KANAIOENSIS	FABACEAE		HI
2		SOPHORA CHRYSOPHYLLA VAR. KAUENSIS	FABACEAE		HI
3A		SOPHORA CHRYSOPHYLLA VAR. LANAIENSIS	FABACEAE		HI
1		SOPHORA CHRYSOPHYLLA VAR. MAKUAENSIS	FABACEAE		HI
1	*	SOPHORA CHRYSOPHYLLA VAR. UNIFOLIATA	FABACEAE		HI
3B		SOPHORA FORMOSA	FABACEAE		AZ
2		SOPHORA GYPSOPHILA VAR. GUADALUPENSIS	FABACEAE		NM TX
2		SOPHORA LEACHIANA	FABACEAE		OR
2		SPHAERALCEA CAESPITOSA	MALVACEAE	GLOBE-MALLOW, JONES	NV UT
3B		SPHAERALCEA FENDLERI VAR. ALBESCENS	MALVACEAE	GLOBE-MALLOW,	AZ
2		SPHAERALCEA PROCERA	MALVACEAE		NM
2		SPHAERALCEA PSORALOIDES	MALVACEAE		UT
2		SPHAERALCEA RUSBYI SSP. EREMICOLA	MALVACEAE	MALLOW, DESERT, RUSBY	CA
1		SPHAEROMERIA COMPACTA	ASTERACEAE	TANSY,	NV
2		SPHAEROMERIA RUTHIAE	ASTERACEAE	TANSY, ZION	UT
2		SPHAEROMERIA SIMPLEX	ASTERACEAE	FALSE SAGEBRUSH, LARAMIE	WY
2		SPHENOSTIGMA COELESTINUM	IRIDACEAE	IXIA, BARTRAM'S	FL
2		SPIGELIA GENTIANOIDES	LOGANIACEAE	PINKROOT,	FL
3C		SPIGELIA LOGANIOIDES	LOGANIACEAE	PINKROOT,	FL
3C		SPIGELIA TEXANA	LOGANIACEAE		TX
2		SPIRAEA VIRGINIANA	ROSACEAE	SPIRAEA, VIRGINIA	GA NC PA TN WV
2		SPIRANTHES DILUVIALIS	ORCHIDACEAE		CO UT
2		SPIRANTHES LANCEOLATA VAR. PALUDICOLA	ORCHIDACEAE	LADIES'-TRESSES,	FL
LE		SPIRANTHES PARKSII	ORCHIDACEAE	LADIES'-TRESSES, NAVASOTA	TX
2		SPIRANTHES POLYANTHA	ORCHIDACEAE		FL PR, Bahamas, Dominican Republic, Guatemala, Mexico
5		SPOROBOLUS NEGLECTUS VAR. OZARKANUS	*** SEE ***	SPOROBOLUS OZARKANUS	
2		SPOROBOLUS OZARKANUS	POACEAE		KS MO
3B		SPOROBOLUS PATENS	POACEAE	DROPSEED,	AZ
2		SPOROBOLUS TERETIFOLIUS	POACEAE		GA NC SC
2		STACHYS HYSSOPIFOLIA VAR. LYTHROIDES	LAMIACEAE		FL
5		STACHYS LYTHROIDES	*** SEE ***	STACHYS HYSSOPIFOLIA VAR. LYTHROIDES	
1		STAHLIA MONOSPERMA	FABACEAE	COBANA NEGRA	PR, Dominican Republic
3B		STANLEYA PINNATA VAR. GIBBEROSA	BRASSICACEAE		WY
5		STEIRONEMA CILIATUM	*** SEE ***	STEIRONEMA LAEVIGATUM	
3C		STEIRONEMA LAEVIGATUM	PRIMULACEAE	LOOSESTRIFE, FRINGED	AL AZ CO CT FL ID LA ME MA MS MT NH NM OR RI TX VT WA, Canada (B.C., N.S.)
5		STELLARIA FONTINALIS	*** SEE ***	ARENARIA FONTINALIS	
3C		STELLARIA IRRIGUA	CARYOPHYLLACEAE	CHICKWEED, (STARWORT,)	CO NM, U.S.S.R.
3C		STENANDRIUM FASCICULARIS	ACANTHACEAE		TX, Mexico
1		STENOGYNE AFFINIS VAR. AFFINIS	LAMIACEAE		HI
1		STENOGYNE AFFINIS VAR. DEGENERI	LAMIACEAE		HI
LE		STENOGYNE ANGUSTIFOLIA VAR. ANGUSTIFOLIA	LAMIACEAE		HI
1		STENOGYNE ANGUSTIFOLIA VAR. HILLEBRANDII	LAMIACEAE		HI
1	*	STENOGYNE ANGUSTIFOLIA VAR. MAUIENSIS	LAMIACEAE		HI
1		STENOGYNE ANGUSTIFOLIA VAR. MEEBOLDII	LAMIACEAE		HI
1	*	STENOGYNE ANGUSTIFOLIA VAR. SPATHULATA	LAMIACEAE		HI
2		STENOGYNE CALAMINTHOIDES VAR. OXYODONATA	LAMIACEAE		HI
1	*	STENOGYNE CINEREA	LAMIACEAE		HI
1		STENOGYNE CRENATA	LAMIACEAE	STENOGYNE, CRENATE-LEAVED	HI
2		STENOGYNE DIFFUSA	LAMIACEAE		HI
2		STENOGYNE GLABRATA	LAMIACEAE		HI
2		STENOGYNE HALIAKALAE	LAMIACEAE	STENOGYNE, HALEAKALA	HI
2		STENOGYNE HIRSUTULA	LAMIACEAE		HI
1		STENOGYNE KANEHOANA	LAMIACEAE		HI
1		STENOGYNE MACRANTHA	LAMIACEAE		HI
1		STENOGYNE MICROPHYLLA	LAMIACEAE		HI
1		STENOGYNE MOLLIS	LAMIACEAE		HI
2		STENOGYNE OXYGONA	LAMIACEAE		HI
2		STENOGYNE PURPUREA VAR. FORBESII	LAMIACEAE		HI
1		STENOGYNE ROTUNDIFOLIA VAR. OBLONGA	LAMIACEAE		HI
5		STENOGYNE RUGOSA VAR. MOLLIS	*** SEE ***	STENOGYNE MOLLIS	
2		STENOGYNE RUGOSA VAR. SUBULATA	LAMIACEAE	MA'OHI'OHI	HI
3B		STENOGYNE SALICIFOLIA	LAMIACEAE		HI
1	*	STENOGYNE SCANDENS	LAMIACEAE		HI
2		STENOGYNE SCROPHULARIOIDES	LAMIACEAE		HI
1		STENOGYNE SESSILIS VAR. HEXANTHA	LAMIACEAE		HI
1		STENOGYNE SESSILIS VAR. LANIENSIS	LAMIACEAE		HI
1	*	STENOGYNE SESSILIS VAR. WILKESII	LAMIACEAE		HI
1		STENOGYNE SHERFFII	LAMIACEAE		HI
1	*	STENOGYNE SORORIA	LAMIACEAE		HI
1	*	STENOGYNE VAGANS	LAMIACEAE		HI
2		STENOGYNE VIRIDIS	LAMIACEAE		HI
5		STEPHANOMERIA BLAIRII	*** SEE ***	MUNZOTHAMNUS BLAIRII	
LE		STEPHANOMERIA MALHEURENSIS	ASTERACEAE	WIRE-LETTUCE, MALHEUR	OR
3C		STEPHANOMERIA SCHOTTII	ASTERACEAE	WIRE-LETTUCE, SCHOTT'S	AZ
2		STILLINGIA SYLVATICA SSP. TENUIS	EUPHORBIACEAE		FL
3C		STIPA CURVIFOLIA	POACEAE		NM

STATUS		SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
3B		STIPA LEMMONII VAR. PUBESCENS	POACEAE	NEEDLE GRASS, HAIRY LEMMON'S	CA
2		STREPTANTHUS ALBIDUS SSP. ALBIDUS	BRASSICACEAE	JEWELFLOWER, METCALF CANYON	CA
2		STREPTANTHUS BATRACHOPUS	BRASSICACEAE	STREPTANTHUS, TAMALPAIS	CA
3C		STREPTANTHUS BERNARDINUS	BRASSICACEAE		CA
2		STREPTANTHUS BRACHIATUS	BRASSICACEAE	STREPTANTHUS, CONTACT MINE	CA
2		STREPTANTHUS BRACTEATUS	BRASSICACEAE		TX
2		STREPTANTHUS CALLISTUS	BRASSICACEAE	JEWELFLOWER, ROYAL	CA
3C		STREPTANTHUS CARINATUS	BRASSICACEAE		TX
2		STREPTANTHUS CORDATUS VAR. PIUTENSIS	BRASSICACEAE		CA
2		STREPTANTHUS CUTLERI	BRASSICACEAE		TX
3C		STREPTANTHUS FARNSWORTHIANUS	BRASSICACEAE	JEWELFLOWER, EVALYN'S	CA
3C		STREPTANTHUS FENESTRATUS	BRASSICACEAE		CA
2		STREPTANTHUS GLANDULOSUS VAR. HOFFMANII	BRASSICACEAE		CA
3C		STREPTANTHUS GLANDULOSUS VAR. PULCHELLUS	BRASSICACEAE		CA
3C		STREPTANTHUS GRACILIS	BRASSICACEAE	STREPTANTHUS, ALPINE	CA
2		STREPTANTHUS HISPIDUS	BRASSICACEAE	JEWELFLOWER, MT. DIABLO	CA
2		STREPTANTHUS HOWELLII	BRASSICACEAE		CA OR
2		STREPTANTHUS INSIGNIS SSP. LYONII /INED.	BRASSICACEAE	JEWELFLOWER, ERUHA RANCH	CA
2		STREPTANTHUS LEMMONII	BRASSICACEAE	JEWELFLOWER, LEMMON'S	AZ
2		STREPTANTHUS MORRISONII	BRASSICACEAE	JEWELFLOWER, MORRISON'S	CA
1		STREPTANTHUS NIGER	BRASSICACEAE	JEWELFLOWER, TIBURON	CA
3C		STREPTANTHUS OLIGANTHUS	BRASSICACEAE	STREPTANTHUS, MASONIC MOUNTAIN	CA NV
2		STREPTANTHUS SPARSIFLORUS	BRASSICACEAE	JEWELFLOWER, SPARSELY-FLOWERED	TX
2		STREPTANTHUS SQUAMIFORMIS	BRASSICACEAE	JEWELFLOWER,	AR OK
2		STROGANOWIA TIEHMII	BRASSICACEAE	STROGANOWIA, TIEHM'S	NV
2		STYLISMA PICKERINGII VAR. PICKERINGII	CONVOLVULACEAE	MORNING-GLORY, PICKERING'S	GA NC NJ SC
S		STYLOPHYLLUM TRASKIAE	*** SEE ***	DUDLEYA TRASKIAE	
3B		STYPHELIA TAMEIAMEIAE VAR. HEXAMERA	EPACRIDACEAE		HI
3C		STYRAX PLATANIFOLIA VAR. STELLATA	STYRACACEAE	SILVERBELLS,	TX
3A		STYRAX PORTORICENSIS	STYRACACEAE	PALO DE JAZMIN	PR
LE		STYRAX TEXANA	STYRACACEAE	SNOWBELLS, TEXAS	TX
2		STYRAX YOUNGAE	STYRACACEAE		TX, Mexico
1	*	SUAEDA DURIPES	CHENOPODIACEAE	SEEPWEED, HARDTOE	TX
3C		SULLIVANTIA HAPEMANII	SAXIFRAGACEAE		MT WY
S		SULLIVANTIA OHIONIS	*** SEE ***	SULLIVANTIA SULLIVANTII	
2		SULLIVANTIA OREGANA	SAXIFRAGACEAE		OR WA
3C		SULLIVANTIA PURPUSII	SAXIFRAGACEAE		CO
2		SULLIVANTIA RENIFOLIA	SAXIFRAGACEAE	SULLIVANTIA, KIDNEY-LEAVED	IL IA MN MO WI
3C		SULLIVANTIA SULLIVANTII	SAXIFRAGACEAE	SULLIVANTIA,	IN KY OH
LE		SWALLENIA ALEXANDRAE	POACEAE	GRASS, EUREKA DUNES	CA
S		SWERTIA COLORADENSIS	*** SEE ***	FRASERA COLORADENSIS	
2		SYMPHORICARPOS GUADALUPENSIS	CAPRIFOLIACEAE		TX
2		SYNANDRA HISPIDULA	LAMIACEAE	SYNANDRA,	AL IL IN KY NC OH TN VA WV
3C		SYNTHYRIS CANBYI	SCROPHULARIACEAE		MT
S		SYNTHYRIS HENDERSONII	*** SEE ***	SYNTHYRIS PINNATIFIDA VAR. CANESCENS	
2	*	SYNTHYRIS MISSURICA SSP. HIRSUTA	SCROPHULARIACEAE		OR
3C		SYNTHYRIS MISSURICA SSP. STELLATA /INED.	SCROPHULARIACEAE		OR WA
3C		SYNTHYRIS PINNATIFIDA VAR. CANESCENS	SCROPHULARIACEAE		ID MT
3C		SYNTHYRIS PINNATIFIDA VAR. LANUGINOSA	SCROPHULARIACEAE		WA
3C		SYNTHYRIS PLATYCARPA	SCROPHULARIACEAE		ID
1		SYNTHYRIS RANUNCULINA	SCROPHULARIACEAE	KITTENTAILS,	NV
3C		SYNTHYRIS SCHIZANTHA	SCROPHULARIACEAE		OR WA
2		TAENIDIA MONTANA	APIACEAE	PIMPERNEL, MOUNTAIN	MD PA VA WV
3C		TAGETES LEMMONII	ASTERACEAE		AZ
3B		TALINUM APPALACHIANUM	PORTULACACEAE		AL
2		TALINUM CALCARICUM	PORTULACACEAE		AL TN
3B		TALINUM GOODDINGII	PORTULACACEAE	FLAMEFLOWER, GOODDING	AZ
2		TALINUM MARGINATUM	PORTULACACEAE		AZ, Mexico
3C		TALINUM MENGESII	PORTULACACEAE		AL GA
3C		TALINUM OKANOGANENSE	PORTULACACEAE		WA, Canada (B.C.)
2		TALINUM RUGOSPERMUM	PORTULACACEAE		IA IL IN MN WI
2		TALINUM VALIDULUM	PORTULACACEAE		AZ UT
2		TANACETUM CAMPHORATUM	ASTERACEAE	TANSY, DUNE	CA
S		TANACETUM COMPACTUM	*** SEE ***	SPHAEROMERIA COMPACTA	
S		TANACETUM SIMPLEX	*** SEE ***	SPHAEROMERIA SIMPLEX	
1		TARAXACUM CALIFORNICUM	ASTERACEAE	DANDELION, CALIFORNIA	CA
2		TARAXACUM CARNEOCOLORATUM	ASTERACEAE		AK, Canada (Yukon)
3C		TAUSCHIA GLAUCA	APIACEAE	TAUSCHIA, GLAUCOUS	CA OR
2		TAUSCHIA HOOVERI	APIACEAE		WA
2		TAUSCHIA HOWELLII	APIACEAE	TAUSCHIA, HOWELL'S	CA OR
3C		TAUSCHIA STRICKLANDII	APIACEAE		OR WA
3C		TAUSCHIA TENUISSIMA	APIACEAE		ID WA
1		TAXUS FLORIDANA	TAXACEAE	YEW, FLORIDA	FL
2		TECTARIA AMESIANA	POLYPODIACEAE		FL, Bahamas
1		TEPHROSIA ANGUSTISSIMA	FABACEAE		FL
2		TEPHROSIA MOHRII	FABACEAE		AL FL GA
1		TERNSTROEMIA LUQUILLENSIS	THEACEAE	PALO COLORADO	PR
1		TERNSTROEMIA SUBSESSILIS	THEACEAE		PR
2		TETRACOCCUS DIOICUS	EUPHORBIACEAE	TETRACOCCUS, PARRY'S	CA, Mexico
2		TETRACOCCUS ILICIFOLIUS	EUPHORBIACEAE	TETRACOCCUS, HOLLY-LEAVED	CA
1	*	TETRAMOLOPIUM ARBUSCULUM	ASTERACEAE		HI
1	*	TETRAMOLOPIUM ARENARIUM	ASTERACEAE		HI

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
1	* TETRAMOLOPIUM CAPILLARE	ASTERACEAE		HI
1	TETRAMOLOPIUM CONSANGUINEUM	ASTERACEAE		HI
1	* TETRAMOLOPIUM CONYZOIDES	ASTERACEAE		HI
1	TETRAMOLOPIUM FILIFORME	ASTERACEAE		HI
1	TETRAMOLOPIUM HUMILE VAR. SUBLAEVE	ASTERACEAE		HI
3B	TETRAMOLOPIUM KAVAIENSIS VAR. KOLOANA	ASTERACEAE		HI
1	TETRAMOLOPIUM LEPIDOTUM	ASTERACEAE		HI
1	TETRAMOLOPIUM POLYPHYLLUM	ASTERACEAE		HI
1	TETRAMOLOPIUM REMYI	ASTERACEAE		HI
1	TETRAMOLOPIUM ROCKII	ASTERACEAE		HI
1	* TETRAMOLOPIUM TENERRIMUM	ASTERACEAE		HI
3B	TETRAMOLOPIUS KAVAIENSIS VAR. KOLOANA	ARALIACEAE		HI
2	TETRAPLASANDRA BISATTENUATA	ARALIACEAE		HI
3B	TETRAPLASANDRA GYMNOCARPA VAR. PUPUKEENSIS	ARALIACEAE	'OHE,	HI
2	TETRAPLASANDRA HAWAIIENSIS VAR. HAWAIIENSIS	ARALIACEAE		HI
1	TETRAPLASANDRA HAWAIIENSIS VAR. MICROCARPA	ARALIACEAE	'OHE,	HI
2	TETRAPLASANDRA KAALAE VAR. MULTIPLEX	ARALIACEAE	'OHE,	HI
3B	TETRAPLASANDRA KAHANANA	ARALIACEAE		HI
1	TETRAPLASANDRA KAVAIENSIS VAR. DIPYRENA	ARALIACEAE	'OHE 'OHE,	HI
1	TETRAPLASANDRA KAVAIENSIS VAR. GRANDIS	ARALIACEAE	'OHE 'OHE,	HI
1	* TETRAPLASANDRA KAVAIENSIS VAR. INTERCEDENS	ARALIACEAE	'OHE 'OHE,	HI
1	TETRAPLASANDRA KAVAIENSIS VAR. KOLOANA	ARALIACEAE		HI
1	* TETRAPLASANDRA KAVAIENSIS VAR. NAHIKUENSIS	ARALIACEAE		HI
1	TETRAPLASANDRA KAVAIENSIS VAR. OCCIDUA	ARALIACEAE		HI
1	TETRAPLASANDRA KOHALAE	ARALIACEAE		HI
1	* TETRAPLASANDRA LANAIENSIS	ARALIACEAE		HI
2	TETRAPLASANDRA LIHUENSIS VAR. GRACILIPES	ARALIACEAE		HI
1	TETRAPLASANDRA LYDGATEI	ARALIACEAE		HI
2	TETRAPLASANDRA MEIANDRA VAR. BISOBTUSA	ARALIACEAE		HI
1	TETRAPLASANDRA MEIANDRA VAR. BRYANII	ARALIACEAE		HI
1	TETRAPLASANDRA MEIANDRA VAR. DEGENERI	ARALIACEAE		HI
1	TETRAPLASANDRA MEIANDRA VAR. HILLEBRANDII	ARALIACEAE		HI
2	TETRAPLASANDRA MEIANDRA VAR. HILOENSIS	ARALIACEAE		HI
1	* TETRAPLASANDRA MEIANDRA VAR. LEPTOMERA	ARALIACEAE		HI
1	TETRAPLASANDRA MEIANDRA VAR. MAKALEHANA	ARALIACEAE		HI
2	TETRAPLASANDRA MEIANDRA VAR. RHYNCHOCARPOIDES	ARALIACEAE		HI
2	TETRAPLASANDRA MEIANDRA VAR. SIMULANS	ARALIACEAE		HI
1	* TETRAPLASANDRA MUNROI	ARALIACEAE		HI
1	TETRAPLASANDRA OAHUENSIS VAR. ERADIATA	ARALIACEAE		HI
2	TETRAPLASANDRA OAHUENSIS VAR. FAURIEI	ARALIACEAE		HI
2	TETRAPLASANDRA OAHUENSIS VAR. HAILIENSIS	ARALIACEAE		HI
2	TETRAPLASANDRA OAHUENSIS VAR. LONGIPES	ARALIACEAE		HI
2	TETRAPLASANDRA OAHUENSIS VAR. PSEUDORHACHIS	ARALIACEAE		HI
2	TETRAPLASANDRA PUPUKEENSIS VAR. NITIDA	ARALIACEAE		HI
1	TETRAPLASANDRA PUPUKEENSIS VAR. PUPUKEENSIS	ARALIACEAE		HI
2	TETRAPLASANDRA PUPUKEENSIS VAR. VENOSA	ARALIACEAE		HI
2	TETRAPLASANDRA TURBANS	ARALIACEAE		HI
2	TETRAPLASANDRA WAIALEALAE VAR. URCEOLATA	ARALIACEAE		HI
1	TETRAPLASANDRA WAIANENSIS	ARALIACEAE	TETRAPLASANDRA, WAIANAE	HI
2	TETRAPLASANDRA WAIMEAE VAR. ANGUSTIOR	ARALIACEAE		HI
1	THALICTRUM COOLEYI	RANUNCULACEAE	MEADOWRUE, COOLEY'S	FL NC
3C	THALICTRUM DEBILE	RANUNCULACEAE		AL AR GA MS TX
2	THALICTRUM HELIOPHILUM	RANUNCULACEAE		CO
2	THALICTRUM STEELEANUM	RANUNCULACEAE	MEADOW-RUE, STEELE'S	DC MD PA VA WV
2	THALICTRUM TEXANUM	RANUNCULACEAE		TX
2	THELESPERMA PUBESCENS	ASTERACEAE		WY
2	THELESPERMA SUBNUDUM VAR. ALPINUM	ASTERACEAE		UT
3C	THELOCACTUS BICOLOR VAR. FLAVIDISPINUS	CACTACEAE		TX, Mexico
2	THELYPODIOPSIS ARGILLACEA	BRASSICACEAE	THELYPODY, CLAY	UT
3C	THELYPODIUM BRACHYCARPUM	BRASSICACEAE	THELYPODY, SHORT-PODDED	CA OR
2	THELYPODIUM EUCOSMUM	BRASSICACEAE		OR
1	THELYPODIUM HOWELLII VAR. SPECTABILIS	BRASSICACEAE		OR
3C	THELYPODIUM LAXIFLORUM	BRASSICACEAE		AZ CO NV UT
1	THELYPODIUM REPANDUM	BRASSICACEAE	THELYPODY, JAEGER'S (WAVY-LEAF)	ID
3C	THELYPODIUM SAGITTATUM VAR. OVALIFOLIUM	BRASSICACEAE		NV UT
LE	THELYPODIUM STENOPETALUM	BRASSICACEAE	MUSTARD, SLENDER-PETALED	CA
3C	THELYPODIUM TENUE	BRASSICACEAE	THELYPODY,	TX
3C	THELYPODIUM TEXANUM	BRASSICACEAE	THELYPODY,	TX
3B	THELYPODIUM VERNALE	BRASSICACEAE		NM
2	THELYPTERIS PILOSA VAR. ALABAMENSIS	POLYPODIACEAE		AL
2	THERMOPSIS MACROPHYLLA VAR. AGNINA	FABACEAE	FALSE LUPINE, SANTA BARBARA	CA
3C	THERMOPSIS MACROPHYLLA VAR. SEMOTA	FABACEAE		CA
1	* THISMIA AMERICANA	BURMANNIACEAE		IL
2	THLASPI ARCTICUM	BRASSICACEAE		AK, Canada (B.C., Yukon)

STATUS		SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
2		THLASPI MONTANUM VAR. CALIFORNICUM	BRASSICACEAE		CA
2		THLASPI MONTANUM VAR. SISKIYOUENSE	BRASSICACEAE		OR
2		THYSANOCARPUS CONCHULIFERUS	BRASSICACEAE	FRINGEPOD, ISLAND	CA
1		TILLANDSIA LINEATISPICA	BROMELIACEAE	PINON	PR VI
3C		TITHONIA THURBERI	ASTERACEAE		AZ, Mexico
5		TITHYMALUS AUSTRINUS	*** SEE ***	EUPHORBIA AUSTRINA	
3B		TOFIELDIA GLUTINOSA SSP. ABSONA	LILIACEAE		ID
LE		TORREYA TAXIFOLIA	TAXACEAE	TORREYA, FLORIDA	FL GA
5		TOUMEYA BRADYI	*** SEE ***	PEDIOCACTUS BRADYI	
5		TOUMEYA KNOWLTONII	*** SEE ***	PEDIOCACTUS KNOWLTONII	
5		TOUMEYA PEEBLESIANUS	*** SEE ***	PEDIOCACTUS PEEBLESIANUS VAR. PEEBLESIANUS	
3C		TOWNSENDIA ALPIGENA VAR. MINIMA	ASTERACEAE		UT
LT		TOWNSENDIA APRICA	ASTERACEAE	TOWNSENDIA, LAST CHANCE	UT
2		TOWNSENDIA JONESII VAR. TUMULOSA	ASTERACEAE		NV
3C		TOWNSENDIA MENSANA	ASTERACEAE		UT
5		TOWNSENDIA MINIMA	*** SEE ***	TOWNSENDIA ALPIGENA VAR. MINIMA	
3C		TOWNSENDIA ROTHROCKII	ASTERACEAE		CO
3C		TOWNSENDIA SMITHII	ASTERACEAE	GROUND-DAISY, BLACK ROCK	AZ
2		TOWNSENDIA SP. NOV. /INED. (NYE CO.)	ASTERACEAE	TOWNSENDIA	NV
2		TOWNSENDIA SP. NOV. INED.	ASTERACEAE		WY
3C		TOWNSENDIA SPATHULATA	ASTERACEAE	TOWNSENDIA, SWORD	WY
2		TRACYINA ROSTRATA	ASTERACEAE	TRACYINA, BEAKED	CA
3C		TRADESCANTIA EDWARDSIANA	COMMELINACEAE		TX
2		TRADESCANTIA OZARKANA	COMMELINACEAE		AR MO OK
3C		TRADESCANTIA WRIGHTII	COMMELINACEAE		NM TX
3C		TRAGIA NIGRICANS	EUPHORBIACEAE		TX
2		TRAGIA SAXICOLA	EUPHORBIACEAE		FL
1		TREMATOLOBELIA WIMMERI	CAMPANULACEAE		HI
1		TRICHILIA TRIACANTHA	MELIACEAE	BARIACO	PR
1		TRICHOMANES DRAYTONIANUM	HYMENOPHYLLACEAE		HI
1		TRICHOSTEMA AUSTROMONTANUM SSP. COMPACTUM	LAMIACEAE	BLUECURLS, HIDDEN LAKE	CA
2	*	TRIFOLIUM AMOENUM	FABACEAE	CLOVER, SHOWY INDIAN	CA
3C		TRIFOLIUM ANDERSONII SSP. BEATLEYAE	FABACEAE	CLOVER, FIVE-LEAF, BEATLEY'S	CA NV
1		TRIFOLIUM ANDERSONII VAR. FRISCANUM	FABACEAE		UT
2		TRIFOLIUM BARNEBYI	FABACEAE		WY
2		TRIFOLIUM BOLANDERI	FABACEAE	CLOVER, BOLANDER	CA
3C		TRIFOLIUM DEDECKERAE	FABACEAE	CLOVER, DEDECKER	CA
2		TRIFOLIUM LEIBERGII	FABACEAE		OR
3C		TRIFOLIUM LEMMONII	FABACEAE	CLOVER, LEMMON'S	CA NV
2		TRIFOLIUM OWYHEENSE	FABACEAE	CLOVER, OWYHEE	ID OR
3C		TRIFOLIUM PLUMOSUM VAR. AMPLIFOLIUM	FABACEAE		ID
3C		TRIFOLIUM PLUMOSUM VAR. PLUMOSUM	FABACEAE		OR WA
2		TRIFOLIUM POLYODON	FABACEAE	CLOVER, PACIFIC GROVE	CA
1		TRIFOLIUM STOLONIFERUM	FABACEAE	BUFFALO-CLOVER, RUNNING	AR IL IN KS KY MO OH WV
2		TRIFOLIUM THOMPSONII	FABACEAE	CLOVER, THOMPSON	WA
2		TRIFOLIUM TRICHOCALYX	FABACEAE	CLOVER, DEL MONTE	CA
2		TRIFOLIUM VIRGINICUM	FABACEAE	CLOVER, KATE'S-MOUNTAIN	MD PA VA WV
3C		TRILLIUM OVATUM SSP. OETTINGERI	LILIACEAE		CA
LE		TRILLIUM PERSISTENS	LILIACEAE	TRILLIUM, PERSISTENT	GA SC
2		TRILLIUM PUSILLUM VAR. MONTICULUM	LILIACEAE		VA WV
2		TRILLIUM PUSILLUM VAR. OZARKANUM	LILIACEAE	TRILLIUM, LEAST, OZARK	AR KY MO TN
2		TRILLIUM PUSILLUM VAR. PUSILLUM	LILIACEAE		AL KY MS NC SC TN
2		TRILLIUM PUSILLUM VAR. VIRGINIANUM	LILIACEAE	TRILLIUM, LEAST, VIRGINIA	MD VA
2		TRILLIUM RELIQUUM	LILIACEAE		AL GA SC
3C		TRILLIUM TEXANUM	LILIACEAE		LA TX
2		TRIPHORA CRAIGHEADII	ORCHIDACEAE	NODDING-CAPS,	FL
2	*	TRIPHORA LATIFOLIA	ORCHIDACEAE	NODDING-CAPS,	FL
1		TRIPSACUM FLORIDANUM	POACEAE	GAMA GRASS,	FL
2	*	TRISETUM ORTHOCHAETUM	POACEAE		MT
2		TRITELEIA CLEMENTINA	LILIACEAE		CA
3C		TRITELEIA DUDLEYI	LILIACEAE		CA
3C		TRITELEIA LEMMONAE	LILIACEAE		AZ
3C		TRITELEIOPSIS PALMERI	LILIACEAE		AZ, Mexico
3C		TROLLIUS LAXUS SSP. LAXUS	RANUNCULACEAE		CT NJ NY OH PA
2		TROPIDOCARPUM CAPPARIDEUM	BRASSICACEAE	TROPIDOCARPUM, CAPER-FRUITED	CA
1		TUCTORIA GREENEI	POACEAE	ORCUTT GRASS, GREENE'S	CA
PE		TUMAMOCA MACDOUGALII	CUCURBITACEAE	GLOBE-BERRY, TUMAMOC	AZ, Mexico
1		URERA KAALAE	URTICACEAE	OPUHE	HI
2		URERA KONAENSIS	URTICACEAE		HI
5		UROSTACHYS HALEAKALAE	*** SEE ***	LYCOPODIUM HALEAKALAE	
5		UROSTACHYS NUTANS	*** SEE ***	LYCOPODIUM NUTANS	
2		URTICA CHAMAEDRYOIDES VAR. RUNYONII	URTICACEAE	ORTIGUILLA,	TX
5		UTAHIA PEEBLESIANUS	*** SEE ***	PEDIOCACTUS PEEBLESIANUS VAR. PEEBLES	ianus
5		UTAHIA SILERI	*** SEE ***	PEDIOCACTUS SILERI	
3B		VACCINIUM COCCINEUM	ERICACEAE	BILBERRY, SISKIYOU MOUNTAINS	CA OR
1		VACCINIUM SEMPERVIRENS	ERICACEAE		SC
3B		VACCINIUM VACILLANS VAR. MISSOURIENSE	ERICACEAE		MO
3C		VALERIANA COLUMBIANA	VALERIANACEAE		WA, Canada (B.C.)
3C		VALERIANA TEXANA	VALERIANACEAE		NM TX
3C		VALERIANA ULIGINOSA	VALERIANACEAE	VALERIAN, MARSH	IL IN ME MI NH NY OH VT WI, Canada (N.B., Ont., Que.)

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
3C	VALERIANELLA TEXANA	VALERIANACEAE	CORNSALAD, EDWARDS' PLATEAU	TX
3C	VANCOUVERIA CHRYSANTHA	BERBERIDACEAE		CA OR
2	VAUQUELINIA PAUCIFLORA	ROSACEAE		AZ NM, Mexico
3C	VERATRUM FIMBRIATUM	LILIACEAE		CA
S	VERATRUM INTERMEDIUM	*** SEE ***	VERATRUM WOODII	
3C	VERATRUM WOODII	LILIACEAE	HELLEBORE, FALSE	AR FL GA IL IN IA KY MO OH OK TN TX
1	VERBENA CALIFORNICA	VERBENACEAE		CA
2	VERBENA MARITIMA	VERBENACEAE		FL
1	VERBENA TAMPENSIS	VERBENACEAE	VERVAIN,	FL
2	VERBESINA CHAPMANNII	ASTERACEAE	CROWNBEARD, CHAPMAN'S	FL
1	VERBESINA HETEROPHYLLA	ASTERACEAE		FL
2	VERNONIA BORINQUENSIS	ASTERACEAE		PR
3C	VERNONIA PULCHELLA	ASTERACEAE		GA
3C	VERONICA COPELANDII	SCROPHULARIACEAE	SPEEDWELL, COPELAND'S	CA
3B	VERONICA SHERWOODII	SCROPHULARIACEAE		OR
2	VIBURNUM BRACTEATUM	CAPRIFOLIACEAE	ARROWWOOD,	AL GA TN
LE	VICIA MENZIESII	FABACEAE	VETCH, HAWAIIAN	HI
1	VICIA OCALENSIS	FABACEAE	VETCH, OCALA	FL
3B	VICIA REVERCHONII	FABACEAE	VETCH, HAIRY POD	OK TX
1	VIGNA OWAHUENSIS	FABACEAE	VIGNA, OAHU	HI
1	VIGNA SANDWICENSIS	FABACEAE		HI
S	VIGUIERA LUDENS	*** SEE ***	HELIANTHUS LUDENS	
3C	VIGUIERA PORTERI	ASTERACEAE		AL GA
3C	VIGUIERA SOLICEPS	ASTERACEAE	SUNFLOWER, PARIA	UT
S	VINCETOXICUM ALABAMENSE	*** SEE ***	MATELEA ALABAMENSIS	
3B	VIOLA ADUNCA VAR. CASCADENSIS	VIOLACEAE		OR WA
1	VIOLA CHAMISSONIANA	VIOLACEAE	'OLOPU	HI
S	VIOLA CHARLESTONENSIS	*** SEE ***	VIOLA PURPUREA VAR. CHARLESTONENSIS	
3C	VIOLA EGGLESTONII	VIOLACEAE		AL GA KY TN
3C	VIOLA FLETTII	VIOLACEAE	VIOLET, FLETT'S	WA
1	VIOLA HELENA	VIOLACEAE		HI
1	VIOLA KAUAIENSIS VAR. WAHIAWAENSIS	VIOLACEAE		HI
3C	VIOLA LANCEOLATA SSP. OCCIDENTALIS	VIOLACEAE		CA OR
2	VIOLA NOVAE-ANGLIAE	VIOLACEAE	VIOLET, NEW ENGLAND	ME MN NY WI, Canada (Man., N.B., Ont.)
1	VIOLA OAHUENSIS	VIOLACEAE		HI
3C	VIOLA PURPUREA VAR. CHARLESTONENSIS	VIOLACEAE	VIOLET, LIMESTONE	NV UT
2	VIOLA ROBUSTA	VIOLACEAE		HI
3C	VIOLA TOMENTOSA	VIOLACEAE	VIOLET, FELT-LEAF	CA
3C	WALDSTEINIA IDAHOENSIS	ROSACEAE		ID
2	WALDSTEINIA LOBATA	ROSACEAE		GA SC
2	WALTHERIA PYROLAEFOLIA	STERCULIACEAE		HI
2	WAREA AMPLEXIFOLIA	BRASSICACEAE		FL
1	WAREA CARTERI	BRASSICACEAE		FL
3C	WAREA SESSILIFOLIA	BRASSICACEAE		AL FL
S	WEDELIA CRISTATA	*** SEE ***	ALLIONIA CRISTATA	
2	WIKSTROEMIA BASICORDA	THYMELAEACEAE		HI
2	WIKSTROEMIA HANALEI	THYMELAEACEAE		HI
2	WIKSTROEMIA ISAE	THYMELAEACEAE		HI
1	WIKSTROEMIA LEPTANTHA	THYMELAEACEAE		HI
2	WIKSTROEMIA MONTICOLA VAR. OCCIDENTALIS	THYMELAEACEAE		HI
2	WIKSTROEMIA PERDITA	THYMELAEACEAE		HI
2	WIKSTROEMIA SKOTTSBERGIANA	THYMELAEACEAE		HI
1	WIKSTROEMIA VILLOSA	THYMELAEACEAE		HI
1	WILKESIA HOBDYI	ASTERACEAE	ILIAU, DWARF	HI
3C	WILLKOMMIA TEXANA	POACEAE		TX
3B	WOODSIA ABBEAE	POLYPODIACEAE		MI MN WI
2	WOODSIA OREGANA VAR. CATHCARTIANA	POLYPODIACEAE	WOODSIA, OREGON	MN NY WI, Canada
S	WULFENIA BULLII	*** SEE ***	BESSEYA BULLII	
2	WYETHIA RETICULATA	ASTERACEAE	MULE-EARS, EL DORADO	CA
S	XANTHOCEPHALUM CALIFORNICUM	*** SEE ***	GUTIERREZIA CALIFORNICA	
S	XANTHOCEPHALUM SAROTHRAE VAR. POMARIENSE	*** SEE ***	GUTIERREZIA SAROTHRAE VAR. POMARIENSIS	
3C	XYLORHIZA COGNATA	ASTERACEAE		CA, Mexico (Baja California)
3C	XYLORHIZA CONFERTIFOLIA	ASTERACEAE		UT
2	XYLORHIZA CRONQUISTII	ASTERACEAE		UT
2	XYLORHIZA ORCUTTII	ASTERACEAE	ASTER, ORCUTT'S	CA, Mexico (Baja California)
2	XYLOSMA CRENATUM	FLACOURTIACEAE		HI
2	XYRIS DRUMMONDII	XYRIDACEAE		AL FL GA LA MS
2	XYRIS ISOETIFOLIA	XYRIDACEAE		FL
3C	XYRIS LONGISEPALA	XYRIDACEAE	YELLOW-EYED-GRASS, KRAL'S	AL FL
2	XYRIS SCABRIFOLIA	XYRIDACEAE		AL FL GA MS
2	XYRIS TENNESSEENSIS	XYRIDACEAE		AL GA TN
3C	YUCCA ANGUSTISSIMA VAR. TOFTIAE	LILIACEAE		UT
S	YUCCA TOFTIAE	*** SEE ***	YUCCA ANGUSTISSIMA VAR. TOFTIAE	
S	ZAMIA FLORIDANA	*** SEE ***	ZAMIA INTEGRIFOLIA	
3C	ZAMIA INTEGRIFOLIA	CYCADACEAE	COONTIE, FLORIDA	FL GA
2	ZANTHOXYLUM BLUETTIANUM	RUTACEAE	A'E (HEA'E)	HI
1	ZANTHOXYLUM DIPETALUM	RUTACEAE	KAWA'U	HI
2	ZANTHOXYLUM GLANDULOSUM	RUTACEAE		HI
1	ZANTHOXYLUM HAWAIIENSE	RUTACEAE		HI

STATUS	SCIENTIFIC NAME	FAMILY	COMMON NAME	RANGE
2	ZANTHOXYLUM KAUAENSE VAR. KOHUANA	RUTACEAE		HI
2	ZANTHOXYLUM MAVIENSE	RUTACEAE		HI
2	ZANTHOXYLUM PARVUM	RUTACEAE	TICKLE-TONGUE, SHINNER'S	TX
1	ZANTHOXYLUM SEMIARTICULATUM	RUTACEAE	A'E (HEA'E)	HI
2	ZANTHOXYLUM SKOTTSBERGII	RUTACEAE	A'E (HEA'E)	HI
PE	ZANTHOXYLUM THOMASIANUM	RUTACEAE	PRICKLY-ASH,	PR VI
3C	ZEPHYRANTHES SIMPSONII	LILIACEAE		FL GA SC
3C	ZEPHYRANTHES TREATIAE	LILIACEAE		FL
3C	ZIGADENUS VAGINATUS	LILIACEAE	DEATHCAMUS, SHEATHED	NV UT
LE	ZIZANIA TEXANA	POACEAE	WILD-RICE, TEXAS	TX
2	ZIZIA LATIFOLIA	APIACEAE		FL
2	* ZIZIPHUS CELATA	RHAMNACEAE		FL

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Part V

Department of Housing and Urban Development

Office of the Assistant Secretary for Housing—Federal Housing Commissioner

24 CFR Part 200

Minimum Property Standards for One and Two Family Dwellings; Final Rule

(Replaces HUD Handbook 4900.1)

DEPARTMENT OF HOUSING AND URBAN DEVELOPMENT

Office of the Assistant Secretary for Housing—Federal Housing Commissioner

24 CFR Part 200

[Docket No. R-85-1183; FR-1655]

Revision of Minimum Property Standards (MPS) for One and Two Family Dwellings

AGENCY: Office of the Assistant Secretary for Housing—Federal Housing Commissioner, HUD.

ACTION: Final rule.

SUMMARY: This final rule changes the basic structure of HUD's Minimum Property Standards (MPS) for one and two family dwellings. The handbook which contained the MPS for such structures is eliminated. The Department will now rely upon acceptable local building codes, acceptable State building codes where there are no acceptable local building codes, or the Counsel of American Building Officials' (CABO) One and Two Family Dwelling Code where there are no acceptable State building codes. State and local codes are acceptable when the HUD Field Office verify them as being comparable to one of the national model codes. Necessary requirements which are unlikely to be contained in State or local codes and not contained in the CABO One and Two Family Dwelling Code are set forth in the Department's regulations as written herein.

These revisions will preserve the quality of one and two family dwellings and protect the Department's insurance fund while simplifying the Department's construction criteria.

EFFECTIVE DATE: November 1, 1985.

FOR FURTHER INFORMATION CONTACT: Mark W. Holman, Manufactured Housing and Construction Standards Division, Room 9156, Department of Housing and Urban Development, 451 7th Street SW., Washington, DC 20410, telephone (202) 755-6584. (This is not a toll-free number).

SUPPLEMENTARY INFORMATION: The National Housing Act, 12 U.S.C. 1701–1749, authorizes the Secretary of Housing and Urban Development (Secretary) to prescribe standards for determining the acceptability of one and two family residential structures for purposes of mortgage insurance. *See, e.g.*, 12 U.S.C. 1715/(f). Some general statutory guidelines govern the nature of these standards. For example, the standards are "to establish the acceptability of. . . . propert[ies] for mortgage insurance . . ." 12 U.S.C. 1715/(f). In addition, the standards must be consistent with the declared national housing policy of realizing "the goal of a decent and suitable living environment for every American family. . . ." *See* 42 U.S.C. 1441.

The Department has prescribed these standards by issuing the Minimum Property Standards (MPS) for One and Two Family Dwellings, which were published as HUD Handbook 4900.1, and incorporated by reference into the Department's regulations by authority of 24 CFR 200.927. Changes in the structure of the MPS are being made to further the indicated statutory purposes.

The Department also has evaluated the revisions to the MPS in light of the recent amendment of Section 526 of the National Housing Act, 12 U.S.C. 1735f–4. *See* Housing and Urban-Rural Recovery Act of 1983, Pub. L. 98–181, sec. 405, 97 Stat. 1153 (1983). As amended, section 526 now permits the Secretary to require, with respect to health and safety, that properties other than manufactured homes comply "with one of the nationally recognized model building codes, or with a State or local building code based on one of the nationally recognized model building codes or their equivalent." The Secretary is "responsible for determining the comparability of the State and local codes to such model codes. . . ."

On October 11, 1984, the Department published a proposed rule to revise the MPS. *See* 49 FR 39855. That rule proposed that the Department rely upon acceptable State and local codes and eliminate the handbook containing the MPS for One and Two Family Dwellings. This final rule adopts as final that proposed rule. It is also similar in many respects to the final rule amending the MPS for Multifamily Housing which was published on May 1, 1984 [49 FR 18690].

Four hundred sixty-one comments were received in response to the proposed rule, and the Department has evaluated the revisions to the MPS in light of those comments.

The Department is aware of the fact that unnecessary or needlessly complex regulations can increase the final cost of a home. This rule is part of the Department's continuing effort to eliminate regulations where possible and to simplify regulations that cannot be eliminated.

I. Description of Final Rule

A. General

The Department is revising the MPS for One and Two Family Dwellings in order to simplify them. Generally, HUD will rely upon State or local building codes or the Council of American Building Officials' (CABO) One and Two Family Dwelling Code to provide the health and safety criteria for its single family construction standards. The Department will rely upon a State or local code only after it has been accepted by the Department as being comparable to one of the nationally recognized model building codes or its equivalent. In some jurisdictions, the Department may partially accept a State or local code. In such areas, the Department will rely upon the State or local code, plus those provisions of the CABO One and Two Family Dwelling Code identified by the local HUD Field Office in accordance with 24 CFR 200.926c. In jurisdictions where a State or local code has not been accepted or partially accepted by the Department, or where no State or local code exists, the Department will require compliance with the CABO One and Two Family Dwelling Code, the Electrical Code for One and Two Family Dwellings and the requirements under Sections 200.926 d and e.

In those instances where the Department will require compliance with the CABO One and Two Family Dwelling Code, the developer or other interested party must also comply with the mandatory codes or standards incorporated by reference therein and the requirements of § 200.926e. In addition, compliance with the Electrical Code for One and Two Family Dwellings, NFPA 70A, 1984 edition, including the appendices, is required. This is identified in 24 CFR 200.926b(a)(2).

In all instances, the Department will require compliance with the standards set forth in § 200.926d, entitled "Construction Requirements". This section contains standards relating to energy and certain other matters.

Section 521 of the National Housing Act, 12 U.S.C. 1735e, requires the Secretary to "adopt a uniform procedure for the acceptance of materials and products to be used in structures approved for mortgages or loans insured under [the National Housing] Act." The Secretary has established such procedures in Handbook 4950.1 and in 24 CFR 200.935. This final rule will not affect these procedures.

Section 100–2 of the now-eliminated MPS for One and Two Family

Dwellings, HUD Handbook 4900.1, provided that "[e]xisting construction shall comply with the Requirements for Existing Housing—One- to Four-Family Living Units, HUD Handbook 4905.1." Under this final rule, this handbook will not be referenced in the Code of Federal Regulations. This handbook will, however, be maintained by the Department as a single family program handbook.

B. Standard for Determining Comparability of State or Local Codes

The Department will determine whether a State or local code is comparable to one of the nationally recognized model codes by conducting an analysis of the comprehensiveness of the State or local code. For use in performing this analysis, the Department has prepared a list of construction-related areas. *See* § 200.926a. This list is based upon the provisions of the nationally recognized model building codes and is made up of major areas and subareas. Each major area has been made a separate paragraph of § 200.926a. For example, Fire Safety is paragraph (a) and Light and Ventilation is paragraph (b). Each major area has been further divided into subareas. For example, the Light and Ventilation paragraph has been divided into two subareas: (1) Habitable rooms and (2) Bath and Toilet rooms.

A State or local code will be found comparable, and therefore acceptable, if it regulates every subarea set forth in § 200.926a. A State or local code will be found partially acceptable if it fails to regulate from one to eight subareas in the same major area; however, no code will be found partially acceptable if it fails to regulate subareas in more than one of the major areas.

Consequently, every State or local code which is partially accepted will be deficient in only one major area. The local HUD Field Office will remedy the deficiency by designating those provisions of the CABO One and Two Family Dwelling Code which regulate the entire major area found deficient. This designation will be made in accordance with the table set forth in § 200.926c.

If a local code is not found to be acceptable or partially acceptable, one and two family residential structures will have to comply, for HUD purposes, with the State code if the State code is considered acceptable or partially acceptable. If the State code is not found acceptable or partially acceptable, one and two family residential structures will have to comply, for HUD purposes, with the CABO One and Two Family Dwelling Code, § 200.926e, and NFPA 70A. In all cases, the requirements set forth in § 200.926d shall apply.

The HUD Field Office, using provisions from the CABO code, will remedy deficiencies in partially acceptable State or local codes so they may be used for HUD purposes. For example, a jurisdiction's code may be deficient in the "Plumbing" major area because it fails to regulate the "Vents and Venting" and the "Cleanouts" subareas. In such a case, the code will be found to be partially acceptable because only subareas from one major area are lacking. The Field Office will remedy the deficiencies in the "Plumbing" major area by designating Part V of the CABO One and Two Family Dwelling Code. Thus, in that jurisdiction, for HUD purposes, properties will have to comply with Part V of the CABO One and Two Family Dwelling Code, the entire local code except for the plumbing requirements, and the requirements set forth in §200.926d.

To determine whether this method of evaluating comparability would protect the Department's interests and satisfy all statutory requirements, the Department conducted a study of representative local codes. Codes from nine jurisdictions were evaluated. Three of the jurisdictions were large cities, three were medium sized cities, and three were small cities. One city of each size was selected from geographically diverse areas of the nation. As a result of this evaluation, it was found that seven of the codes regulated the areas identified in § 200.926a.

These seven codes were then subjected to a more detailed evaluation. This detailed analysis was performed to determine whether the local codes were in fact comparable to one of the nationally recognized model codes. In each case where the Department subjected a code to detailed analysis, we determined that it was comparable to one of the model codes.

C. Review Process

Before a State or local code can be relied upon to provide health and safety requirements for HUD purposes, it will have to be accepted or partially accepted by the Department. The review process for State codes, however, differs from that for local codes. With respect to State codes, the HUD Field Offices will immediately review them, without requests or submissions from lenders or other interested parties.

The Department will review a local code when it receives a request from a lender or other interested party. The person requesting review must submit to the HUD Field Office a copy of the local building code and a copy of the statute, ordinance, order or regulation establishing the code, if any. The submitting party will not be required to submit copies of any part of a code already in the possession of the particular HUD Field Office. If the Department accepts the local code, it will issue a letter stating that the code is acceptable. If the Department declines to accept a local code, the submitting party will be notified, and given an opportunity to present its views as to why the local code should be accepted.

If the local code has been neither previously accepted nor partially accepted and if a lender or other interested party wishes to have it accepted, then it must submit the material described in the preceding paragraph. If a local code has been previously accepted or partially accepted, then lenders or other interested parties must submit either a certificate stating that the local code has not been changed since the date of its acceptance or partial acceptance by the Secretary, or a copy of all changes that have been made since the date of the Secretary's acceptance or partial acceptance. All submissions must be made by the time of application for mortgage insurance or other benefits.

Each Regional and Field Office will maintain a current list of States and jurisdictions with accepted or partially accepted codes, including the dates of acceptances.

D. Use of the Council of American Building Officials (CABO) One and Two Family Dwelling Code

In those jurisdictions without acceptable or partially acceptable local codes, one and two family dwellings must comply, for HUD purposes, with an acceptable or partially acceptable State code. In those jurisdictions without acceptable or partially acceptable State or local codes, one and two family dwellings will have to comply, for HUD purposes, with the CABO One and Two Family Dwelling Code identified in 24 CFR 200.926b(a) and with the requirements of § 200.926e. In all cases, the requirements set forth in § 200.926d shall apply.

In those jurisdictions with a partially acceptable local code or a partially acceptable State code, certain portions of the CABO code identified in 24 CFR 200.926b(a) will be used. The HUD Field Office will identify, in the written notice of partial acceptance, those portions of the CABO code with which the dwelling must comply. In so doing, the HUD Field Office will rely upon the table at § 200.926c to determine which portions of the CABO code will supplement the partially acceptable State or local code.

The requirement to comply, for HUD purposes, with all or part of the CABO code or any State or local code applies only to the particular one or two family dwelling in question and has no applicability to other buildings in the community.

E. Differences Between the Proposed and Final Rules

Under the proposed rule, if the code which was applicable in a particular jurisdiction was found to be neither acceptable nor partially acceptable, then one and two family dwellings would have had to comply, for HUD purposes, with one of three nationally recognized model codes. The code to be evaluated was the code applicable in the jurisdiction, whether it was a local code, a State code, or a combination of the two. The final rule changes this system in two respects. First, the Department will evaluate a local code, if there is one, to determine its acceptability. If it is found to be neither acceptable nor partially acceptable, then one and two family dwellings will have to comply, for HUD purposes, with an acceptable or partially acceptable State code, if one exists. If no acceptable or partially acceptable State code exists, then one and two family dwelling will have to comply, for HUD purposes, with the CABO One and Two Family Dwelling Code. Reliance upon the CABO code is the second change in the system which would have been established under the proposed rule. Under the proposed rule, the Department would have permitted reliance upon the BOCA Basic/National Building Code; the Standard Building Code; or the Uniform Building Code. In addition to these changes, the final rule adds references to Appendices C and F of HUD Handbook 4910.1, "Minimum Property Standards for Multifamily Housing." Finally, a number of minor editorial changes have been made in the rule in order to clarify its meaning and application.

II. Public Comments

A. General

The Department received 461 comments. Most of these were concerned with the thermal energy requirements set forth in § 200.926d. Many of the comments indicated support for the policies underlying the rule change. Other comments suggested changes to specific provisions of the proposed rule.

The Department received several comments with respect to the rule's general policy of relying on State or local codes to the greatest extent possible. One commenter complained that the rule would allow an "unnecessary and excessive proliferation" of different requirements throughout the country. All housing, whether conventionally or HUD financed, has always had to comply with local building codes. Only HUD financed housing had to comply with an additional set of standards, the MPS. The Department believes that the use of State or local codes will significantly reduce the burden of conflicting requirements that several layers of building standards tend to create. Moreover, this rule is consistent with both the Administration's policy of federalism and the Department's statutory mandate.

Another commenter argued that the existing MPS should be retained because model and State or local codes are primarily concerned with health and safety issues. They do not regulate performance and durability, as the existing MPS do. According to this commenter, the elimination of HUD's MPS will enable builders and developers to increase their profits and in the process build shoddier houses, which, in the long run, will reduce the quality of housing. For several reasons, the Department believes that the new system will not be deleterious to HUD-financed or other housing. First of all, the elimination of some criteria does not mean that builders will eliminate quality materials or good design. Conventionally financed housing has for years been built to meet specifications that exceed HUD's standards, because HUD has historically set only minimum standards. Consumer taste has often dictated more than the MPS requires. While elimination of the MPS will not reduce the quality of construction, it will eliminate the adherence to duplicative building codes, which can increase the cost of construction. Additionally, elimination of a single federal standard will allow for greater flexibility in addressing local needs through the use of local or regional codes. Further, buyers in today's market place are better educated and able to determine for themselves whether they wish to purchase homes containing marketability or liveability fixtures.

Moreover, the rule addresses only the construction criteria of home building, not the valuation procedures. If a buyer chooses to accept smaller spaces or fewer features in a home so that he or she can save money, the valuation procedures will reflect this choice. These valuation procedures establish the maximum mortgage amount the Department will insure.

Finally, the primary purpose for the MPS throughout its history was to reduce the risk to the Department's insurance fund by ensuring that HUD could dispose of homes acquired through foreclosure at a minimum of loss to the Department. The Department has concluded that because of the increasing acceptability of local codes, buyer education, and builder interest, the use of model codes and the additional criteria of the Rule are sufficient to produce construction which will be adequate collateral to support an acceptable mortgage insurance risk. In sum, the Department is confident that construction meeting acceptable State or local codes or the CABO code, plus the additional requirements set forth herein, is adequate for HUD purposes and ensures that the quality of construction will remian high for the homebuyer.

The Department solicited comment on six subjects. *See* the proposed rule to revise the Minimum Property Standards for one and two family dwellings published on October 11, 1984 at 49 FR 39858. The discussion of the comments below corresponds to these six inquiries. Comments not pertaining to any of these areas are discussed in section II.g.

B. Energy

In the proposed rule, the Department solicited comment concerning the energy requirements of the MPS:

> The Department is considering making changes to the energy requirements discussed above. In particular, the Department is considering whether to adopt the energy standards contained in the January 29, 1984 Addenda to ANSI/ASHRAE/IES 90A-1980 or the standards contained in the 1983 edition of the Model Energy Code. The Department seeks comment on these alternatives and on the availability and the propriety of other options. 49 FR 39858.

In response, the Department received numerous comments relating to energy requirements. Many commenters expressed concern that adoption of a model code or standard would eliminate the special consideration for masonry construction granted by HUD's Local Acceptable Standard for Arizona and Florida. Many other commenters suggested that we adopt one of the versions of the Model Energy Code or the ANSI/ASHRAE/IES Standard. Finally, the Department received many comments suggesting specific changes to the MPS energy standards.

After reviewing these comments, the Department has decided not to proceed to a final rule with respect to the energy requirements of the MPS for one and two family dwellings. Rather, the Department will continue to evaluate the comments and to study possible alternatives, and will issue a final rule for energy criteria in the future.

The energy criteria will, therefore, remain unchanged from those currently in effect. To be consistent with the new structure of the MPS, however, the current energy criteria will be published in the Code of Federal Regulations, at 24 CFR 200.926d(d). One commenter suggested that the energy criteria should remain in handbook form because the Code of Federal Regulations (CFR) is not available to builders. The Department believes that publication in the CFR ensures that any standards contained therein are widely available. Moreover, the HUD Field Offices will reprint and make available to builders the construction requirements in § 200.926 d and e.

C. Burden on Small Homebuilders

The second subject on which the Department solicited comment was whether this rule would "reduce burdens on small homebuilders, who often find it especially difficult to comply with two different sets of construction standards." 49 FR 39858. The Department also sought comment as to "whether the proposed arrangements will lead to any particular difficulties for small builders in learning of the standards to which they must comply, and as to what additional measures the Department might adopt to alleviate the disadvantages inherent in small operations." 49 FR 39858. Only a few commenters responded directly concerning this matter. In general, the commenters who responded believed that the Department's proposal would reduce regulatory conflicts, minimize waste and bring home costs down. They also commented that neither lenders nor builders would have problems in complying with the simplified requirements.

One commenter recognized that lenders and builders will need some type of education program to acquaint them with the changes. The Department realizes there will be some uncertainty as to the correct procedures during the transition period from the current system to the new system. During this transition, the Department's staff will be trained in code acceptance and use and will be instructed to give as much assistance to lenders and builders as possible. Formal or organized training by the Department for lenders and builders is not possible because of staff and funding limitations. However, if problems arise, appropriate information will be disseminated to ensure a full understanding of the necessary program functions by private sector participants.

D. Applicable Construction Standards

The Department requested comment on whether the proposed approach to correcting deficiencies in State or local codes, when they are judged partially acceptable, would impose any serious burdens upon builders. The Department also requested comment on whether there was an alternative, less burdensome approach that is consistent with the Department's mandate.

One commenter expressed concern that when local codes are supplemented by referencing portions of model codes (in the final rule, only the CABO code), there is a possibility that conflicts between the local and CABO codes could occur. The commenter suggested that when conflicts arise, the local code should prevail.

The Department's intent is to allow the use, where possible, of State or local codes as HUD's health and safety criteria. When the State or local code is deficient in that it does not cover subareas in only one area, CABO code criteria will be used only to supplement the deficient State or local code. The Department recognizes that there may be times when the State or local code criteria are not compatible with the portion of the CABO code designated to supplement the deficient code. When conflict occurs, the local HUD Field Office will determine, on a case-by-case basis, which criteria will apply. *See* § 200.926(b). The Department believes that this approach provides sufficient flexibility for lenders and builders, yet will ensure that the Department's interest in providing decent, safe and sanitary housing is met.

Another commenter pointed out that, under the Proposed Rule, the local HUD Field Office would have been required to designate an appropriate model code to be used when a jurisdiction has a partially accepted code. This could result in more than one model code being designated by a HUD Field Office, depending upon which part of the local code was deficient. Further, in neighboring local jurisdictions under the same or a different HUD Field Office jurisdiction, a different model code could be referenced for the same deficient part of a local code. Finally, it was pointed out that when there is no acceptable local code, the Proposed Rule directed the lender or other interested party to select the model code to be used. The commenter concluded that this system could yield a maze of construction requirements. It would have been very difficult for the small home builder, fee inspectors, direct endorsement mortgagees, and HUD Field Office staff to know which criteria would be applicable in every local jurisdiction, to keep current editions of the local and model codes, and to build, review plans and specifications and inspect construction to so many different combinations of local and model codes.

To eliminate potential conflicts or confusion, we are eliminating the options that permit the HUD Field Office and the lender or other interested party to choose which model code applies. The Final Rule now incorporates by reference only the CABO One and Two Family Dwelling Code as a model building code. Where there is no acceptable local code, the lender or other interested party is required to comply with the State building code, if it is acceptable or partially acceptable. If there is not an acceptable or partially acceptable State building code, then the CABO One and Two Family Dwelling Codes applies. Where the State or local code is partially acceptable, the HUD Field Office will supplement it with portions of the CABO code.

The Department has decided to use only the CABO code for several reasons other than the simplicity that the use of only one code will provide. First, the CABO code has been widely adopted throughout the country. Second, it is a model code created strictly for the regulation of single family dwellings. Third, it was created and is referenced by the three major model code organizations as single family dwelling criteria. Fourth, referencing only the CABO code will obviate the need for including a mechanism in the rule to ensure that all of the model code references are current. Finally, the CABO code regulates the construction areas identified in § 200.926a in a way that the Department has concluded adequately protects its interests.

One commenter argued that the Department should not supplement a deficient subarea of a State or local code by substituting an entire major area of a model (now CABO) code. Another commenter suggested that HUD should specify, on a case-by-case basis, what modifications would be necessary. The Department believes, however, that the system as proposed will effectively supplement partially acceptable codes. It has been the Department's experience that if a State or local code is deficient in one subarea, there are likely to be other deficiencies in other subareas within the major area involved. Thus, substitution of the entire major area ensures that all relevant criteria are adequately addressed. Further, by establishing a uniform system under which partially acceptable codes will be supplemented, the Department can both minimize confusion and attain some uniformity within a State or local jurisdiction.

E. The Developer Submission of "No Change" Certificate

Comment was solicited as to whether the Department's requirement for a certificate of "no change" and the requirement to submit all changes made to a local code since it was previously accepted or partially accepted were overly burdensome to small builders. We asked if there were alternatives to this approach that would be less burdensome but still meet HUD's objectives.

In response, the Department received several comments. One commenter stated that the certification of "no change" would in fact increase the burden on builders and on lenders and other interested parties. The commenter also argued that the Department should maintain, and that lenders or others should not be required to submit, this information. Another commenter believed that it is not unreasonable to require that all current local amendments to the codes be submitted to the HUD Field Office by the builder, lender or other interested party.

The Department believes that the certificate of "no change" is the least burdensome way of ensuring that a code has remained acceptable or partially acceptable. The Department does not have the resources to monitor the status of the thousands of building codes that exist in the country. In contrast, a builder must always comply with local codes whether or not he participates in HUD programs. Thus, he must know what the latest edition of an applicable code requires and is in a better position to know whether any changes have been made on the code since he last used it.

F. Procedures for Acceptance of Products and Materials

The Department also solicited comments concerning how its procedures for acceptance of materials and products under section 521 of the National Housing Act could be streamlined so that the Department would not duplicate functions and analyses performed by adjuncts to the national recognized model code organizations.

Several commenters approved of HUD's moving toward private sector product acceptance systems, such as those of the model code organizations and the National Evaluation Service (NES). These comments however, failed to discuss any other means of acceptance of materials and products available to HUD that would not be duplicative of the functions of the code organizations or the NES. In reply to our question on how HUD duplicates NES or model code procedures, one commenter suggested that internal HUD procedures be developed to permit HUD recognition of CABO National Evaluation Reports (NER) or new products, thus avoiding duplication. The commenter pointed out that the present HUD system requires the product manufacturer to pay Material Release and Structural Engineering Bulletin fees for the same product for which he has previously paid a fee for CABO analysis and issuance of an NER. The commenter concluded that this additional Federal agency expense and overlap should be avoided.

Similarly, in response to the Department's inquiry regarding how product durability might be ensured, several commenters suggested that the long-term durability of products and materials which are evaluated by NES and the model code organizations is assured because the reports are prepared on the basis of compliance with the code criteria and with nationally recognized industry standards. The commenters were not aware of any long-term durability problem with products, materials and housing systems that are evaluated by the model code organizations.

On the other hand, some commenters wanted to continue with HUD's Technical Suitability of Products Program, particularly the certification of products program. To do otherwise, the commenters argued, would compromise HUD's acceptance/assurance activity as well as cause considerable disruption to the current industry-wide certification process.

The Department has carefully evaluated these comments, and has decided to make no changes to the Technical Suitability of Products Program at this time. Although the Proposed Rule set forth no changes in the program, it requested comment on the issue to determine whether there was more information available since our last request for comment on this issue, on March 1, 1984. At that time, the Department published a proposed rule, at 49 FR 7587, that would have instituted a system of fees to cover the costs of services provided by the Technical Suitability of Products Program. On August 9, 1984, at 49 FR 31854, the Final Rule was published. The preamble of that Final Rule explained that, for several reasons, the Department decided not to make any changes in the present system for evaluating new products and materials.

As explained therein, the Department is required by statute to maintain such a system. Section 521 of the National Housing Act, 12 U.S.C. 1735e, states, in part, that "[t]he Secretary shall adopt a uniform procedure for the acceptance of materials and products to be used in structures approved for mortgages or loans insured under this Act. Under such procedure any material or product which the Secretary finds is technically suitable for the use proposed shall be accepted." The Department has complied with the statute by instituting the Technical Suitability of Products Program, and the system cannot, in accordance with the statute, be eliminated.

Because the Department received no new information since its previous determination to maintain current procedures, we are not changing the Technical Suitability of Products Program at this time.

The Department also received comments on the manner, if any, in which the new structure of the MPS would affect the current system of products and materials acceptance. Some commenters suggested that HUD, in view of the necessity to maintain durability criteria, should maintain in the MPS for One and Two Family Dwellings a list of product standards similar to that found in the MPS for Multifamily Housing. By so doing, it was suggested, HUD could assure that products and materials would at least meet an industry standard of quality and safety. It was also pointed out that some standards will never be listed in CABO because they relate to components or products such as cabinets, furnishings and carpet, which are not subject to building code enforcement. The commenters concluded that the omission of these standards from CABO necessitated HUD's referencing the standards, because homes with mortgages insured by HUD contain those components and products, and HUD should therefore ensure that they would meet acceptable levels of quality, safety and durability.

We agree that many products standards, and particularly HUD Use of Materials Bulletins, are not and probably will never be listed in model codes such as CABO. Because of our need to ensure the continued durability of products which are likely to be used in HUD-insured dwellings, we will reference, in § 200.926d, Appendices C and F of HUD Handbook 4910.1, MPS for Multifamily Housing. These appendices are lists of standards referenced by the Department.

Finally, a commenter recommended that HUD management coordinate any changes regarding the HUD Technical Suitability of Products Program with the Farmers Home Administration, Veterans Administation, and the Office of Management and Budget. The commenter noted that various agencies have conflicting requirements with respect to materials acceptance, and suggested that three Federal agencies and OMB jointly decide on workable acceptance procedures for new products, to be followed by the three agencies in the future. The Department agrees that this idea is worthwhile, and will investigate the possibility of coordinating acceptance procedures among these agencies.

G. Design Criteria for Housing for the Elderly

The last subject on which the Department solicited comment was its proposal to eliminate criteria for housing for the elderly. The Department asked whether any of these requirements should remain and whether other requirements should be incorporated.

Only a few commenters responded to the question. One commenter agreed that the Department should delete the design criteria for housing for the elderly. The commenter suggested that the free market should dictate design criteria, and that private sector committees under AARP, ANSI and others provide adequate guidelines.

Another commenter questioned whether the Department intended to adopt Appendix M of the Standard Building Code (SBC), which contains criteria for the handicapped. Because the final rule adopts only the CABO code and does not reference the SBC, it is inappropriate to adopt Appendix M of the SBC.

The Final Rule remains unchanged on this issue. We will therefore eliminate specific criteria for housing for the elderly.

H. Other Comments

The Department received many comments which suggested that the various appendices or supplements to the model codes be incorporated or excluded. One commenter also suggested that the Department designate the model code predominantly in use in any given area when supplementing partially acceptable codes. Because the final rule references only the CABO One and Two Family Dwelling Code, and not the four model codes, these issues are no longer of concern.

Several commenters expressed concern over the rule's impact on the Technical Suitability of Products Program. Some urged the Department to retain Use of Materials Bulletin No. 44, which establishes a standard for carpets. Another commenter suggested that HUD retain certain Federal Specifications for the tile and sheet vinyl flooring, as was done for the MPS for multifamily structures. As discussed above, we have incorporated Appendices C and F of the MPS for Multifamily Housing, HUD Handbook 4910.1, which list material standards and Use of Materials Bulletins that may be used in HUD-insured housing. Another commenter stated that § 200.926d(d), which covers Special Construction and Product Acceptance, was unclear with reference to whether HUD will continue to accept existing certification programs for HUD-approved products. This section has been rewritten to clarify that the procedures of HUD Handbook 4950.1 and Use of Materials Bulletins shall be applicable to the MPS for One and Two Family Dwellings.

One commenter suggested that the list of construction comparison items at §200.926a be rewritten so as to require a State or local code to regulate certain subareas only if local conditions warrant criteria on those subareas. The Department finds this comment persuasive, and has revised § 200.926a by requiring a code to regulate certain subareas only if certain conditions of ANSI A58.1–82 are present in the specific jurisdiction.

One commenter suggested that the number of major areas listed in § 200.926a be reduced, arguing that the Model Codes are not broken down in the same manner as the list of major areas. The Department based this list on the Model Codes themselves, and we believe that our list is reasonably consistent with the codes. The Department, however, has modified the list, which is also used to review codes for multifamily use, by deleting some subareas that apply primarily to multifamily-type construction. For example, the "Mechanical" major area no longer contains the "Boiler and Pressure Vessels" subarea.

One commenter suggested that the Department exempt modular construction from the rule. Although the Department is sympathetic to modular home manufacturers' concern regarding inconsistent local requirements, we see no reason to exempt modular construction from the system set forth in this rule, because modular housing always must comply with local requirements, whether it is being financed through HUD or through other means. The Department has no authority to promulgate a preemptive national code for modular housing.

One commenter suggested that local building officials should serve as the model code enforcement officials, instead of the Secretary's designee, as set forth in § 200.926b(b)(2). The Department, however, has no means of ascertaining the qualifications of local officials; therefore, to ensure that this function is adequately performed, the final rule is unchanged in this regard.

Another commenter suggested that the local code acceptance system could be simplified by using the certification of a State or local official or other qualified individual that the local or State code regulates the areas and subareas identified in § 200.926a.

Section 526 of the National Housing Act, however, states that the "Secretary shall be responsible for determining the acceptability of the State and local codes to . . . model codes." The Department therefore cannot delegate this responsibility. Moreover, the HUD Field Offices are responsible for determining whether a house complies with the applicable requirements. They must therefore be familiar with the codes, and should have responsibility for their acceptance.

One commenter argued that the system for code acceptance is too complex and should be simplified. The Department believes that, as set forth in the final rule, the system for acceptance is as simple as possible, considering the need for flexibility and thoroughness. The result of the system—reliance on State or local codes as much as possible—will ease the burden of unnecessary standards on builders and lenders. The Department believes that this goal justifies the complexity of the acceptance process.

Finally, the Department received many comments of an editorial nature. We have evaluated these comments, and have made changes in the Final Rule as appropriate.

III. Procedural Requirements

The collection of information requirements contained in this rule have been approved by the Office of Management and Budget (OMB) and assigned approval number 2502–0338.

This final rule does not constitute a "major rule" as that term is defined in section 1(b) of Executive Order 12291 on Federal Regulation issued by the President on February 17, 1981. The rule does not: (1) Have an annual effect on the economy of one hundred million dollars or more; (2) cause a major increase in costs or prices for consumers, individual industries, Federal State, or local government agencies or geographic regions; or (3) have significant adverse effect on competition, employment, investment, productivity, innovation or on the ability of United States-based enterprises to compete with foreign-based enterprises in domestic or export markets.

Consistent with the provisions of 5 U.S.C. 605(b) (The Regulatory Flexibility Act), the Secretary hereby certifies that this rule does not have a significant economic impact on a substantial number of small entities. In all cases, one and two family residential structures must be built in compliance with State or local codes. Upon the effective date of this rule, the Department generally will accept such compliance as satisfying the Department's concerns relating to the health and safety aspects of those structures. Further, this rule will generally reduce the burden of compliance which already exists for both small and large entities.

A Finding of No Significant Impact with respect to the environment has been made in accordance with HUD regulations in 24 CFR Part 50, which implement section 102(2)(C) of the National Environmental Policy Act of 1969. The Finding of No Significant Impact is available for public inspection during regular business hours at the Office of Rules Docket Clerk at Room 10276, Department of Housing and Urban Development, 451 Seventh Street SW., Washington, D.C. 20410.

This rule is listed as item H-74-82 in the Department's Semiannual Agenda of Regulations published at 50 FR 17286, 17298 on April 29, 1985 pursuant to Executive Order 12291 and the Regulatory Flexibility Act.

The catalog of Federal Domestic Assistance does not apply to this Rule.

Paperwork Reduction Act

Information collection requirements contained in this regulation (§ 200.926) have been approved by the Office of Management and Budget under the provisions of the Paperwork Reduction Act of 1980 (Pub. L. 96-511) and have been assigned OMB control number 2502-0338.

List of Subjects in 24 CFR Part 200

Administrative practice and procedure, Claims, Equal employment opportunity, Fair housing, Housing standards, Loan programs: Housing and community development, Mortgage insurance, Organization and functions (Government agencies), Reporting and recordkeeping requirements, Minimum Property Standards, Incorporation by reference.

Accordingly, HUD proposes to amend 24 CFR Part 200 as follows:

PART 200—INTRODUCTION

Subpart S—Minimum Property Standards

1. The authority citation for Part 200 continues to read as follows:

Authority: Titles I and II of the National Housing Act (12 U.S.C. 1701 through 1715a-18); sec. 7(d), Department of Housing and Urban Development Act (42 U.S.C. 3535(d)).

2. § 200.927 is revised as follows:

§ 200.927 Incorporation by reference of minimum property standards.

The Minimum Property Standards as contained in the handbooks identified in § 200.929(b) are incorporated by reference into this section as though set forth in full in accordance with 5 U.S.C. 552(a) and 1 CFR Part 51.

3. Section 200.929 is amended by revising the introductory paragraph of paragraph (b) and paragraph (b)(1) as follows:

§ 200.929 [Amended]

* * * * *

(b) *Identification.* The Minimum Property Standards have been published as described below:

(1) MPS for One and Two Family Dwellings. *See* §§ 200.926, 200.926a–e.

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4. 24 CFR Part 200 is amended by adding §§ 200.926, 200.926a, 200.926b, 200.926c, 200.926d and 200.926e as follows:

§ 200.926 Minimum property standards for one and two family dwellings.

(a) *Construction standards.*—(1) *Applicable structures.* The standards identified or contained in §§ 200.926 and 200.926a–200.926e shall apply to single family detached homes, duplexes, triplexes and to living units in a structure where the units are located side by side in townhouse fashion.

(2) *Applicability of standards to new construction.* The standards referenced in paragraph (a)(1) of this section are applicable to:

(i) Structures approved for insurance or other benefits prior to the start of construction;

(ii) Structures which are approved for insurance or other benefits based upon participation in an insured warranty program;

(iii) Structures which are insured as new construction based upon a Certificate of Reasonable Value issued by the Veterans Administration; and

(iv) Proposed construction insured under the Direct Endorsement program.

(b) *Conflicting standards.* The requirements contained in § 200.926d do not preempt local or State standards, nor do they alter or affect a builder's obligation to comply with any local or State requirements. However, a property shall be eligible for benefits only if it complies with the requirements of this subpart, including any referenced standards. When any of the requirements identified in § 200.926c are in conflict with a partially accepted local or state code, the conflict will be resolved by the HUD Field Office servicing the jurisdiction in which the property is to be located.

(c) *Standard for evaluating local or state building codes.* The Secretary shall compare a local building code submitted under § 200.926(d) or a State code to the list of construction related areas contained in § 200.926a.

(1) A local or State code will be accepted if it regulates each area and subarea on the list.

(2) A local or State building code will be partially accepted if it regulates most of the areas on the list. Provided, however, that no code may be partially accepted if it fails to regulate one or more subareas in more than one of the major areas. The major areas are: fire safety, light and ventilation, structural loads, foundation systems, materials standards, construction components, glass, mechanical, plumbing and electrical. *See* § 200.926a.

(3) For purposes of this paragraph, a local or State code regulates an area or subarea if it establishes a standard concerning that area or subarea.

(d) *Code selection.* Any materials required to be submitted under this section must be submitted by the time the lender or other interested party applies for mortgage insurance or other benefits.

(1) *Jurisdictions without previously accepted building codes.* The following submission requirements apply to lenders and other interested parties in jurisdictions without building codes, jurisdictions with building codes which have never been submitted for acceptance, and jurisdictions with building codes which previously have been submitted for acceptance and have not been accepted or partially accepted by the Secretary.

(i) In jurisdictions without local building codes:

(A) If the State building code is acceptable, the lender or other interested party must comply with the State building code and the requirements of § 200.926d;

(B) If the State building code is partially acceptable, the lender or other interested party must comply with:

(*1*) The acceptable portions of the partially acceptable code; and

(*2*) Those portions of the CABO One and Two Family Dwelling Code or the Electrical Code for One-and-Two-Family Dwellings designated by the HUD Field Office in accordance with § 200.926c; and

(*3*) The requirements of § 200.926d.

(C) If there is no State building code or if the State building code is unacceptable, the lender or other interested party must comply with:

(*1*) The CABO One and Two Family Code and the Electrical Code for One and Two Family Dwellings, as identified in § 200.926b(a); and

(*2*) The requirements of § 200.926d.

(ii) In jurisdictions with local building codes which have never been submitted for review, lenders or other interested parties must:

(A) Comply with the requirements of § 200.926(d)(1)(i) (A), (B) or (C), as appropriate; or

(B) Request the Secretary's acceptance of the local building code in accordance with § 200.926(d)(1)(iv).

(*1*) If the Secretary determines that the local building code is unacceptable, then the lender or other interested party must comply with the requirements of § 200.926(d)(1)(i) (A), (B) or (C) as appropriate.

(*2*) If the Secretary determines that the local code is partially acceptable, then the lender or other interested party must comply with:

(*i*) The acceptable portions of the partially acceptable local code; and

(*ii*) Those portions of the CABO One and Two Family Dwelling Code or the Electrical Code for One and Two Family Dwellings designated by the HUD Field Office in accordance with § 200.926c; and

(*iii*) The requirements of § 200.926d.

(*3*) If the Secretary determines that the local code is acceptable, then the lender or other interested party must comply with the local building code and the requirements of § 200.926d.

(iii) In jurisdictions with local building codes which previously have been submitted for review and which have been found unacceptable by the Secretary:

(A) If the local code has not been changed since the date the code or changes thereto were submitted to the Secretary, the lender or other interested party must comply with the requirements of § 200.926(d)(1)(i) (A), (B) or (C), as appropriate; or

(B) If the local code has been changed since the date when the code or changes thereto were submitted to the Secretary, the lender or other interested party must submit a copy of all changes to the local building code, including all applicable service codes and appendices and a copy of the statute, ordinance, regulation or order making such changes in the code, which have been made since the date when the code or other changes thereto were last submitted to the Secretary. However, the lender or other interested party need not submit any part already in the possession of the HUD Field Office. Based upon the Secretary's determination concerning the acceptability of the local code as changed, the lender or other interested party must comply with the requirements of § 200.926(d)(1)(ii)(B) (*1*), (*2*) or (*3*), as appropriate.

(iv) In order to obtain the Department's approval of a local code, the lender or other interested party must submit the following material to the HUD Field Office serving the jurisdiction in which the property is to be constructed:

(A) A copy of the jurisdiction's local building code, including all applicable service codes and appendices; and

(B) A copy of the statute, ordinance, regulation, or order establishing the code, if such statute, ordinance, regulation or order is not contained in the building code itself.

However, the lender or other interested party need not submit any document already on file in the HUD Field Office.

(2) *Jurisdictions with previously accepted or partially accepted building codes.* The following submission requirements apply to lenders or other interested parties in any jurisdiction with a building code which has been accepted or partially accepted by the Secretary:

(i) The lender or other interested party shall submit to the HUD Field Office serving the jurisdiction in which the property is to be constructed:

(A) A certificate stating that, since the date when the code or any changes thereto were last submitted to the Secretary, the jurisdiction's local building code has not been changed; or

(B) (*1*) A copy of all changes to the jurisdiction's building code, including all applicable service codes and appendices, which have been made since the date when the code or other changes thereto were last submitted to the Secretary. However, the lender or other interested party need not submit any part already in the possession of the HUD Field Office; and

(*2*) A copy of the statute, ordinance, regulation, or order making such changes in the code.

(ii) If, based upon changes to the local building code, the Secretary determines that it is unacceptable, the lender or other interested party must comply with the requirements of § 200.926(d)(1)(i)(A), (B) or (C), as appropriate.

(iii) If the local building code was previously found by the Secretary to be partially acceptable and there have been no changes to it or if the local building code was previously found by the Secretary to be partially acceptable and if, based upon changes to it, the Secretary determines that it is still partially acceptable or if the local building code was previously found by the Secretary to be acceptable and if, based upon changes to it, the Secretary determines that it is partially acceptable, then the lender or other interested party must comply with § 200.926(d)(1)(ii)(B)(*2*) (*i*), (*ii*) and (*iii*).

(iv) If the local building code was previously found by the Secretary to be partially acceptable and if, based upon changes to it, the Secretary determines that it is acceptable, or if the local building code was previously found by the Secretary to be acceptable and there have been no changes to the code, or if the local building code was previously found by the Secretary to be acceptable and if, based upon changes to it, the Secretary determines that it is still acceptable, then the lender or other interested party must comply with the local building code and the requirements of § 200.926d.

(3) *Notification of decision.* The Secretary shall review the material submitted under § 200.926(d). Following that review, the Secretary shall issue a written notice (except where there is a previously accepted or partially accepted code which has not been changed) to the submitting party stating whether the local building code is acceptable, partially acceptable, or not acceptable. Where the local building code is not acceptable, the notice shall also state whether the State code is acceptable, partially acceptable or not acceptable. The notice shall also contain the basis for the Secretary's decision and a notification of the submitting party's right to present its views concerning the denial of acceptance if the code is neither accepted nor partially accepted. The Secretary may, in his discretion, permit either an oral or written presentation of views.

(4) *Department's responsibilities.* (i) Each Regional and Field Office will maintain a current list of jurisdictions with accepted local or State building codes, a current list of jurisdictions with partially accepted local or State building codes and a current list of jurisdictions

with local or State building codes which have not been accepted. For local codes, the lists will state the most recent date when the code or changes thereto were submitted to the Secretary. The lists, which shall be prepared by the Field Offices and submitted to the Regional Offices, will be available to any interested party upon request. In addition, the list of jurisdictions whose codes have been partially accepted shall identify in accordance with § 200.926c those portions of the codes listed at § 200.926b(a) with which the property must comply.

(ii) The Department is responsible for obtaining copies of the State codes and any changes thereto.

§ 200.926a Residential building code comparison items.

HUD will review each local and State code submitted under this subpart to determine whether it regulates all of the following areas and subareas:

(a) *Fire Safety.*

(1) Allowable height;

(2) Fire separations;

(3) Fire resistance requirements;

(4) Egress doors and windows;

(5) Unit smoke detectors;

(6) Flame spread.

(b) *Light and ventilation.*

(1) Habitable rooms;

(2) Bath and toilet rooms.

(c) *Structural loads.*

(1) Design live loads;

(2) Design dead loads;

(3) Snow loads (for jurisdictions with snow loading conditions identified in Section 7 of ANSI A58.1–82);

(4) Wind loads;

(5) Earthquake loads (for jurisdictions in seismic zones 3 or 4 as identified in Section 9 of ANSI A58.1–82).

(d) *Foundation systems.*

(1) Foundation depths;

(2) Footings;

(3) Foundation materials criteria.

(e) *Materials standards.*

(1) Materials standards.

(f) *Construction components.*

(1) Steel;

(2) Masonry;

(3) Concrete;

(4) Lumber;

(5) Roof construction and covering;

(6) Chimneys and fireplaces.

(g) *Glass.*

(1) Thickness/area requirements;

(2) Safety glazing.

(h) *Mechanical.*

(1) Heating, cooling and ventilation systems;

(2) Gas, liquid and solid fuel piping and equipment;

(3) Chimneys and vents;

(4) Ventilation (air changes).

(i) *Plumbing.*

(1) Materials standards;

(2) Sizing and installing drainage systems;

(3) Vents and venting;

(4) Traps;

(5) Cleanouts;

(6) Plumbing fixtures;

(7) Water supply and distribution;

(8) Sewage disposal systems.

(j) *Electrical.*

(1) Branch circuits;

(2) Services;

(3) Grounding;

(4) Wiring methods;

(5) Cable;

(6) Conduit;

(7) Outlets, switches and junction boxes;

(8) Panelboards.

§ 200.926b Model codes.

(a) *Incorporation by reference.* The following model code publications are incorporated by reference in accordance with 5 U.S.C. 552(a) ahd 1 CFR Part 51. The incorporation by reference of these publications has been approved by the Director of the Federal Register. The locations where copies of these publications are available are set forth below.

(1) *CABO One and Two Family Dwelling Code,* 1983 edition, with the 1984 and 1985 Amendments, excluding Chapter 1—Administrative; Part VI—Electrical; and Part VII—Energy Conservation, but including Appendices A and B of the Code. Available from Council of American Building Officials, 5203 Leesburg Pike, Falls Church, VA 22041.

(2) *Electrical Code for One and Two Family Dwellings,* NFPA 70A, 1984 Edition, including appendices. Available from the National Fire Protection Association, Batterymarch Park, Quincy, Massachusetts 02269.

(b) *Model code compliance requirements.* (1) When a one or two family dwelling is to comply with the model codes set forth in § 200.926b(a), the following requirements of those model codes shall not apply to those properties:

(i) Those provisions of the model codes that establish energy requirements for one and two family dwellings; and

(ii) Those provisions of the model codes that require or allow the issuance of permits of any sort.

(2) Where the model codes set forth in § 200.926b(a) designate a building, fire, mechanical, plumbing or other official, the Secretary's designee in the HUD Field Office serving the jurisdiction in which the dwelling is to be constructed shall act as such official.

(c) *Designation of Model Codes.* When a one or two family dwelling is to comply with portions of a model code or the entire model code, it shall comply with the model codes in paragraph (c)(1) and/or (c)(2) of this section as designated by the HUD Field Office serving the jurisdiction in which the property is located. In addition, such property shall comply with all of the standards which are referenced in such code or codes.

(1) *CABO One and Two Family Dwelling Code/1983 with 1984 and 1985 Amendments.*

(2) *Electrical Code for One and Two Family Dwellings, NFPA 70A/1984.*

§ 200.926c Model code provisions for use in partially accepted code jurisdictions.

If a lender or other interested party is notified that a State or local building code has been partially accepted, then the properties eligible for HUD benefits in that jurisdiction shall be constructed in accordance with the applicable State or local building code, plus those additional requirements identified below. Depending upon the major area identified in § 200.926a which is not adequately regulated by the State or local code, the HUD Field Office will designate, in accordance with the schedule below, those portions of one of the model codes with which the property must comply.

SCHEDULE FOR MODEL CODE SUPPLEMENTS TO LOCAL OR STATE CODES

Deficient major from § 200.926a as determined by field office review	Portions of the CABO 1- and 2-family dwelling code/1983 with 1984 and 1985 amendments with which property must comply
(a) Fire safety	Chapters 2, 9; Section R-402.
(b) Light and ventilation	Chapter 2; Section R-309.
(c) Structural loads	Chapter 2.
(d) Foundation systems	Chapter 3.
(e) Materials standards	Chapter 26.
(f) Construction components	Part III.
(g) Glass	Chapter 2.
(h) Mechanical	Part IV.
(i) Plumbing	Part V.
(j) Electrical	Electrical code for 1- and 2-family dwellings (NFPA 70A–1984).

§ 200.926d Construction requirements.

(a) *Application.* (1) *General.* These standards cover the actual site, the immediate site environment for the dwellings, including streets, storm water disposal, and other services and facilities for the site.

(2) *Requirements for accessibility to physically handicapped people.* The HUD Field Office will advise project sponsors as to the extent accessibility will be required for new construction of one- and two-family dwellings on a project-by-project basis.

(i) *Technical standards. See* HUD Handbook, 4910.1, Sections 100–1.3b and 100–1.3c.

(3) *Variations to standards.*—

(i) *New materials and technologies. See* § 200.926d(d). Alternatives, nonconventional or innovative methods and materials shall be equivalent to these standards in the areas of structural soundness, durability, economy of maintenance or operation and usability.

(ii) *Variation procedures.* Variations from the requirements of any standard with which the Department requires compliance shall be made in the following ways:

(A) For a particular design or construction method to be used on a single case or project, the decision is the responsibility of the Field Office. Headquarters concurrence is not required.

(B) Where a variation is intended to be on a repetitive basis, a recommendation for a Local Acceptable Standard, substantiating data, and background information shall be submitted by the Field Office to the Director, Office of Manufactured Housing and Regulatory Functions.

(iii) Variances which require individual analysis and decision in each instance are not considered as repetitive variances even though one particular standard is repeatedly the subject of variation. Such variances are covered by § 200.926d(a)(3)(ii)(A).

(b) *General acceptability criteria.*—(1) *Real estate entity.* The property shall comprise a single plot except that a primary plot with a secondary plot for an appurtenant garage or for other use contributing to the marketability of the property will be acceptable provided the two plots are in such proximity as to comprise a readily marketable real estate entity.

(2) *Service and facilities.*—(i) *Trespass.* Each living unit shall be one that can be used and maintained individually without trespass upon adjoining properties, except when the windowless wall of a detached dwelling is located on a side lot line. A detached dwelling may be located on a side lot line if:

(A) legal provision is made for permanent access for the maintenance of the exterior portion of the lot line wall, and

(B) the minimum distances from the dwelling to the dwellings on the abutting properties are not less than the sum of the side yard distances computed as appropriate for the type of opposing walls. (minimum distance 10 ft).

(ii) *Utilities.* Utility services shall be independent for each living unit, except that common services such as water, sewer, gas and electricity may be provided for living units under a single mortgage or ownership. Separate utility service shut-off for each unit shall be provided. For living units under separate ownership, common utility services may be provided from the main to the building line when protected by an easement or convenant and maintenance agreement acceptable to HUD, but shall not pass over, under or through any other living unit. Individual utilities serving a living unit may not pass over, under or through another living unit under the same mortgage unless provision is made for repair and maintenance of utilities without trespass or when protected by an easement or covenant providing permanent access for maintenance and repair of the utilities. Building drain cleanouts shall be accessible from the exterior where a single drain line within the building serves more than one unit.

(3) *Site conditions.* (i) The property shall be free of those foreseeable hazards and adverse conditions which may affect the health and safety of the occupants or the structural soundness of the improvements, or which may impair the customary use and enjoyment of the property. The hazards include toxic chemicals, radioactive materials, other pollution, hazardous activities, potential damage from soil or other differential ground movements, ground water, inadequate surface drainage, flood, erosion, or others located on or off site. The site must meet the standards set forth at 24 CFR Part 51.

(ii) When special conditions exist or arise during construction which were unforeseen and which necessitate precautionary or hazard mitigation measures, the HUD Field Office shall require corrective work to mitigate potential adverse effects from the special conditions as necessary. Special conditions include rock formations, unstable soils or slopes, high ground water levels, springs, or other conditions which may adversely affect a property. It shall be the builder's responsibility to ensure proper design, construction and satisfactory performance where these conditions are present.

(4) *Access.* (i) Each property shall be provided with vehicular or pedestrian access by a public or private street. Private streets shall be protected by permanent easement.

(ii) Each living unit shall have a means of access such that it is unnecessary to pass through any other living unit.

(iii) The rear yard shall be accessible without passing through any other living unit.

(iv) For a townhouse type dwelling, access to the rear yard may be by means of alley, easement, passage through the dwelling, or other means acceptable to the HUD Field Office.

(c) *Site design.*—(1) *General.* (i) A site design shall be provided which includes an arrangement of all site facilities necessary to create a safe, functional, healthful, durable and energy efficient living environment.

(ii) These site design standards are applicable only in communities which have not adopted criteria for site development applicable to one and two family dwellings.

(iii) Single family detached houses situated on individual lots located on existing streets with utilities need not comply with the requirements of § 200.926d(c) (2), (3) and (4)(ii).

(2) *Streets.* (i) Existing or proposed streets on the site shall connect to private or public streets and shall provide all-weather access to all buildings for essential and emergency use, including access needed for deliveries, service, maintenance and fire equipment.

(ii) Streets shall be designed for dedication for public use and maintenance or, when approved by the HUD Field Office, may be retained as private streets where protected by permanent easements.

(3) *Dedication.* Utilities shall be located to permit dedication to the local government or appropriate public body.

(4) *Drainage and flood hazard exposure.* (i) The minimum grades at buildings and at openings into basements shall be at elevations which prevent adverse effect by water or water entering basements from flood levels equivalent to a 50 year return frequency after full development. The floor elevations of all habitable space shall be above runoff and flood levels equivalent to a 100 year return frequency after full development.

(ii) Streets shall be usable during runoff equivalent to a 10 year return frequency. Where drainage outfall is inadequate to prevent runoff equivalent to a 10 year return frequency from ponding over 6 inches deep, streets shall be made passable for commonly used emergency vehicles during runoff equivalent to a 25 year return frequency, except where an alternate access street not subject to such ponding is available.

(iii) Crawl spaces shall not pond water or be subject to prolonged dampness.

(d) *Special construction and product acceptance.*—(1) *Structural features of factory produced (modular or panelized) housing or components.*

(i) For factory fabricated systems or components, HUD Handbook 4950.1, "Technical Suitability of Products Program Technical and Processing Procedures" shall apply.

(ii) The requirements of this Part shall apply to structural features, consisting of factory fabricated systems or components assembled either at the factory or at the construction site, if the total construction is covered by these standards and can be inspected on-site for determination of compliance.

(2) *Non-structural or non-standard features.* These features include methods of construction, systems, sub-systems, components, materials and processes which are not covered by these requirements. *See* HUD Handbook 4950.1 for procedures to be followed in order to obtain acceptance of non-structural components or materials. *See* HUD Handbook 4910.1, Appendix F for a list of Use of Materials Bulletins. Products and methods shall conform to the appropriate Use of Materials Bulletin.

(3) *Standard features.* These features include methods of construction, systems, sub-systems, components, materials and processes which are covered by national society or industry standards. For a list of standards to which compliance is required, *see* HUD Handbook 4910.1, Appendix C.

(e) *Thermal requirements.*—(1) *Building insulation.*

(i) *General.* Buildings shall be insulated so as to ensure conservation of energy, economy of operation and comfort to the occupants.

(ii) *Overall coefficient of heat transmission.* (A) All buildings which are heated or cooled mechanically shall be constructed to comply with the U values shown in the table at § 200.926d(e)(1)(iii). The U values shown do not include adjustments for framing in walls, ceilings or floors, nor for the sash frame in windows or glass doors.

(B) Where the stated U value of any one component of roof deck, ceiling, wall or floor cannot be practically obtained, such U value may be increased to the minimum figure attainable and the U value for other components decreased until the overall heat gain or heat loss does not exceed the total attained by conformance to the stated U values. (See Note 2 of the table at § 200.926d(e)(1)(iii)).

(iii) *Component coefficient values.* For ceilings, walls, floors and openings, U values shall not exceed those shown in the following table:

MAXIMUM U VALUES FOR CEILING, WALL AND FLOOR SECTIONS FOR ELECTRIC RESISTANCE HEAT (E.R.) AND HEAT PUMP OR FOSSIL FUEL HEAT (F.F.)[1]

Heating degree days (65 °F base)	Ceilings[2,3]		Walls[2]		Floors[4]		Windows[5]		Sliding glass doors[5]		Storm doors[6]	
	E.R.	F.F.	E.R.	F.F.	E.R.	F.F.	E.R.	F.F.	E.R.	F.F.	E.R.	F.F.
0 to 1000	.05	.05	.08	.08			1.13	1.13	1.13	1.13	No	No.
1001 to 2500	.04	.05	.07	.08			.69	1.13	.69	1.13	No[7]	No.
2501 to 3500	.03	.04	.05	.08	.07		.69	1.13	.69	1.13	No[7]	No.
3501 to 4500	.03	.03	.05	.07	.05	.05	.69	.69	.69	.69	No[7]	No.
4501 to 6000	.03	.03	.05	.07	.05	.07	.47	.69	.69	.69	Yes	No.[7]
6001 to 7000	.026	.03	.05	.07	.05	.07	.47	.69	.69	.69	Yes	No.[7]
70001 +	.026	.026	.05	.05	.05	.05	.47	.47	.69	.69	Yes	Yes.

Notes

[1] For areas of 5,000 heating degree days (HDD) or less, houses using heat pumps may be insulated to levels required for fossil fuels. In areas above 5,000 HDD, houses using air-to-air heat pumps shall be insulated to levels required for electric resistance (E.R.) heating, except where the following are used:

a. Water source heat pumps.
b. Fossil fuel supplement heat.
c. Units with multiple capacity.
(1) Dual compressors
(2) Modulating compressor speed
(3) Dual speed compressor
d. Uni-directional heat pumps (such as annual cycle energy systems (ACES)).

e. Units with balanced heating and cooling load.

[2] The following combinations of wall and ceiling values are considered to provide annual heating and cooling consumption comparable to that predicted for values in the above Table and may be substituted accordingly. Other components shall conform to the values shown below for the specific heating degree day (HDD):

HDD (65°-F	ER		FF	
	CLG	Wall	CLG	Wall
0 to 1000	.04	.14	.04	.14
	.03	.15	.03	.15
	.026	.16	.026	.16
1001 to 1500	+	+	.04	.13
	.03	.13	.03	.14
	.026	.14	.026	.16
1501 to 2500	+	+	.04	.12
	.03	.11	.03	.13
	.026	.12	.026	.14
2501 to 3000	+	+	.03	.12
	.026	.07	.026	.13

(+See Table at § 200.926d(e)(1)(iii).

[3] Includes roof/ceiling assemblies, in which the finished ceiling is the underside of the roof deck.

[4] For floors of heated spaces over unheated basements, unheated garages or unheated crawl spaces. A basement, crawl space or garage shall be considered unheated unless it is provided with a positive heat supply sufficient to maintain a minimum temperature of 50°F. A positive heat supply is defined as heat supplied to a space by design or by heat losses occurring from energy-consuming systems or components associated with that space. Where the walls of an unheated basement or crawl space are insulated in lieu of floor insulation, the total heat loss attributed to the floor from the heated area shall not exceed the heat loss calculated for floors with required insulation.

[5] Maximum glass area shall not exceed 15 percent of the gross area of all exterior walls enclosing heated spaces, except when it can be demonstrated that the winter daily solar heat gain exceeds the 24 hour heat loss and the glass area is properly screened from summer solar heat gain. In areas where cooling is the predominant load and the heating load is insignificant (as an example, 2000 or more cooling hours and 2000 or less heating degree days), the maximum glazing area stated above may be waived when glass area is properly screened from solar heat gain. Any additional glass area shall have a significant portion of operable sash in order to provide natural ventilation.

[6] A 1-¾ inch metal faced door system with an insulated core and durable weatherstripping providing a U value equal or better than 0.32, and an infiltration rate not greater than .50 cfm per foot of crack length, tested accordingly to ASTM E 283 at 1.567 psf of air pressure, may be substituted for a conventional door and storm door. All exterior doors shall be weatherstripped.

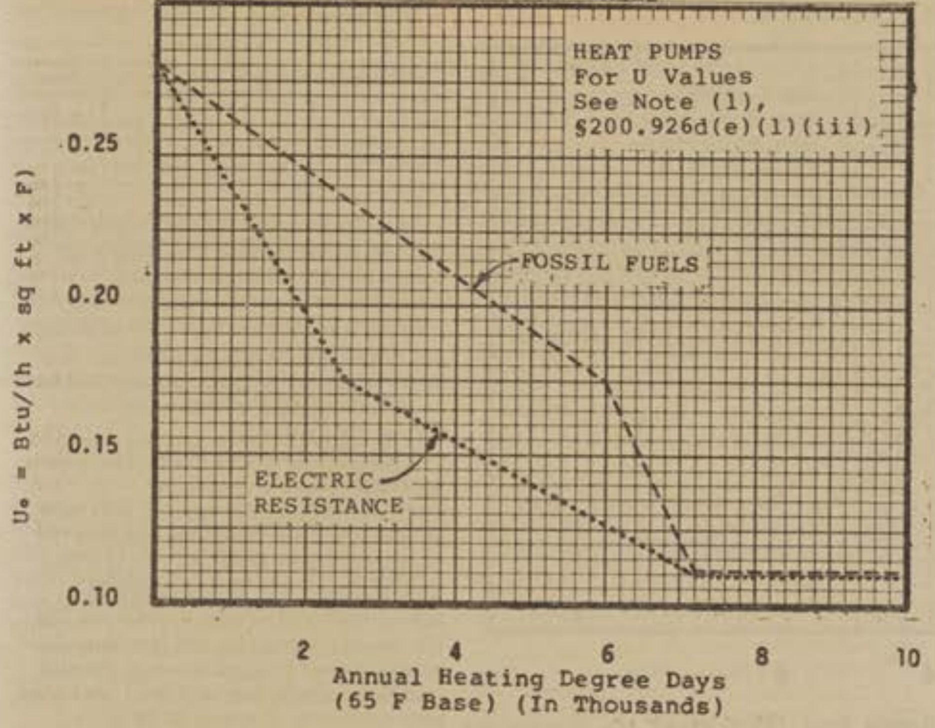
[7] In areas with 1501 or more heating degree days, a storm door is required when the primary door is a hollow core door or is over 25% glass.

(iv) *Alternate performance criteria.* (A) As an alternative to conformance with the table at § 200.926d(e)(1)(iii), dwellings which conform to the performance criteria of this section shall be considered acceptable.

(B) U_o (gross wall)—Total exterior wall area (opaque wall and window and door) shall have a combined thermal transmittance value (U_o value) not to exceed the values shown in Figure 1. Equation 1 shall be used to determine acceptable combinations to meet the requirements of Figure 1.

(C) U_o (gross ceiling)—Total ceiling area (opaque ceiling and skylights) shall have a combined thermal transmittance value (U_o value) not to exceed the values shown in Figure 2. Equation 2 shall be used to determine acceptable combinations to meet the requirements of Figure 2.

FIGURE 1 - GROSS WALLS



Equation 1 Formula for Determining Combinations (See Figure 1)

where

${}^{U}0 = ({}^{U}\text{wall}\ {}^{A}\text{wall} + {}^{U}\text{window}\ {}^{A}\text{window} + {}^{U}\text{door}\ {}^{A}\text{door})/A$

${}^{U}0$ = the average thermal transmittance of the gross wall area, Btu/(h x sq. ft. x F)

${}^{A}0$ = the gross area of all exterior walls enclosing heated spaces, sq. ft.

${}^{U}\text{wall}$ = the thermal transmittance of all elements of the opaque wall area, Btu/(h x sq. ft. x F)

${}^{A}\text{wall}$ = opaque wall area enclosing heated spaces, sq. ft.

${}^{U}\text{window}$ = the thermal transmittance of the window area, Btu/(h x sq. ft. x F)

${}^{A}\text{window}$ = window area (including sash), sq. ft.

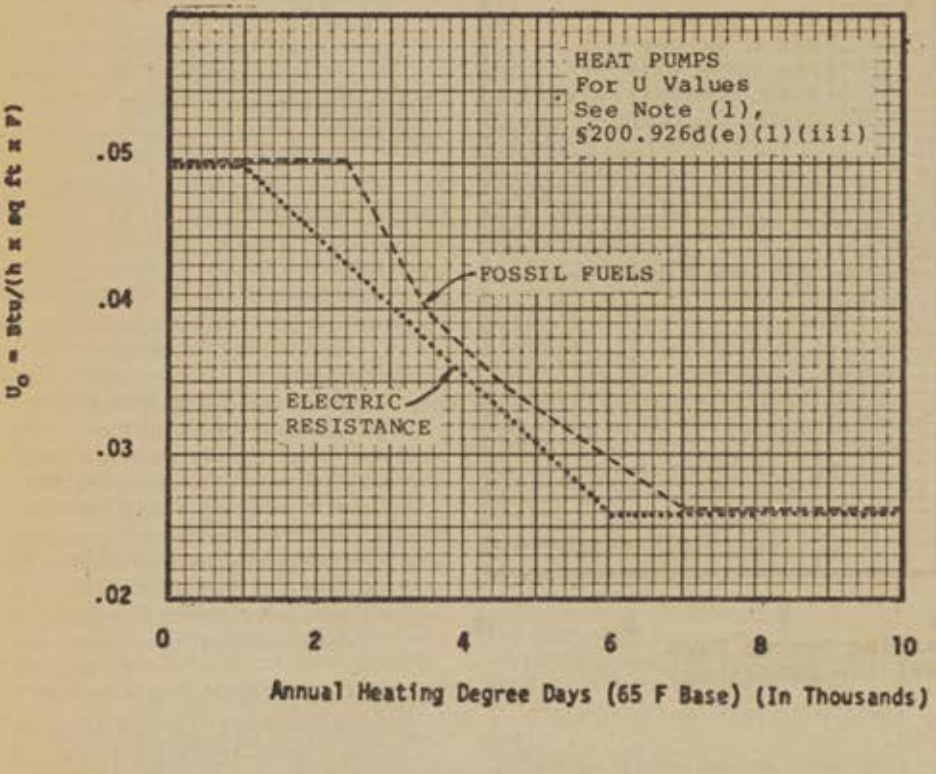
${}^{U}\text{door}$ = the thermal transmittance of the door area, Btu/(h x sq. ft. x F)

${}^{A}\text{door}$ = door area (including sash), sq. ft.

Note.—Where more than one type of wall, window and/or door is used, the U x A term for that exposure shall be expanded into its sub-elements, as follows:

${}^{U}\text{wall}_1{}^{A}\text{wall}_1 + {}^{U}\text{wall}_2{}^{A}\text{wall}_2$, etc.

GROSS CEILING - FIGURE 2



Equation 2 Formula for Determining Roof/Ceiling Combinations

where:

$U_o = ({}^{U}roof{}^{A}roof + {}^{U}skylight{}^{A}skylight/A$.

U_o = the average thermal transmittance of the gross roof/ceiling area, Btu/(h x sq. ft. x F)

A_o = the gross area of a roof/ceiling assembly, sq. ft.

${}^{A}roof$ = opaque roof/ceiling area, sq. ft.

${}^{U}roof$ = the thermal transmittance of all elements of the opaque roof/ceiling area, Btu/(h x sq. ft. x F).

${}^{U}skylight$ = the thermal transmittance of all skylight elements in the roof/ceiling assembly, Btu/h x sq. ft. x f)

${}^{A}skylight$ = skylight area (including frame), sq. ft.

Note to Equation 2.—Where more than one type of roof/ceiling and/or skylight is used, the U x A term for that exposure shall be expanded into its subelements, as:

${}^{U}roof_1\ {}^{A}roof_1 = {}^{U}roof_2\ {}^{A}roof_2$, etc.

(v) *Overall structure performance alternative.* Structures which can be shown by accepted engineering practice to have energy consumption equal to or less than that which would be obtained by conformance to the criteria of § 200.926(e)(1)(iii) or (iv) shall be considered acceptable. The contribution of passive solar energy and the related storage and reradiation capacity of masonry, water and other mass may be recognized in computing energy consumption under this alternate method. The following requirements shall govern in determining comparability:

(A) The methodology shall be cost effective to the energy consumer.

(B) The methodology shall not adversely affect the structural capacity, durability, or safety aspects of the structure.

(C) All data and calculations must show valid performance comparison between the proposed option and a structure comparable in size, configuration, orientation and occupant usage designed in accordance with § 200.926d(e)(1) (iii) or (iv).

(vi) *Basement or crawl space foundation walls.* Insulation may be omitted from floors over heated basement areas or heated crawl spaces if foundation walls are insulated. Foundation walls of heated areas below grade need not be insulated except where recreation or similar use rooms or habitable rooms are provided, or where more than 50 percent of the wall is exposed to outside air. The U value of foundation wall sections shall not exceed the values shown in the following table except where the alternative methods shown in § 200.926d(e)(1) (iv) or (v) are employed and foundation walls are included in the determination of the average thermal transmittance of the gross wall area.

MAXIMUM U VALUES OF THE FOUNDATION WALL SECTIONS OF HEATED BASEMENT OR HEATED CRAWL SPACE

Heating degree days (65 °F base)	Maximum U value
2500 or less	No requirement.
2501 to 4500	0.17
4501 or more	0.10

(vii) *Crawl space plenum walls.* When a crawl space is used as a supply or return plenum, the crawl space perimeter wall shall be insulated to provide a maximum heat loss of 35 Btuh per lineal foot of perimeter wall, assuming a crawl space air temperature of 70 °F for return plenums and 110 °F for supply plenums.

(viii) *Slab-on-grade floors.* For slab-on-grade floors of heated or mechanically cooled spaces, the thermal resistance of the insulation around the perimeter of the floor shall be not less than shown in the following table. Insulation shall extend downward from the top of the slab for not less than 24 in. or downward to the bottom of the slab and horizontally beneath the slab for a minimum total distance of 24 in.

MINIMUM R VALUES OF PERIMETER INSULATION FOR SLABS-ON-GRADE

Heating degree days (65 °F base)	Minimum R values [1]	
	Heated slab	Unheated slab
500 or less	2.8	
1,000	3.5	
2,000	4.0	
2,500	4.4	2.5
3,000	4.8	2.8
4,000	5.5	3.5
5,000	6.3	4.2
6,000	7.0	4.8
7,000	7.8	5.5
8,000	8.5	6.2
9,000	9.2	6.8
10,000 or greater	10.0	7.5

[1] For increments between degree days shown, U values may be interpolated, or the values shown in Figure 3 of ASHRAE 90A-80 may be substituted.

(ix) *Heat loss and heat gain calculations.* (A) Calculations of heat loss and heat gain shall be made in accordance with the data and procedures contained in the American Society of Heating Refrigerating and Air-conditioning Engineers' (ASHRAE) Handbook of Fundamentals–1985, the Hydronics Institute's "Heat Loss Calculation Guide" H-21-1984 and "Cooling Load Calculation Guide" C-30-1965, and the Air Conditioning Contractors of America's "Load Calculation for Residential Winter and Summer Air Conditioning" Manual J-1981.

(B) Inside design temperature shall be 70 °F for heating and 75 °F for cooling. The outside design temperature for heating shall be that established by the ASHRAE Handbook of Fundamentals at the 97.5% winter design dry bulb temperature for the location involved. The outside design temperature for cooling shall be that established by the ASHRAE Handbook of Fundamentals at the 2.5% summer design dry bulb temperature for the location involved.

(f) *Water supply systems.*— (1) *General.* (i) Each living unit shall be provided with a continuing and sufficient supply of safe water under adequate pressure and of appropriate quality for all household uses. This system shall not impair the function or durability of the plumbing system or attachments.

(ii) The chemical and bacteriological standards of the local health authority shall apply. In the absence of such standards, the maximum contaminant levels of EPA shall apply. A water analysis may be required by either the health authority or the HUD Field Office.

(iii) Whenever feasible, connection shall be made to a public water system. When a public system is not available, connection shall be made to a community system which complies with HUD Handbook 4940.2, if feasible.

(2) *Individual water systems.* (i) The system should be capable of delivering a flow of 5 gpm over at least a 4 hour period.

(ii) Water that requires continuing or repetitive treatment to be safe bacterially or chemically is not acceptable. Individual dwelling water purification units are not an acceptable alternative but may be used to improve acceptable water.

(iii) After installation, the system shall be disinfected in accordance with the recommendations or requirements of the local health authority. In the absence of a health authority, system cleaning and disinfection shall conform to the current EPA Manual of Individual Water Supply Systems.

(iv) Bacteriological or chemical examination of a water sample collected by a representative of the local or state health authority shall be made when required by that authority or the HUD Field Office.

(3) *Location of wells.* (i) A well located within the foundation walls of a dwelling is not acceptable except in arctic or subarctic regions.

(ii) Water which comes from any soil formation which may be polluted, contaminated, fissured, creviced or less than 20 ft. below the natural ground surface is not acceptable, unless acceptable to the local health authority.

(iii) Individual water supply systems are not acceptable for individual lots in areas where chemical soil poisoning has been or is practiced if the overburden of soil between the ground surface and the water bearing strata is coarse grained sand, gravel, or porous rock, or is creviced in a manner which will permit the recharge water to carry the toxicants into the zone of saturation.

(iv) The following table shall be used in establishing the minimum acceptable distances between wells and sources of pollution located on either the same or adjoining lots. These distances may be increased by either the health authority having jurisdiction or the HUD Field Office.

DISTANCE FROM SOURCE OF POLLUTION

Source of pollution	Minimum horizontal distance (feet)
Property Line	10
Septic Tank	50
Absorption Field	[1] 100
Seepage Pit	[1] 100
Absorption Bed	[1] 100
Sewer Lines w/Permanent Watertight Joints	10
Other Sewer Lines	50
Chemically Poisoned Soil	[3] 25
Dry Well	50
Other	([2])

[1] This clearance may be increased or decreased depending upon soil and rock penetrated by the well and aquifer conditions. The clearance may be increased in creviced limestone and permeable strata of gravel and sand. The clearance may be reduced to 50 ft. only where the ground surface is effectively separated from the water bearing formation by an extensive, continuous and impervious strata of clay, hardpan, or rock. The well shall be constructed so as to prevent the entrance of surface water and contaminants.

[2] The recommendations or requirements of the local health authority shall apply.

[3] This clearance may be reduced to 15 feet only where the ground surface is effectively separated from the water bearing formation by an extensive, continuous and impervious strata of clay, hardpan, or rock.

(4) *Well construction.* (i) The well shall be constructed so as to allow the pump to be easily placed and to function properly.

(ii)(A) All drilled wells shall be provided with a sound, durable and watertight casing capable of sustaining the loads imposed.

(B) The casing shall extend from a point several feet below the water level at drawdown or from an impervious strata above the water level to 12 in. above either the ground surface or the pump room floor. The casing shall be sealed at the upper opening to a depth of at least 15 feet.

(iii) Bored wells shall be lined with concrete, vitrified clay or equivalent materials.

(iv) The space between the casing or liner and the wall of the well hole shall be sealed with cement grout.

(v) The well casing shall not be used to convey water except under positive pressure. A separate drop pipe shall be used for the suction line.

(vi) When sand or silt is encountered in the water-bearing formation, the well shall either be compacted and gravel packed, or a removable strainer or screen shall be installed.

(vii) The surface of the ground above and around the well shall be compacted and graded to drain surface water away from the well.

(viii) Openings in the casing, cap, or concrete cover for the entrance of pipes, pumps or manholes shall be watertight.

(ix) If a breather is provided, it shall extend above the highest level to which surface water may rise. The breather shall be watertight, and the open end shall be screened and positioned to prevent entry of dust, insects and foreign objects.

(5) *Pump and equipment.* (i) Pumps shall be capable of delivering the volume of water required under normal operating pressure within the living unit. Pump capacity shall not exceed the output of the well.

(ii) Pumps and equipment shall be mounted to be free of objectionable noises, vibrations, flooding, pollution, and freezing.

(iii) Suction lines shall terminate below maximum drawdown of the water level in the well.

(iv) Horizontal segments of suction line shall be placed below the frost line in a sealed casing pipe or in at least 4 in. of concrete. The distance from suction line to sources of pollution shall be not less than shown in the table at § 200.926d(f)(3)(iv).

(6) *Storage tanks.* (i) A pressure tank having a minimum capacity of 42 gallons shall be provided. However, prepressured tanks and other pressurizing devices are acceptable provided that delivery between pump cycles equals or exceeds that of a 42 gallon tank.

(ii) Tanks shall be equipped with a clean-out plug at the lowest point, and a suitable pressure relief valve.

§ 200.926e Supplemental information for use with the CABO One and Two Family Dwelling Code.

The following shall be used in Table No. R-202, Climatic and Geographic Design Criteria of the CABO One and Two Family Dwelling Code.

(a) *Roof live loads.*

Roof slope 3 in 12 or less: 20 psf
Roof slope over 3 in 12: 15 psf
Roof used as deck: 40 psf

(b) *Roof snow load.* The roof snow load shall be in accordance with section 7 of ANSI A58.1–82.

(c) *Wind pressures.* The minimum Design Wind Pressures (net pressures) set forth below apply to areas designated as experiencing basic wind speeds up to and including 80 mph, as shown in ANSI A58.1–82, Figure 1, Basic Wind Speed Map. These pressures also apply to buildings not over 30 ft. in height above finish grade, assuming exposure C or defined in ANSI A58.1–82.

(1) *Minimum design wind pressure criteria.*

(i) Buildings (for overturning racking or sliding): p=20 psf.

(ii) Chimneys, p=30 psf.

(iii) Exterior walls, p=15 psf inward or outward. Local pressure at corners of walls shall be not less than p=30 psf outward. These local pressures shall not be included with the design pressure when computing overall loads. The pressures shall be applied perpendicularly outward on strips of width equal to 10 percent of the least width of building.

(iv) Partitions, p=10 psf.

(v) Windows, p=20 psf inward or outward.

(vi) Roof, p=20 psf inward or outward.

Roofs with slopes greater than 6 in 12 shall be designed to withstand pressures acting inward normal to the surface, equal to the design wind pressure for exterior walls. Overhanging eaves, cornices, and ridges, 40 psf upward normal to roof surface. These local pressures shall not be included with the design pressure when computing overall loads. The pressures shall be applied perpendicularly outward on strips of width equal to 10 percent of the least width of building.

Net uplift on horizontal projection of roof shall not be less than 12 psf.

(2) *Severe wind design pressures.* If the construction is higher than 30 ft., or if it is located in an area experiencing wind speeds greater than 80 mph, higher design wind pressures than shown above are required. Use Section 6 of ANSI A58.1–82 for higher criteria and for determining where wind speeds greater than 80 mph occur.

Pressures are assumed to act horizontally on the gross area of the vertical projection of the structure except as noted for roof design.

(d) Seismic conditions shall be in accordance with Section 9 of ANSI A58.1–82.

(e) *Subject to damage from: weathering.* A jurisdiction's weathering region shall be as established by the map in ASTM C 62–83.

(f) *Subject to damage from: frost line depth.* Exterior wall footings or foundation walls including those of accessory buildings shall extend a minimum of 6 in. below the finished grade and, where applicable, the prevailing frost line.

(g) *Subject to damage from: termites.* "Yes" shall be used in locations designated as Regions I, II or III. "No" shall be used in locations designated as Region IV. The map for Termite Infestation Probability in Appendix A of CABO, One and Two Family Dwelling Code shall be used to determine the jurisdiction's region.

(h) *Subject to damage from: decay.* "Yes" shall be used in locations designated as moderate to severe and slight to moderate. "No" shall be used in locations designated as none to slight. The Decay Probability map in Appendix A of CABO, One and Two Family Dwelling Code shall be used to determine the jurisdiction's decay designation.

(Approved by the Office of Management and Budget under OMB control number 2502–0338.)

5. The Appendix to Part 200 is revised as follows:

Appendix to Part 200—Standards Incorporated by Reference in the Minimum Property Standards for One and Two Family Dwellings

The following publications are incorporated by reference in the HUD Minimum Property Standards (MPS). The MPS are in turn incorporated by reference in 24 CFR Part 200, Subpart S. The MPS may be purchased from the U.S. Government Printing Office, Washington, DC 20402. It is also available for public inspection at the HUD Program Information Center, Room 1104, 451 Seventh Street, S.W., Washington, DC, at each HUD Regional, Area, and Service Office, and at the Office of the Federal Register, 1100 L Street, NW, Washington, DC. The individual standards referenced in the MPS are available at the addresses contained in the following table. They are also available for public inspection at HUD, Manufactured Housing and Construction Standards Division, Room 9156, 451 Seventh Street, SW, Washington, DC and the Office of the Federal Register.

Air Conditioning Contractors of America, 1228 17th Street, N.W., Washington, DC 20036.
  Load Calculation for Residential Winter and Summer Air Conditioning, Manual J 1981.
American National Standards Institute, 1430 Broadway, New York, New York 10018.
  ANSI A58.1–82 Minimum Design Loads in Building and other Structures.
American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pennsylvania 19103.
  ASTM C 62–83 Standard Specification for Building Brick (Solid masonry units made from clay or shale).
American Society of Heating, Refrigerating and Air Conditioning Engineers, 1791 Tullie Circle, NE, Atlanta, Georgia 30329.
  ASHRAE Handbood of Fundamentals—1985.
  ASHRAE Energy Conservation in New Building Design 90A–80.
Council of American Building Officials, 5203 Leesburg Pike, Falls Church, Virginia 22041.
  CABO One and Two Family Dwelling Code, 1983 edition, with the 1984 and 1985 Amendments, excluding chapter 1—Administrative; Part VI—Electrical; and Part VII—Energy Conservation, but including appendices A and B of the Code.
The Hydronics Institute, 35 Russo Place, Berkeley Heights, New Jersey 07922.
  Heat Loss Calculation guide, H–21–1984.
The National Fire Protection Association, Batterymarch Park, Quincy, Massachusetts 02269.
  Electrical Code for One and Two Family Dwellings, NFPA 70A, 1984 Edition, including appendices.
Department of Housing and Urban Development, 451 Seventh Street, S.W., Washington, DC 20410.
  Handbooks:
  4940.2–8/73 Minimum Design Standards for Community Water Supply.
  4950.1–8/79 Technical Suitability of Products Program, Technical and Processing Procedures.
Environmental Protection Agency, 401 M Street, S.W., Washington, DC 20460.
  EPA 430/9–74/007 Manual of Individual Water Supply Systems (Reprinted 1975) (PB 258402).

Dated: September 18, 1985.

Janet Hale,

Acting General Deputy Assistant Secretary for Housing—Federal Housing Commissioner.

[FR Doc. 85–22935 Filed 9–26–85; 8:45 am]

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Friday
September 27, 1985

Part VI

Department of Justice

Office of Juvenile Justice and Delinquency Prevention

Program Announcement; School Crime and Discipline Research and Development; Notice

DEPARTMENT OF JUSTICE

Office of Juvenile Justice and Delinquency Prevention

Program Announcement: School Crime and Discipline Research and Development Program

AGENCY: Office of Juvenile Justice and Delinquency Prevention, Justice.

ACTION: Notice of issuance of a solicitation of applications to conduct a research and development program on school crime.

SUMMARY: The Office of Juvenile Justice and Delinquency Prevention (OJJDP), pursuant to section 243 of the Juvenile Justice and Delinquency Prevention Act of 1974, as amended, announces a new OJJDP initiative entitled, "School Crime and Discipline Research and Development Program." The primary goal is to test promising strategies for the development, implementation, and monitoring of disciplinary and crime control policies and procedures which are intended to reduce school crime and disorder to provide for a safer school environment.

OJJDP's National Institute for Juvenile Justice and Delinquency Prevention (NIJJDP) invites public or private agencies to submit competitive grant applications to design, develop and test the efficacy of improved disciplinary policies and procedures for the reduction of school crime and disorder in secondary schools. Targeted schools must be experiencing a high level of disciplinary or crime problems. Emphasis will be placed on the assessment of the effects of legislation, case law, regulations and lawsuits related to discipline and crime control. This initiative will require a collaborative effort among researchers, program planners, school administrators, faculty, and law enforcement personnel, as appropriate.

The deadline for submission of applications is December 6, 1985.

FOR FURTHER INFORMATION CONTACT: Deborah A. Wysinger, Office of Juvenile Justice and Delinquency Prevention, 633 Indiana Ave, N.W., Room 742, Washington, D.C. 20531, telephone (202) 724-7560.

SUPPLEMENTARY INFORMATION:

Request for Proposals—School Crime and Discipline Research and Development Program

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I. Introduction

This solicitation for applications to conduct a School Crime and Discipline Research and Development Program is issued by the National Institute for Juvenile Justice and Delinquency Prevention (NIJJDP), of the Office of Juvenile Justice and Delinquency Prevention (OJJDP), U.S. Department of Justice. The OJJDP and its NIJJDP were established by the Juvenile Justice and Deliquency Prevention Act of 1974, as amended (JJDP Act).

The JJDP Act authorizes NIJJDP to: "encourage the development of demonstration projects in new, innovative techniques and methods to prevent and treat juvenile delinquency;" (Section 243(2)) and to "provide for the evaluation of all juvenile delinquency programs assisted under this title in order to determine the results and the effectiveness of such programs," (Section 243(3)).

OJJDP's National Institute for Juvenile Justice and Delinquency Prevention invites public or private agencies to submit applications designed to develop and test promising strategies for the reduction of school crime and improvement of disciplinary procedures and school environment.

OJJDP has allocated a total of $300,000 for the first phase of this Initiative. Up to three projects not to exceed $100,000 each for the initial twelve month budget period will be funded as a result of this competition. It is anticipated that this research and development initiative will entail three to four years of program activity to conduct the necessary planning, implementation and testing. Therefore project periods will not exceed four years. The second phase of this initiative will consist of awards to each of the three selected R&D projects for up to $200,000 for each subsequent twelve month budget period.

This competition will be conducted according to the OJJDP Competition and Peer Review Policy, 28 CFR Part 34, Subpart A, published August 2, 1985 at 50 FR 31365–31366.

II. Background

Creating a safe and orderly school environment is a prerequisite for students to focus their attention on learning. Many of the recent reports on educational reform—while agreeing on the need for improved curricula and graduation standards—insist that little reform can occur unless schools become safer. President Reagan put the problem in perspective while speaking to secondary school principals, "As long as one teacher is assaulted, one classroom disrupted, or one student attacked, then I must and will speak out to give you the support you need to enforce discipline in our schools. I can't say it too forcefully, to get learning back into our schools, we must get crime and violence out." The issue of student rights and administrative control have become a focal point for political discussion.

The most comprehensive recent national study specifically devoted to school crime is the *Safe School Study*, which was conducted by the National Institute of Education (NIE) in the mid 1970's. The major objectives of that study were to determine the frequency and seriousness of crime in elementary and secondary schools in the United States and what schools are doing to prevent it. The findings, published in 1978, indicated that 8% of the nation's schools had a serious problem with crime, that one-fourth of all schools in the country were vandalized in a given month and 10% were burglarized.

Based on student reports, NIE estimated that 2.4 million secondary school students (11%) had something stolen from them in a typical month. Approximately 1.3% of the students (282,000) reported being attacked in a typical month, with more than two-fifth of the attacks involving some injury. Junior high school students were twice as likely to report being attacked as high school students. The risk of serious attack was greater in urban areas. Teacher victimization rates reported in the NIE study were roughly similar to those of students.

A 1983 report on school violence by Jackson Toby, Director of Rutgers University's Institute for Criminological Research, concluded that the NIE data had probably under estimated the actual instances of school violence at the time the survey was conducted ("Violence in School," *Crime and Justice: An Annual Review of Research*, Vol. 4).

Problems with school discipline are not only recognized by the general public—teachers also list discipline problems as a major concern. The National Education Association (1977) reported that 66% of responding teachers viewed managing student behavior as the main problem faced in teaching. Many teachers felt they were unable to even cope with, much less resolve, the discipline problems they faced each day (Kindsvetter, 1978).

The operation of public schools has traditionally been largely a matter for local determination, subject to state control in certain respects. Recently, however, a number of federal statues and judicial decisions have regulated, in one way or another, the activities of public schools,[2] and some of these have engendered state legislative action also affecting local practices.[3] The complexity of this regulatory enterprise is apparent. On the one hand, legislative and judicial decisions set policies at a remote level that are expected to produce consistent behavior in schools. This requires collaboration and cooperation among various levels. The rule or desired practice must be clearly expressed and understood by those charged with implementation. Local administrators must be able and willing to carry out the policy,[4] and where several agencies are responsible for administration, they must agree about their obligations and discharge them in a coordinated and uniform fashion for policy implementation to be complete.

On the other hand, regulation has significance for local officials whether or not they accurately follow specific commands. While appellate courts usually address these discipline and crime control issues on a piecemeal basis, program administrators ordinarily cannot.

The relationship between judicial or legislative regulation and the behavior of those affected by regulatory activity is a complicated matter from a number of perspectives. Courts are mandated to make decisions of specific issues and in contrast school officials need to develop comprehensive policies. For example, based on the United States Supreme Court decision in *Goss v. Lopez*, 419 U.S. 565 (1975), the Indiana State legislature applied that decision through the imposition of procedural rules for short disciplinary supensions of students.[5] School districts have gone somewhat further by specifying procedures for expulsion and exclusion from school, or for inschool suspension of students and other forms of discipline.

However, school administrators must also decide what procedures are required for other alternative methods of discipline they employ. Another example is the recent analysis of the court decision of the *New Jersey v. T.L.O.*, 105 S. Ct. 733 (1985), where the court provided general guidelines for permissible student searches by school officials, but did not specifically address issues such as: whether students have a legitimate expectation of privacy in lockers, desks or other school property in which school supplies are stored; or standards governing searches of those areas by school officials or other public authorities acting at school officials' request.[6] School administrators and teachers must either abandon procedures for which clear guidance is lacking, which may be undesirable or even impracticable, or they must consider additional safeguards in terms of what will probably be required by courts when they ultimately address the procedural requirements for those alternatives. This latter approach carries with it some significant risks, including the possiblity that the action taken will not only be declared invalid but will give rise to a lawsuit for damages predicated upon violation of a student's civil rights.[7]

Some commentators have suggested that certain legislative code provisions and case law may prevent the use of disciplinary and crime control techniques perceived as necessary to maintain a safe education environment. One problem appears to be the effectiveness of the punishment that is both legally acceptable and educationally sound. Several reports indicate that student lawsuits filed against teachers and administrators, the threat of such suits, and the belief that they may be filed inhibit effective discipline in schools. Specifically, lawsuits brought pursuant to 42 U.S.C. Section 1983, as interpreted by *Wood v. Strickland*, 420 U.S. 308 (1975), are believed to influence disciplinary and crime control practices, although empirical evidence is virtually nonexistent. In addition, those practices are influenced by court decision such as *Goss v. Lopez*, 419 U.S. 565 (1975), and *New Jersey v. T.L.O.*, 105 S.Ct. 733 (1985). The following questions merit further investigation: whether changes in the law, including court decisions, have resulted in more or less effective school discipline and crime control; to what extent does the perception that lawsuits might be filed actually inhibit implementation of certain approaches to school discipline and their effectiveness; to what extent do lawsuits (potential suits and those actually filed and decided) have an impact on the effectiveness of school discipline and how widespread are these perceptions and effects; what changes, if any, in the law are desirable.

Opinions differ regarding whether the justice system standards and procedures of due process are appropriate for schools, or, whether they interfere with effective disciplining of disruptive students. Certain studies, supporting the incorporation of justice system due process, associate observance of these standards with more positive student attitudes towards school rules, the legal system and academic achievement. Other educators would argue that following the burdensome procedures of due process unduly inhibits their ability to discipline a disruptive student in a timely and effective fashion.

Preliminary findings based on a 12 percent return from a study currently being conducted by the National Center for Education Statistics on school principals' perceptions of school discipline policies and practices show that only a small percentage of the principals surveyed consider the Supreme Court rulings to be a moderate or large operational burden. The findings also indicated that the principals believed that lack of security personnel, or fear of being sued for disciplining students had little effect on the ability of teachers or themselves to maintain order and discipline students. Other constraints, such as inadequate teacher training in discipline procedures and school law and inadequate alternative placement programs for disruptive students, had a greater effect on the ability to maintain order than those previously discussed. It should be noted, however, that the study findings are very preliminary and that the survey was only of school principals and not teachers.

In assessing the impact of federal case precedents on local school operations, it is important to assess the level of knowledge of administrators and teachers about relevant legislation and major court decisions. A recent survey conducted by Julius Menacker and Ernest Pascarella of teachers and administrators of inner city and suburban Chicago public schools on their knowledge of education-related Supreme Court rulings revealed that administrators scored significantly higher (74%) than teachers (63%) on a questionnaire about 13 major Supreme Court cases. Findings from this survey also revealed an ineffective and haphazard communication network for transmitting important Supreme Court decisions to the schools where they have the greatest impact—at the classroom level.

While there does not appear to be a consensus among educators, researchers and parents regarding the impact of legislation, case law and litigation on school disciplinary policies and procedures, there is national recognition that some secondary schools are experiencing unacceptably high levels of disruption. The magnitude of this problem appears to be greatest among those schools located in urban areas serving junior high populations.

OJJDP has sponsored demonstrations of a variety of innovative approaches designed to reduce school crime, to improve disciplinary policies and practices, to improve teacher and student safety, and to enhance academic achievement. Practitioners in the field have also identified a number of promising approaches that attempt to deal with problems of school crime, discipline and school environment. While there has been considerable attention to various aspects of disciplinary and crime control policies—e.g., the role of law enforcement in schools, appropriate strategies to respond to classroom disruption, improvement of the school environment to prevent delinquency—less attention has been given to the relationship between Federal and State legislation, case law and litigation, and development of comprehensive school disciplinary and crime control policies and procedures.

III. Program Goal and Objectives

A. Program Goal

To test promising strategies for the development, implementation, and monitoring of disciplinary and crime control policies and procedures, which are intended to reduce school crime and disorder to provide for a safer school environment.

B. Major Objectives

1. To identify significant Federal and State legislation, case law, and litigation that are relevant to the development of disciplinary and crime control policies, procedures and practices in selected schools/school districts.
2. To develop clear, consistent discipline and crime control codes, based on analysis of legislation, case laws state regulations, and lawsuits.
3. To develop strategies to implement discipline and crime control codes.
4. To implement procedures that translate the codes into practice.
5. To document the planning and implementation of approaches to discipline and to school crime in order to identify the essential components of this process.
6. To measure the impact of strategy implementation on the reduction of disciplinary infractions and school crime.
7. To assess the strategies in terms of cost effectiveness and suitability for replication and institutionalization within school systems.

IV. R&D Strategy

This Research and Development (R&D) model is designed to develop and test the efficacy of improved disciplinary policies and procedures for the reduction of school crime and disorder in secondary schools. It calls for the development of policies and procedures based on a careful analysis of relevant legislation, case law and litigation against the schools. This program will consist of three separate R&D projects, which will be coordinated by OJJDP. This initiative requires a collaborative effort at each site among researchers, program planners, school administrators, faculty, and law enforcement personnel, as appropriate. This working group will be referred to as the "program team" throughout this solicitation.

The program team will first conduct a thorough assessment of the school crime and discipline problems, taking into account relevant legislation, case law, and previous lawsuits. This should include the identification of significant legislative provisions and case law that may promote or inhibit the development of effective policies and procedures for discipline and crime problems. It should also include an assessment of the perceived effects of previous lawsuits on discipline and crime control practices. The program team will generate a strategy for crime reduction and improvement of disciplinary policies and procedures in target school(s). The program team should develop clear policy statements and should communicate these policies to those responsible for implementation. Finally, implementation of disciplinary policies and practices should be monitored by school administrators and evaluated by the program team.

Under this initiative each program team *will be required* to conduct a review of existing discipline codes and establish revised school-wide disciplinary policies and procedures based on assessment of legislation, case law and lawsuits. These revisions should be characterized by clear stipulation of sanctions for disciplinary infractions, consistent and fair enforcement of rules, and differentiation between disciplinary violations and criminal acts; Local jurisdictions will be allowed considerable flexibility in determining which specific program strategies offer the greatest potential for improvement of disciplinary practices in their target schools. Examples of strategies for implementing revised policies which can be tested:

1. Involvement of students and parents in the development of disciplinary codes;
2. development of written guidelines and/or statements of understanding with police and courts;
3. development of in-service training for teaches and administrators on their legal rights and responsibilities related to discipline and crime control, including an emphasis on protecting themselves from lawsuits as appropriate;
4. improved communications procedures for day-to-day operations, delineation of respective roles and responsibilities, establishment of cooperative working relationships through written policies and procedures for working with law enforcement agencies and the courts;
5. training of teachers in techniques of effective classroom management and control of disruptive behavior;
6. improved school incident reporting procedures and utilization of crime analysis techniques.

All projects are required to utilize a systematic program development process in both the planning and implementation stages. This process provides a structure for identifying possible relationships between legislation, case law, and lawsuits and discipline and crime control strategies, assessing the nature and extent of discipline and crime problems, establishing clear measurable goals, designating measurable objectives, developing appropriate strategied and interventions, identifying potential obstacles and resources for program implementation, developing work plans, and establishing an on-going data collection and feedback system. It is expected that the assessment and planning process will require six to nine months. *Successful completion of the assessment, policy revisions and action plan will be a requirement for continued funding (by special condition).* The following outline is provided to serve as a conceptual framework for all members of the working team involved in the collaborative effort of the program development process. The ultimate aim of this collaboration is to generate a common focus and shared vision of what the program is being designed to accomplish.

A. Problem Statement

Clear articulation of the problem being addressed requires identification of the various relevant legislation, case law and data sources and utilization of this information to define the nature and extent of the problem.

B. Clear Goals

Researchers and program implementers focus their activities on the problem at hand by delineating measurable goals. Goal statements describe what the organization is ultimately trying to achieve.

C. Theory

A theoretical framework must be developed which clearly outlines why the problem exists and provides guidance for project implementers' use in building their strategies.

D. Measurable Objectives

The statement of the project objectives must specify the time frame for achievement of these intermediary outcomes, the anticipated level of improvement, as well as the basis for measurement of change. Clearly delineated objectives provide the foundation for the evaluation design.

E. Strategies

Interventions and strategies are those selected activities designed to achieve an objective or set of objectives. For the purposes of this initiative, applicants will be allowed considerable local discretion in choosing to test a variety of strategies for policy implementation. The selection and development of strategies or interventions must be based on systematic analysis of crime and discipline problems and relevant laws and regulations.

F. Work Plans

Work plans are to be developed with specific strategies for implementation of project interventions. Fully articulated strategies include specification of two elements: critical bench marks and tasks. Critical bench marks are those key decisions, agreements, or actions necessary to progress with implementation of the plan. Failure to accomplish critical bench marks in a timely fashion will signal program planners to devise a new strategy. To effectively execute strategies, action planners should also specify time frames for task completion and individuals responsible. The action plan serves as a management tool and provides objective standards for achievement.

G. Evaluation

During theprogram development process, researchers and program managers work together to specify key research questions and develop a process and impact evaluation design. Data collection activities are jointly planned and carefully executed. Researchers provide timely interim feedback to program managers on: (1) progress toward executing program strategies as well as (2) success or failure in meeting measurable objectives.

The program development process begins with the initial conceptualization of the problem and continues throughout the duration of this program test. For the purpose of responding to this RFP, applicants must include all components listed in section V, "Application Requirements." The work plan included in the application will be considered preliminary and subject to continued development and refinement during the planning period to be scheduled for the first six to nine months following the grant award.

NIJJDP will encourage coordination among individual R&D projects in terms of developing and utilizing similar concepts and comparable "core" measures. With cross-site adoption of comparable measures, a substantial data base could be generated. This coordinated effort is expected to enhance the potential contribution of this research initiative to the state-of-the-art of developing safe school environments.

V. Eligibility Criteria

Eligible applicants include public or private research agencies or organizations. In order to implement this program design, it is required that a collaborative relationship exist between researchers, school administrators and justice system representatives, as appropriate. The intent of this initiative is to assess the effects of legislation, case law, regulations and lawsuits related to discipline and crime control; and to develop methods for establishing comprehensive, and consistent discipline and crime control codes, and to determine if the policy has been effectively implemented. School districts targeted for program change must be experiencing a high level of disciplinary or crime problems within the school. They should also have experienced several lawsuits or significant threats of lawsuits related to disciplinary actions in the past couple years. Research organizations will apply directly for grant awards and may choose to provide limited support through subcontracts to schools to cover on-site costs critical for program implementation. The primary cost of implementing the intervention strategies must be supported by available community and school system resources. This requirement is in keeping with the intent of this program to test cost effective program models which would be suitable for replication in other jurisdictions without external funding.

In order to maximize open competition in the award of this R&D grant, "for-profit" organizations are eligible to apply, provided they certify compliance with the following two requirements:

(1) The OJJDP grant award will not be used to support the normal profit-making operations of the organization, but will serve to support or stimulate the legislatively authorized research and evaluation objectives of NIJJDP.

(2) For at least one year following the termination of this award, the recipient will not compete for or accept any procurement or assistance award supported by OJJDP funds which may have resulted or been derived from the original award.

VI. Minimum Program Application Requirements

Applicants must complete all parts of the application for Federal Assistance (Standard Form 424). Particular attention should be paid to the Budget Information, Part III, and Program Narrative, Part IV. In addition to the requirements set forth under the Program Narrative, the following information must be included in the application:

(1) A succinct statement of your understanding of the goal and objectives of the School Crime and Discipline R&D Program.

(2) A problem statement which clearly documents the nature and extent of school crime and disciplinary problems in the schools or school systems targeted for change. Identify the criteria utilized in school site selection and provide data to justify this selection. Document recent experiences with actual lawsuits or significant threats of lawsuits related to disciplinary actions.

(3) A description of those long-range measurable goals which your program is designed to reach. Specify how goals will be measured, when a substantial level of goal accomplishment is expected, and how progress in meeting goals can be attributed to program interventions.

(4) A review of the theoretical and empirical literature relevant to relationship of legislation and case law to discipline and crime control problems. Include a discussion of the theoretical framework developed to specifically guide the identification and assessment of problems identified at the targeted school system(s).

(5) A description of program objectives which specifies what measurable changes are expected, and when change is anticipated.

(6) A description of assessment and planning process.

(7) A work plan which describes time frames for accomplishing critical bench

marks and specifies responsibilities for tasks for the planning process. This work plan must provide a detailed description of the six to nine month planning period and a preliminary plan for implementation of program interventions, evaluation activities and for the development of research products. It should also include a plan for delivering training and technical assistance to local school administrators, faculty, and justice system personnel, as appropriate, to assist them in incorporating the program development process into their routine operations.

(8) A preliminary evaluation design which includes research questions, key concepts and variables, research methodology (including sampling procedures and measurement of key variables), and a plan for data analysis and feedback. Applicants must provide evidence that the most rigorous experimental design possible will be utilized in order to increase the strength of attributions of program impact. Applicants must address the issue of coordination with other research sites funded under this program investigating and developing comparable measures of similar concepts across schools.

A Privacy Certificate must be submitted with the application in accordance with funding agency regulations, copies of which are available upon request.

(9) Written verification from authorized officials of all parties involved in the R&D program team (e.g., school administrators, faculty, and justice system personnel) of commitment to collaborate in the program planning, implementation, data collection, and program refinement processes. Access to specific types of data (official school records, police arrest records, student and teacher interviews) must also be verified in writing.

(10) If it is determined to be necessary for the research organization grantee to provide financial support from the grant award to another organization to cover costs critical for program implementation, the application must include: a statement of work for the proposed contract; and the procedures to be followed for competitive selection *or* a justification for noncompetitive award for these support services.

(11) A detailed budget must be provided for the initial twelve months budget period including travel funds for coordination meetings with other program grantees (estimate costs for three two-day meetings to be hosted by various participating grantees), and any costs which might be required to serve as a host site for one meeting. An annual estimate of costs to complete the R&D program must also be included.

(12) A description of the project management structure which includes proposed staffing plan, brief position descriptions which delineate roles and responsibilities, description of relevant staff experience and expertise, and resumes of key project staff (include as an appendix to the application). The project director must devote a minimum of fifty percent (50%) of his/her time to this effort.

(13) An organizational capability statement which describes relevant organizational experience and demonstrates that the applicant has the substantive and financial capability to effectively administer the project.

(14) The applicant must indicate a willingness to host an on-site visit by OJJDP staff and/or Peer Review Panel.

VII. Selection Procedures and Criteria

In general, all applications received in response to this solicitation will be reviewed in terms of the rigor and feasibility of the R&D design, and thoroughness regarding approaches to planning, implementation and evaluation problems. Applications will be technically evaluated by Department of Justice (DOJ) employees who are qualified by training and experience and by a peer review panel (a group of experts other than officers and employees of the DOJ). Site visits may be conducted by peer review panel and/or OJJDP staff to verify information provided by those applicants ranked as best qualified for further consideration.

Specifically, applications will be rated according to the following criteria and weights:

(1) The problem to be addressed is clearly stated including evidence of knowledge of related literature and justification for site selection (refer to Section V (3) and (4))	15
(2) An understanding of the goal and objectives of this research and development program is clearly articulated, including a description of the goals and objectives of the proposed research (refer to Sections V (3), (4), (5) and (6))	20
(3) The research and development design and methodology is sound and contains program elements directly linked to the achievement of project objectives including written verification of commitment to collaborate and accessibility of data (refer to Sections V (8) and (9))	20
(4) The project management structure is adequate to the successful conduct of the project (refer to Sections V(12))	10
(5) Organizational capability is demonstrated at a level sufficient to successfully support the project (refer to Section V(14))	10
(6) Budget costs are reasonable, complete and appropriate in comparison to the activities proposed to be undertaken (refer to Sections V (11), (12) and (10))	10
(7) The workplan is adequate, clear and feasible and will support the development of useful products (refer to Section V(7))	15

In order to examine variations in state level impact on school crime and discipline only one site will be funded in a given state.

Applications receiving the highest total score on the above criteria will be recommended for funding to the Administrator, OJJDP. Peer review recommendations in conjunction with the results of internal review and any necessary supplementary review will assist the Administrator's consideration of competing applications and selection of applications for funding. The final award decision will be made by the OJJDP Administrator.

VIII. Submission Requirements

All applicants responding to this solicitation should be aware of the following requirements for submission:

1. Applicants which plan to respond to this announcement are requested to submit written notification of their intent to apply to NIJJDP/OJJDP by October 21, 1985. Such notification should specify: the name of the applicant organization, mailing address, telephone number, and primary contact person. This notification should be forwarded to Deborah Wysinger, NIJJDP/OJJDP, U.S. Department of Justice, 633 Indiana Ave., N.W., Washington, D.C. 20531.

2. Applicants must submit the original signed application and three copies to NIJJDP/OJJDP. The necessary forms for applications (Standard Form 424) will be provided upon request.

3. The NIJJDP/OJJDP will notify applicants in writing of the receipt of their application. Subsequently, applicants will be notified by letter as to the decision made regarding whether or not their submission will be recommended for funding. It is anticipated that the grant may be awarded as early as April, 1986.

4. Applications must be received by mail or hand delivered to the NIJJDP/OJJDP by 5:00 p.m. e.s.t. on December 6, 1985. Those applications sent by mail should be addressed to Deborah Wysinger, NIJJDP/OJJDP, U.S.

Department of Justice, 633 Indiana Avenue, NW., Washington, D.C. 20531. Hand delivered applications must be taken to the NIJJDP/OJJDP, Room 784, 633 Indiana Avenue, N.W., Washington, D.C. between the hours of 8:00 and 5:30 p.m. except Saturdays, Sundays or federal holidays.

IX. Civil Rights Compliance

A. All recipients of OJJDP assistance must comply with the non-discrimination requirements of the Juvenile Justice and Delinquency Prevention Act of 1974 as amended; Title VI of the Civil Rights Act of 1964; Section 504 of the Rehabilitation Act of 1973 as amended; Title IX of the Education Amendments of 1972; the Age Discrimination Act of 1975; and the Department of Justice Non-Discrimination Regulations (28 CFR Part 42, Subparts C, D, E, and G).

B. In the event a Federal or State court of Federal or State administrative agency makes a finding of discrimination after a due process hearing on the grounds of race, color, religion, national origin or sex against a recipient of funds, the recipient will forward a copy of the finding to the Office of Civil Rights Compliance (CRC) of the Office of Justice Programs.

C. Applicants shall maintain such records and submit to the OJJDP upon request timely, complete and accurate data establishing the fact that no person or persons will be or have been denied or prohibited from participation in, benefits of, or denied or prohibited from obtaining employment in connection with any program activity funded in whole or in part with funds made available under this program because of their race, national origin, sex, religion, handicap or age. In the case of any program under which a primary recipient of Federal funds extends financial assistance to any other recipient or contracts with any other persons(s) or groups(s), such other recipient, persons(s) or groups(s) shall also submit such compliance reports to the primary recipient as may be necessary to enable the primary recipient to assure its civil rights compliance obligations under any grant award.

X. References

(See appendices A & B.)

Alfred S. Regnery,

Administrator, Office of Juvenile Justice and Delinquency Prevention.

Appendix A

Notes

1. E.g., *Goss v. Lopez*, 419 U.S. 565 (1975) (school disciplinary procedures); Tinker v. Des Moines Indep. Community School District, 393 U.S. 503 (1969) (School discipline-free spreech); Education of All Handicapped Children Act, 20 U.S.C. .1401.1461 (1976); Rehabilitation Act of 1973, 29 U.S.C. SS 701–794 (1976).

2. E.g., Ind. Code .20–8.1–5–1 to –17 (1982).

3. See S. Wasby, the Impact of the United States Supreme Court 231–32 (1970).

4. Indiana Due Process and Pupil Discipline Code, Ind. Code .20–8.1–5–8, –10 (1982).

5. National School Safety Center News Journal, Spring 1985, New Jersey v. T.L.O., The Lay of the Law, Diane Mahoney, (p. 31).

6. See e.g., Wood v. Strickland, 420 U.S. 308 (1975).

Appendix B

References

Cabinet Council on Human Resources (1983). *Disorder in Our Public Schools.* (Available from Cabinet Council Working Group on School Violence/Discipline, Washington, D.C.)

Epley, Glen B. Crime and Punishment: The Judicial Role in School Discipline and Substantive Due Process. *Education Law Reporter, Vol. 19* (p. 765), 1983.

Gallup, George H. The 14th Annual Gallup Poll of the Public's Attitudes Toward Public Schools. *Phi Delta Kappan*, September 1982.

Kindsvetter, R. A new view of the dynamics of discipline. *Phi Delta Kappan*, January 1978, 59(5), 322–324.

Lufler, Henry S., Jr. Past Court Cases and Future School Discipline. *Education and Urban Society, Vol. 14, No. 2*, February 1982, 169–1984.

Menacker, Julius and Pascarella, Ernest. How Aware Are Educators of Supreme Court Decisions That Affect Them? *Phi Delta Kappan Journal*, 1983.

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Schimmel, David. Discipline, Due Process and Religion in the Schools: Recent Court Decisions Affecting Education (or Controversial Issues of the 80's) submitted to Phi Delta Kappan Journal.

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Friday
September 27, 1985

Part VII

Department of Justice

Office of Juvenile Justice and Delinquency Prevention

Program Announcement; Program of Research on the Causes and Correlates of Delinquency; Notice

DEPARTMENT OF JUSTICE

Program Announcement; Program of Research on the Causes and Correlates of Delinquency

AGENCY: Office of Juvenile Justice and Delinquency Prevention, Justice.

ACTION: Notice of issuance of solicitation of applications to conduct research on the causes and correlates of delinquent behavior.

SUMMARY: The Office of Juvenile Justice and Delinquency Prevention (OJJDP), pursuant to Part C, Section 243 of the Juvenile Justice and Delinquency Prevention Act of 1974, as amended, hereby announces a new OJJDP initiative entitled "Program of Research on the Causes and Correlates of Delinquency." The primary research goal is to improve our understanding of the development of prosocial and antisocial behavior patterns.

OJJDP's National Institute for Juvenile Justice and Delinquency Prevention (NIJJDP) invites public or private agencies to submit applications to design and carry out longitudinal investigations of the development of prosocial and antisocial behavior patterns in communities characterized by high rates of serious delinquency.

Under this program of research, each project will establish an interdisciplinary research team capable of examining the etiology of delinquency in the context of the community, family, and individual differences. Emphasis will be placed on developing innovative techniques for measurement of those delinquency risk factors which offer the greatest utility in terms of being suitable targets for change through prevention and intervention strategies.

OJJDP has allocated a total of $1,000,000 for initial awards to four to six grantees competitively selected under this program of research. These initial awards will provide financial assistance for each research organization to: Conduct planning activities; coordinate their efforts with other grantees involved in this program of research; develop refined research designs and data collection instruments; produce baseline data on the study community and youth population; and to support longitudinal data collection. Under the initial award, the timeframe for conduct of the above tasks is estimated to be 15 to 18 months. Due to the longitudinal nature of this initiative, total project periods will be up to five years. This competition will be conducted according to the OJJDP Competition and Peer Review Policy, 28 CFR Part 34, Subpart A, published August 2, 1985 at 50 FR 31365–31366. The deadline for submission of applications is December 2, 1985.

FOR FURTHER INFORMATION CONTACT: Barbara Tatem Kelley, Research and Program Development Division, NIJJDP, OJJDP, 633 Indiana Avenue, NW., Room 784, Washington, DC 20531; telephone (202) 724-5929.

SUPPLEMENTARY INFORMATION:

Request for Proposals—Program of Research on the Causes and Correlates of Delinquency

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VIII. Procedures and Criteria for Selection
IX. Procedures and Deadline for Submission of Applications
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I. Introduction and Background

The Office of Juvenile Justice and Delinquency Prevention (OJJDP), hereby invites applications for a Program of Research on the Causes and Correlates of Delinquency. To understand the goal, objectives and strategy of this initiative, it is imperative to first consider previous research on this topic. Substantial effort has been expended by researchers as they attempted to isolate those risk factors which were associated with an increased propensity for involvement in delinquent behavior. Overall, this research supports the conclusion that no single cause accounts for all delinquency, and no single pathway or developmental progression leads to a life of crime.

Certain studies focused on the identification of early behavioral problems which might indicate that a child is especially "high risk" for subsequent delinquency. In the family setting, high risk children challenged parental authority or were difficult to control at home. In the school setting, high risk children displayed what might be considered nonadaptive or aggressive behavior patterns in the elementary classroom. While such early problem behavior may not have had origins of hostility and defiance, if these disruptive behavior patterns produce negative peer and angry adult reactions, antisocial and delinquent behaviors were likely to emerge with increasing age.

Other researchers have targeted for investigation those youth entering adolescence. At this stage of a youth's socialization, examples of delinquency risk factors include: Association with delinquent peers; alienation from parents and school authorities; disrespect for the law; lack of educational achievement; evidence of learning disabilities; failure to achieve age-appropriate, psychosocial developmental milestones; presence of psychiatric difficulties; impairment of neurological/psychomotor functioning; residence in a high crime neighborhood; and experimentation with alcohol and drugs.

Several long-term studies have been supported to identify risk factors related to substantial serious and violent delinquency. These studies have been conducted in a variety of jurisdictions to identify correlates and causes of delinquency and the development of serious delinquent careers. Factors such as violence in the family, involvement in law-violating gangs and groups, use of alcohol and other drugs, and residence in a high crime neighborhood all seem to contribute to the chronic involvement of a small proportion of offenders in serious crime.

The most common pattern of delinquent behavior appears to be one of declining seriousness and discontinuation after the teen-age period. However, it is apparent that those juvenile offenders characterized by an early age of delinquency onset, frequent police contacts, numerous court referrals, more serious offense histories, and commitment to juvenile corrections are more likely to pursue adult criminal careers. A number of researchers have investigated the career criminal phenomenon (Petersilia, 1980).

If one considers that delinquency research falls on a continuum from investigations of correlates to causes, then empirical findings produced to date have largely clustered on the "correlate" end of the scale. While it is likely that researchers have detected virtually all of the major risk factors correlated with a proclivity for involvement in delinquency, researchers reporting their findings have frequently had to stop short of attributing causality to any given factor or combination of factors. This hesitation to draw causal inferences was largely a function of the limitations of the research methodologies employed as well as the lack of an explicit articulation of the theoretical bases for the causal relationships.

It is exceedingly difficult to determine causality in a nonlaboratory setting lacking experimental controls. Even when delinquency is described in behavioristic terms, there is no simple statement of a given stimulus producing a particular response. Rather, the individual brings to a given situation a biological/genetic makeup which has been influenced by a host of reinforcement contingencies in the social learning arena of the family, the schools, peer associations and community environment.

Asserting delinquency causality also implies that a temporal relationship has been established, with the casual variable preceding or precipitating the onset of antisocial behavior. Interpretation of causal sequencing of events involves the application of repeated measures of the same individuals over that span of time relevant to the development of antisocial patterns. With the exception of such research as the Cambridge Study in Delinquent Behavior (Farrington, 1983), very few delinquency investigations have fulfilled the criteria of prospective longitudinal research.

Another key consideration in the attribution of causality is evidence that in the absence of a given risk factor, a youth will be less inclined to engage in antisocial behavior. Much of the previous research has failed to establish adequate comparison samples to demonstrate that the nonoccurrence of a presumed relevant condition results in a nondelinquent outcome.

The emphasis in this discussion on causality is intended to challenge the research community to creatively approach the "why" of the development of antisocial behavior in order to provide a sound, empirical basis for advances in delinquency prevention strategies and juvenile justice system intervention.

Previous research provides a basis for the identification of which groups of youth are at greater risk for delinquency involvement. Under this program of research, children growing up in high crime communities will be the focus of investigation. By virtue of this design parameter, the generalizability of findings will necessarily be limited to populations exposed to comparable risk factors. A critical research question which remains largely unanswered is why many children growing up in high crime communities experience positive socialization and thrive in spite of exposure to many delinquency risk factors. A significant challenge is to distinguish which of the risk factors appear to have the most direct impact on antisocial behavior and what naturally occurring phenomenon support prosocial development. Full exploration of this question will entail an interdisciplinary examination of the child's development, with consideration of individual differences, family experiences, socio-cultural influences, and environmental factors.

Another practical concern in prevention programming is the need to establish a sounder basis for identifying at an early age those children at greatest risk of negative social development. It is of limited utility, and certainly more costly, to wait until an individual adopts an antisocial or delinquent lifestyle and then attempt to reverse a well entrenched behavior pattern. Recent literature on prediction technology is promising in terms of more clearly specifying base rates, relative improvement over chance, and multiple gating techniques (Monahan, 1981; Loeber and Dishion, 1983). Also, with the advent of computerized sophistication in data analyses, previously insurmountable data management issues can now be addressed.

Under this program of research, OJJDP is committed to supporting innovative research designs which will yield useful findings regarding the causes and correlates of delinquency. Utility, in this instance, refers to the applicability of theoretical and empirical advances to the improvement of cause-focused strategies for delinquency prevention (Hawkins, Pastor, Bell and Morrison, 1980) and intervention.

II. Program Goals and Objectives

A. Purpose and Goal Statement

The purpose of this Program of Research on the Causes and Correlates of Delinquency is to expand the existing body of knowledge. The primary research goal is to improve our understanding of the development of prosocial and antisocial behavior patterns.

B. Major Research Objectives

The following is a listing of those objectives which are considered most critical to this program research:

1. To identify the sequential ordering of different developmental processes and life experiences, including social, psychological, behavioral and environmental, which lead to either positive socialization or adaptation of a delinquent or criminal lifestyle.

2. To identify those predisposing factors, of whatever nature, which characterize a child at risk for involvement in antisocial behavior, delinquency and crime.

3. To examine the etiology of crime and delinquency in the context of the community, family and individual differences.

4. To enhance our ability for early identification and intervention with high risk children for the prevention of delinquency.

5. To advance the development of a sound theoretical framework for the development of effective strategies for delinquency prevention and justice system intervention.

III. Research Strategy

This program of research is intended to function as a coordinated effort by multiple grantees. Applicants may propose to initiate a new study under this announcement or they may propose to supplement ongoing or existing research. In either case, applicants must demonstrate that their proposed research fulfills the objectives and design requirements of this initiative.

A. Theoretical Considerations

The theoretical framework for many previous studies tended to be rather open-ended, with researchers often capturing those risk factor data which were readily available. This approach often limited the applicability of findings to theory testing or theory building. Under this initiative, researchers will be required to delineate the theoretical foundation which guides their inclusion of factors to be investigated. Applicants are urged to approach the etiology of antisocial behavior from an interdisciplinary perspective and to take into consideration existing theories of delinquency causality as well as possible integrations of theories across disciplines.

B. Design Requirements

The research strategy does not call for replication of prior studies, rather for innovation in overcoming the methodological shortcomings of previous research. For example, due to technological limitations and funding constraints, past studies have frequently focused on a narrow range of factors, have lacked an interdisciplinary approach to the investigation of child development, and have not involved the collection of data at such points in time that would allow for interpretation of causal sequencing. Under this initiative, emphasis will be placed on conducting contemporary or prospective longitudinal studies, instead of relying exclusively on retrospective and archival data sources. Researchers should not only address those demographic factors which are easy to measure and relatively immutable, but also devise assessment techniques to capture data on those factors which are more difficult to measure but more suitable targets for change through prevention and intervention strategies.

Researchers are encouraged to approach creatively the very complex design issues related to the longitudinal investigation of the etiology of delinquency in the context of the individual, family, and community. Under this program of research, each research project should have the following characteristics:

1. Selection of study communities characterized by serious delinquency in order to have a sufficient number of the most high risk families and youth.

2. Longitudinal design which provides for data collection at critical stages of the developmental process and allows for interpretation of causal sequencing of events.

3. Careful delineation of a sampling plan to answer specific research and policy questions.

4. Emphasis on studying those delinquency risk factors which offer the greatest utility in terms of being suitable targets for change through prevention and intervention strategies.

5. Innovative techniques for measurement of those factors related to the development of prosocial and antisocial behavior patterns.

6. Sensitivity to cultural and ethnic distinctions in the etiology of delinquency.

7. Establishment of an interdisciplinary research team.

C. Restrictions on Bio-Medical or Behavior Control Experimentation

While OJJDP encourages researchers to assess a broad range of delinquency risk factors, it should be noted that this office is legislatively restricted from conducting research involving any biomedical or behavior control experimentation on individuals. As described in section 261(d) of the JJDP Act, the term "behavior control" refers to methods which involve a substantial risk of physical or psychological harm to the individual and which are intended to modify or alter antisocial behavior.

D. Coordination of Program of Research

OJJDP recognizes the need for all researchers involved in this program of research to coordinate their activities. Purposeful coordination will serve:

1. To ensure comprehensive coverage of key research questions.

2. To provide a forum for collaboration among the various researchers in the development and subsequent utilization of comparable measures.

3. To establish multiple longitudinal data bases which could be subjected to aggregate secondary analysis on selected issues.

While coordination activities will be scheduled throughout the duration of this initiative, the initial six to nine months will be an intensive coordinated planning period. During this planning period, all researchers will convene to familiarize each other with their proposed research designs, and to exchange ideas on state-of-the-art interdisciplinary advances in such areas as factor measurement and predictive modeling.

IV. Major Responsibilities of Successful Applicants

Each organization selected to participate in this program of research will be responsible for all aspects of the project design, implementation, and product development.

A. Phase I

Initial awards will support Phase I project activities. Under Phase I, each research organization will be responsible for the following tasks:

1. Conduct of planning activities.

2. Coordination of efforts with other grantees involved in this program of research, as discussed in section III. D.

3. Development of refined and detailed research design.

4. Development of comprehensive workplan for the implementation of the refined design.

5. Development and pretesting of data collection instruments.

6. Securement of full access to all study data sources.

7. Development of detailed plan for protection of confidentiality of data.

8. Production of baseline data on the study community and youth population.

9. Initiation of longitudinal data collection.

There is no exact timeframe for the completion of these tasks, however, Phase I awards will provide support for 15 to 18 months of activity. For those reseachers initiating a new study, it is estimated that a considerable amount of time under the initial Phase I award will be devoted to tasks 1 through 8. For those researchers supplementing ongoing studies, more time under Phase I may be available for proceeding with actual data collection. All participants in this initiative must successfully complete tasks 1 through 8 prior to receiving consideration for continuation funding under Phase II.

B. Phase II

All subsequent continuation awards will support Phase II activities, which include the following:

1. Full implementation of longitudinal data collection.

2. Development and maintenance of a computerized data base.

3. Analysis of data.

4. Continued coordination and data sharing with other researchers involved in this initiative.

5. Ongoing assessment of the validity and reliability of measurement techniques and instruments.

6. Refinement of data collection approaches and research design, as appropriate.

7. Communication of findings through the production of interim reports, issue papers, and final reports.

With respect to task 7, researchers are strongly urged to surface interesting findings as they become available. Throughout the life of these projects, researchers should strive to produce documents which clearly convey significant findings and practical applications for policy-makers, practitioners, and other researchers.

V. Eligibility Requirements

Applicants who fail to satisfy the following requirements will be ineligible for funding consideration. Funding agency determination of eligibility will be based solely upon OJJDP review of documentation provided in the application.

A. Eligible applicants include public or private agencies or organizations.

B. In order to maximize open competition in the award of these research grants, "for-profit" organizations are eligible to apply provided they certify compliance with the following two agency policy requirements:

1. The OJJDP grant award must not be used to support the normal profit-making operations of the organization, but must serve to support or stimulate the legislatively authorized research and evaluation objectives of NIJJDP.

2. For at least one year following the termination of this award, the recipient will not complete or accept any procurement or assistance award supported by OJJDP funds which may have resulted or been derived from the original award.

C. Applicant organizations may choose to submit joint proposals with other organizations as long as one organization is designated in the application as the primary applicant and any co-applicants are designated as such. Further discussion of co-applicants is provided under section VII. Together co-applicants must meet the eligibility requirements specified in section V. D and E.

D. The applicant must have experience in the following areas in order to be eligible for consideration:

1. Prior experience in the design and implementation of research on the causes and correlates of delinquency or crime.

2. Prior experience in the conduct of longitudinal research.

3. Prior experience in the development, maintenance and analysis of large computerized data bases.

E. The applicant must establish a project research team with interdisciplinary expertise necessary for the design and conduct of research on the child developmental, family, and community risk factors for anti-social behavior.

F. The applicant must have the management and financial capability to effectively implement a project of this scope and complexity.

VI. Duration and Dollar Amount

A. Due to the longitudinal nature of this research, project periods will be up to five years. For the purposes of responding to this solicitation, applicants are advised that the initial awards will provide support for Phase I activities (refer to Section IV.A). The timeframe for the conduct of Phase I tasks is estimated to be 15 to 18 months.

B. OJJDP has allocated a total of $1,000,000 for initial Phase I awards to four to six grantees competitively selected under this program of research. It is anticipated that a series of Phase II (see section IV.B) grants will be made available to support the continuation of individual research projects. Funding of a noncompeting continuation grant within a previously approved project period may be withheld for justifiable reasons, which include:

1. There is no continued need for further research.

2. A grantee is delinquent in submitting required reports.

3. Adequate grantor agency funds are not available to support the project.

4. A grantee fails to show satisfactory progress in achieving the objectives of the project or otherwise fails to meet the terms and conditions of the award.

5. A grantee's management practices fail to provide adequate stewardship of the grantor agency funds.

6. Outstanding audit exceptions have not been cleared.

7. Any reason which would indicate that continued funding would not be in the best interests of the Government.

C. Recognizing the considerable expense involved in conducting longitudinal research of this nature, OJJDP strongly encourages applicants to seek funding from additional sources, both public and private.

VII. Minimum Program Application Requirements

All applicants must submit a completed Application for Federal Assistance (Standard Form 424), including a program narrative, a detailed budget and a budget narrative. The program narrative shall not exceed 75 double-spaced pages in length.

In submitting applications which contain more than one organization, the relationships among the parties must be set forth in the application. As a general rule, organizations which describe their working relationship in the development of products and the delivery of services as primarily cooperative or collaborative in nature will be considered as co-applicants. Those organizations which are primarily procuring services or products from another organization would not be considered as co-applicants. In the event of a co-applicant submission, one co-applicant must be designated as the payee to receive and disburse project funds and be responsible for the supervision and coordination of the activities of the other co-applicants. Under this arrangement each organization would agree to be jointly and severally responsible for all project funds and services. Each co-applicant must sign the SF-424 and indicate their acceptance of the conditions of joint and serveral responsibility with the other co-applicants.

In addition to the requirements specified in the instructions for preparation of Standard Form 424, the following information must be included in the application:

A. A statement of the problem to be addressed, including the review of the theoretical and empirical literature on the development of prosocial and antisocial behavior patterns, causes and correlates of delinquency, and implications of research findings for prevention and intervention.

B. A succinct statement of your understanding of the goal and objectives of the Program of Research on the Causes and Correlates of Delinquency.

C. A complete discussion of the proposed research design and methodology including:

1. Delineation of theoretical framework which guides your study.

2. Presentation of your specific study's goals, objectives, and research questions to be addressed.

3. Discussion of what theory-based factors will be addressed and how these will be measured.

4. Description of the high crime community selected for investigation including socio-demographic information, documentation of the delinquency problem, cultural and ethnic context, and location/boundaries.

5. Description of proposed sample, discussion of expected rate of attrition, and justification that the proposed sample size is adequate for longitudinal design.

6. Presentation of the longitudinal design for data collection.

7. Specification of data sources and verification of data access.

8. A Privacy Certificate describing procedures to be followed to assure confidentiality of data in accordance with funding agency regulations, copies of which are available upon request.

9. Discussion of plans for data analysis.

10. Suggestions for ways to coordinate with other researchers involved in this initiative.

D. A detailed workplan for Phase I activities which includes identification of major milestones, designation of organizational responsibility, and a schedule for completion of tasks and products. The nature and utility of products should be discussed.

E. A description of the project management structure which includes proposed staffing plan, brief positions descriptions which delineate roles and responsibilities, identification of proposed members of interdisciplinary research team, description of relevant staff experience and expertise, and résumés of key project staff (include as an appendix to the application). The project director must devote a minimum of fifty percent (50%) of his/her time to this effort.

F. An organizational capability statement which describes relevant organizational experience and demonstrates that the applicant has the substantive and financial capability to effectively administer the project.

G. A detailed budget for the Phase I period, including travel funds for coordination meetings with other program grantees (estimate costs for three three-day meetings to be hosted by various participating grantees), and any costs which might be required to serve as a host site for one meeting.

H. An estimated budget of annual costs for conducting Phase II activities through the conclusion of the project period.

I. Applications which propose noncompetitive contracts for the provision of specific services must contain a sole source justification for any procurement in excess of $10,000.

VIII. Selection Procedures and Criteria

In general, all applications received in response to this solicitation will be reviewed in terms of their potential contribution to the state-of-the-art, the appropriateness and feasibility of the research design, and innovative measurement of risk factors.
Applications will be evaluated by a peer review panel according to the OJJDP Competition and Peer Review Policy, 28 CFR Part 34, Subpart B, published August 2, 1985 at 50 FR 31366–31367. Site visits may be conducted by peer review panelists and/or OJJDP staff to verify information provided by those applicants ranked through peer review as best qualified for further consideration.

Specifically, applications will be rated according to the following criteria and weights:

A. The problem to be addressed by this research project is clearly stated, including evidence of knowledge of related literature (refer to section VII, A)	10
B. An understanding of the goals and objectives of this program of research is clearly articulated, including assessment of the degree to which proposed research would further these objectives (refer to section VII, B and C.2)	10
C. The research design and methodology is sound and contains program elements directly linked to the achievement of project objectives (refer to section VII, C)	40
D. The project management structure is adequate to the successful conduct of the project (refer to section VII, E)	15
E. Organizational capability is demonstrated at a level sufficient to successfully support the project (refer to section VII, F)	5
F. Budget costs are reasonable, complete and appropriate in comparison to the activities proposed to be undertaken (refer to section VII, G, H, and I)	10
G. The workplan is adequate, clear and feasible and will support the development of useful products (refer to section VII, D)	10

The results of peer review under a competitive program will be a relative aggregate ranking of applications in the form of "Summary Ratings." These will ordinarily be based on numerical values assigned by individual peer reviewers, and as set forth in the OJJDP *Peer Review Manual*.

OJJDP intends to sponsor a number of individual projects which, in combination, serve to investigate a broad range of developmental issues related to the etiology of delinquency. To achieve this end, individual projects sponsored under this program of research should be complementary in scope and approach rather than duplicative. In addition to the scores based upon the above weighted criteria, the final selection process will also include consideration of diversity of: Research approach and developmental risk factors targeted for investigation. A final selection consideration will also be the estimated long-range costs of conducting the proposed research for the duration of the project period. OJJDP may choose to negotiate with the finalists to make certain adjustments to their applications. Peer review recommendations in conjunction with the results of internal review and any necessary supplementary review, will assist the Administrator's consideration of competing applications and selection of applications for funding. The final award decision will be made by the OJJDP Administrator.

IX. Procedures and Deadlines for Submission of Applications

A. Organizations which plan to respond to this announcment are requested to submit written notification of their intent to apply to NIJJDP/OJJDP by October 25, 1985. Such notification should specify: The name of the applicant organization, mailing address, telephone number, and primary contact person. In the event that organizations intend to apply as co-applicants (as described in section VII of this solicitation), each of the co-applicants are to provide the above information. The submission of this notification is optional. It is requested to assist NIJJDP in estimating the workload associated with the review of applications and for notifying potential applicants of any supplemental information related to the preparation of their applications.

B. Applicants must submit the original signed application and three copies to NIJJDP/OJJDP. The necessary forms for applying (Standard Form 424) will be provided upon request.

C. The deadline for submission of applications is *December 2, 1985*. All applications must be delivered or mailed to NIJJDP/OJJDP by 5:30 p.m. on that date. Those applications which are mailed must be postmarked on or before December 2, 1985, by the U.S. Postal Service.

D. The mailing address for all correspondence (e.g., applications, notification of intent to apply, requests for forms) related to this program announcement is as follows: Research and Program Development Division, National Institute for Juvenile Justice and Delinquency Prevention, Office of Juvenile Justice and Delinquency Prevention, Room 784, 633 Indiana Avenue, NW, Washington, D.C., 20531.

X. Civil Rights Compliance

A. All recipients of OJJDP assistance must comply with the nondiscrimination requirements of the Juvenile Justice and Delinquency Prevention Act of 1974 as amended; Title VI of the Civil Rights Act of 1964; section 504 of the Rehabilitation Act of 1973 as amended; Title IX of the Education Amendments of 1972; the Age Discrimination Act of 1975; and the Department of Justice Non-Discrimination Regulations (28 CFR Part 42, Subparts C, D, E, and G).

B. In the event a Federal or State court or Federal or State administrative agency makes a finding of discrimination after a due process hearing on the grounds of race, color, religion, national origin or sex against a recipient of funds, the recipient will forward a copy of the finding to the Office of Civil Rights Compliance (CRC) of the Office of Justice Programs.

C. Applicants shall maintain such records and submit to the OJJDP upon request timely, complete and accurate data establishing the fact that no person or persons will be or have been denied or prohibited from participation in, benefits of, or denied or prohibited from obtaining employment in connection with any program activity funded in whole or in part with funds made available under this program because of their race, national origin, sex, religion, handicap or age. In the case of any program under which a primary recipient of Federal funds extends financial assistance to any other recipient or contracts with any other person(s) or group(s), such other recipient, person(s) or group(s) shall also submit such compliance reports to the primary recipient as may be necessary to enable the primary recipient to assure its civil rights compliance obligations under any grant award.

XI. References

Farrington, D. *Further Analyses of a Longitudinal Survey of Crime and Delinquency.* Unpublished final report to the National Institute of Justice, Washington, D.C., 1983.

Loeber, R., and Dishion, T. Early predictors of male delinquency: A review. *Psychological Bulletin,* 1983, Volume 94, Numer 1, pp. 68–99.

Monahan, J. *Predicting Violent Behavior: An Assessment of Clinical Techniques.* Beverly Hills, California: Sage Publications, Inc., 1981.

Petersilia, J. Criminal career research: Morris, N., and Tonry, M., eds. *Crime and Justice: An Annual Review of Research.* Chicago: University of Chicago Press, 1980, Volume 2, pp. 321–379.

Alfred S. Regnery,

Administrator, Office of Juvenile Justice and Delinquency Prevention.

[FR Doc. 85-23085 Filed 9-26-85; 8:45 am]

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federal register

Friday
September 27, 1985

Part VIII

Department of Transportation

Federal Aviation Administration

14 CFR Part 61

Duration of Airman Medical Certificates; Withdrawal of Notice of Proposed Rulemaking

DEPARTMENT OF TRANSPORTATION

Federal Aviation Administration

14 CFR Part 61

[Docket No. 19176; ref. Notice 82-15]

Duration of Airman Medical Certificates

AGENCY: Federal Aviation Administration (FAA), DOT.

ACTION: Withdrawal of notice of proposed rulemaking.

SUMMARY: This notice withdraws Notice 82-15, published in the **Federal Register** on December 2, 1982 (47 FR 54414), which proposed to extend the duration of third-class airman medical certificates for operations requiring a private or student pilot certificate. Notice 82-15 was in response to a petition for rulemaking by the Aircraft Owners and Pilots Association (AOPA) dated May 11, 1979. This petition asks that the duration of all third-class certificates be changed to 3 years. As proposed in Notice 82-15, the new rule would have required periodic third-class medical examinations at 2- to 5-year intervals based on the age of the airman rather than every 2 years as now required. Notice 82-15 elicited significant opposition. A number of concerns were expressed by the medical community. After careful consideration, Notice 82-15 is being withdrawn.

FOR FURTHER INFORMATION CONTACT: William H. Hark, M.D., Aeromedical Standards Division, AAM-200, Federal Aviation Administration, 800 Independence Avenue, SW., Washington, D.C. 20591, telephone number (202) 426-3802.

SUPPLEMENTARY INFORMATION:

Background

On December 2, 1982 (47 FR 54414), in response to a petition by the AOPA, the Federal Aviation Administration (FAA) published Notice of Proposed Rulemaking (NPRM) No. 82-15. That notice proposed to extend the duration of third-class airman medical certificates for operations requiring a private or student pilot certificate. The proposal would have required periodic third-class medical examinations at intervals based on the age of the airman. It also would have relaxed the requirements for a large number of private or student pilots to undergo a medical examination every 24 months.

Reasons for the Withdrawal

A majority of the commenting private physicians and an aviation-oriented medical association experessed to opposition to the proposal. A number of physicians, though expressing support for the intent of the proposal, suggest alternative examination intervals or changes in the scope of examination. Several note their belief that the periodic medical certification examination is the only reason many airmen visit a physician. They believe that health consciousness is desirable in airmen and that the periodic encounters with designated physicians (Aviation Medical Examiners) encourage it. They argue that a reduction in examination frequency may be construed as a downgrade of the importance of health to flight safety.

The majority of individual airmen and their organizations are in favor of a reduction in frequency of examination. One large organization of airline pilots expresses support but emphasizes the need for limiting certificate validity periods in questionable cases where the airman's medical condition requires evaluation to determine if the standards are met. Some commenters want the elimination of all medical examinations.

Whereas Notice No. 82-15 dealt solely with the duration of airman medical certificates, the FAA has announced and is conducting a complete review of the medical standards for airmen and of its certification practices and procedures (47 FR 16298, April 15, 1982; 47 FR 30795, July 15, 1982). As part of that review the American Medical Association (AMA) is reviewing these standards and procedures and is expected to report its recommendations to the FAA in February 1986. Given the imminent issuance of the AMA's report, and the fact that the report may well provide the FAA with better data on which to base an evaluation of the safety concerns regarding the proposals which were raised by the medical community, the FAA has decided to withdraw the notice and reconsider this matter in the context of its review of the AMA's recommendation. Any future consideration of examination frequency will be given within the context of this study's outcome.

Withdrawal of NPRM

Accordingly, Notice No. 82-15 (47 FR 54414, December 2, 1982) is withdrawn. This action does not preclude the FAA from considering similar proposals in the future or commit it to any further or future course of action on this subject.

Authority: 49 U.S.C. 1354(a), 1355, 1421, 1422, and 1427; 49 U.S.C. 106(g) (revised, Pub. L. 97-449, January 12, 1983).

Issued in Washington, D.C., on September 23, 1985

William T. Brennan,

Acting Director of Flight Operations.

[FR Doc. 85-23103 Filed 9-24-85; 12:16 pm]

federal register

Friday
September 27. 1985

Part IX

Environmental Protection Agency

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40 CFR Part 3

Employee Responsibilities and Conduct; Final Rule

ENVIRONMENTAL PROTECTION AGENCY

40 CFR Part 3

[OGC FRL-2874-6]

Employee Responsibilities and Conduct

AGENCY: Environmental Protection Agency.

ACTION: Final Rule.

SUMMARY: EPA is revising the Employee Responsibilities and Conduct regulations at 40 CFR Part 3. These regulations are necessary to reflect changes in the Agency's organization, to implement Rule 1.11 of the American Bar Association's 1983 Model Rules of Professional Conduct, to exempt tax-exempt bond funds from the prohibition of 18 U.S.C. § 208(a), to make minor changes based on the Agency's experience in administering the earlier regulation and to clarify other provisions. The effect of these changes is to allow employees who own tax-exempt bond funds to participate in matters affecting the financial interests of state and local governments and, in jurisdictions where Rule 1.11 of the American Bar Association's 1983 Model Rules of Professional Conduct applies, to bar former EPA attorneys from participating in court challenges to rules they helped to make. In addition, Deputy Ethics Officials are authorized to require certain employees below GS/GM-13 to file confidential financial statements.

DATE: This regulation is effective September 27, 1985.

ADDRESS: Office of General Counsel (LE-132G), Environmental Protection Agency, 401 M Street, SW., Washington, DC 20460.

FOR FURTHER INFORMATION CONTACT: Donnell L. Nantkes, (202) 382-4550.

SUPPLEMENTARY INFORMATION:

Background

On June 26, 1985, EPA published a Notice of Proposed Rulemaking at 50 FR 26506 to revise regulations published February 29, 1985 (49 FR 7528). Comments were due by July 29, 1985. No comments were received. Accordingly, these regulations are published as proposed, except that 40 CFR 3.302(e) is revised to make it clear that the employment interests of employees' spouses and minor children are required to be reported on EPA Form 3120-1, Confidential Statement of Employment and Financial Interests.

EPA published a complete revision of its regulation regarding Employee Responsibilities and Conduct on February 29, 1984. Since then, parts of the revised regulation have become outdated as a result of changes in the Agency's organizational structure. For example, the list of officials in Appendix B to Subpart C who are subject to special requirements under Section 318(d) of the Clean Air Act is out-of-date. This list is updated without substantive change. In addition, § 3.302(b)(1) currently refers to the "Enforcement Counsel." This position has been replaced by an "Assistant Administrator for Enforcement and Compliance Monitoring."

Section 3.301(b)(1) is revised to exempt tax-exempt bond funds from the prohibition of 18 U.S.C. 208(a). Such funds are analogous to the diversified mutual funds which are currently exempt.

Section 3.302 is revised to authorize Ethics Officials to require certain employees below GM/GS-13 to file EPA Form 3120-1, Confidential Statement of Employment and Financial Interests, where their duties directly affect the financial interests of specific parties. This authority applies only to employees who are contracting officers, project officers, inspectors, auditors or On-Scene Coordinator representatives. Such employees may have serious conflicts, and Ethics Officials should be able to monitor their financial and employment interests. Directing other employees below GM/GS-13 to file continues to require the specific approval of the Office of Government Ethics.

Section 3.302 is also revised to make it clear that employees who are required to file EPA Form 3120-1, Confidential Statement of Employment and Financial Interests, must provide information concerning the employment interests of their spouses and minor children.

Since in EPA's experience there is no basis for distinguishing outside employment involving holders of assistance agreements from such employment involving EPA contractors and subcontractors, the provisions regarding approval of outside employment are expanded to include outside employment involving holders of assistance agreements.

The regulation also implements Rule 1.11 of the American Bar Association's 1983 Model Rules of Professional Conduct by providing that, where the local jurisdiction has adopted Rule 1.11, former EPA attorneys are prohibited from participating in judicial challenges to rules which they helped to develop. This change applies only where a complaint has not been filed by the effective date of this regulation or the date Rule 1.11 became effective in the jurisdiction, whichever occurs later.

The following changes are intended to clarify existing provisions:

(1) The *Discussion* paragraph after the verbatim reprint of 18 U.S.C. 208 in Appendix A to Subpart A is revised to make it clear that a vested right to funds in a State retirement system does not create a disqualification under the statute where the funds are invested in a separate trust account and are not controlled by the former employer.

(2) Paragraph (1) of Appendix A to Subpart C is revised to make it clear that interests in mutual funds must be reported on EPA Form 3120-1. This is desirable because the exemption for mutual funds at § 3.301(b)(1) does not apply to funds which concentrate their investments in particular industries, and Ethics Officials should be able to judge whether mutual funds are within the exemption.

(3) Paragraph (3) of Appendix A to Subpart C is revised to specify the information to be included in the Deputy Ethics Officials' annual certifications to the Designated Agency Ethics Official. This information includes: (1) the number of employees at GM/GS 13-15; (2) the number required to file; (3) the number of other employees required to file; (4) the number of remedial actions by type; (5) a certification that no employee who performs any "functions or duties" under the Surface Mining Control and Reclamation Act holds any prohibited interests; and (6) a certification that all required reports have been received, reviewed and signed and that any necessary remedial actions have been completed. This paragraph has also been reworded to clarify the procedures for submission of annual supplemental statements and (where employees interests have changed) submission of updates every four months.

(4) Paragraph (4) of Appendix A to Subpart C is revised to make it clear that a Confidential Statement of Employment and Financial Interests may be disclosed to the Office of Inspector General and, on the written request of the chairman, to a committee or subcommittee of Congress. This provision is implicit in the February 29, 1984 regulation and does not change existing practice.

(5) Section 3.508(e) is revised to make it clear that the requirement for approval of outside employment applies to employment involving firms regulated by the "EPA program Office or Regional Office" in which an employee serves.

(6) Appendix A to Subpart E is revised to make it clear that requests for approval of outside employment are to

be routed through employees' supervisors.

Executive Order 12291

Under Executive Order 12291, EPA is required to judge whether a regulation is "major" and therefore subject to the regulatory impact analysis requirements of the Order or whether it may follow other development procedures. We have determined that this regulation is not "major" as it will not have a substantial impact on the economy. Consequently, the regulation is not subject to the impact analysis requirements of Executive Order 12291.

List of Subjects in 40 CFR Part 3

Conflicts of interests.

Dated: September 11, 1985.

Lee M. Thomas,
Administrator.

Therefore, 40 CFR Chapter I, Part 3 is amended as follows:

PART 3—EMPLOYEE RESPONSIBILITIES AND CONDUCT

1. The authority citation for Part 3 continues to read as follows:

Authority: Executive Order 11222, 30 FR 6460; 3 CFR 1964–1965, p. 306; 5 CFR Parts 734, 735 and 737.

2. Redesignate § 3.105 as 3.106 and add a new § 3.105 to read as follows:

§ 3.105 Post-employment restrictions affecting former EPA Attorneys.

This section applies where a jurisdiction in which a former EPA employee holds bar membership has adopted Rule 1.11 of the American Bar Association's Model Rules of Professional Conduct dated August 1983, in particular Rule 1.11(d)(2) which provides that the term "matter" includes "any other matter covered by the conflict of interest rules of the appropriate government agency." In such cases, the term "matter" includes participation (in the form of drafting, providing advice or making recommendations) in the development of EPA regulations. Where a former EPA employee participated in the development of an EPA regulation while employed by EPA, he or she may not represent or assist in representing any party or parties as an attorney in any judicial proceeding to contest the validity of the rule. However, this section applies only where the complaint was not filed before the effective date of this regulation or the date when Rule 1.11 became effective in the jurisdiction, whichever occurs later.

3. In Appendix A to Subpart A, add the following sentences at the end of the Discussion paragraphs within the discussion of 18 U.S.C. 207:

Appendix A to Subpart A—Conflict of Interest Statutes and Examples.

* * * * *

Retirement plans maintained by a former employer may also amount to a financial interest, depending on the circumstances. For example, a fund managed by a former employer which includes company stock would create a personal financial interest, whereas a pension plan of a State government which is managed by a separate trustee and in which the former employee's right have vested would not create a personal financial interest in the State government in which an EPA employee formerly served. Employees are encouraged to seek the advice of the Designated Agency Ethics Official regarding pension plans.

4. Section 3.300 is amended by revising paragraphs (a) and (b)(7) to read as follows:

§ 3.300 Prohibitions against acts affecting a personal financial interest.

(a) As discussed in Appendix A to Subpart A, section 208(a) of Title 18, United States Code, prohibits an employee from knowingly participating in an EPA matter in which the employee, the employee's spouse, minor child, present or prospective employer, or organization in which the employee is an officer, has a financial interest.

(b) * * *

(7) Rulemaking and policy matters which have a direct and predictable effect on the financial interests of companies in which the employee owns stock or has an official or employment relationships. Examples are discussed in Appendix A to Subpart A.

5. In § 3.301 revise paragraph (b)(1) to read as follows:

§ 3.301 Waiver.

* * * * *

(b) * * *

(1) Mutual funds (including tax-exempt bond funds), except those which concentrate their investments in particular industries;

* * * * *

6. In § 3.302 revise paragraph (b)(1) and redesignate paragraphs (c)(2) and (d) as paragraphs (d) and (e) respectively. Add a new paragraph (c)(2) and revise redesignated paragraph (d) and redesignated paragraph (e), to read as follows:

§ 3.302 Financial Disclosure Reports and Confidential Statements of Employment and Financial Interests.

* * * * *

(b) * * *

(1) All employees who report to the General Counsel or the Assistant Administrator for Enforcement and Compliance Monitoring, and Regional Counsel employees;

(c) * * *

(2) Those in positions classified at GS–12 or below (or comparable levels under other pay systems) if their duties directly affect the financial interests of specific parties. Such positions are limited to contracting officers, project officers, inspectors, auditors and On-Scene Coordinator representatives.

(d) Other employees classified below the GM or GS–13 level (or comparable levels under other pay systems) who are in positions which otherwise meet the criteria of paragraph (c)(1) of this section may also be required to file, provided the Office of Government Ethics has approved in writing. Deputy Ethics Officials should consult with the Designated Agency Ethics Official in seeking such approval.

(e) *Financial and employment interests of employees' relatives.* In completing Confidential Statements of Employment and Financial Interests, the financial and employment interests of a spouse or minor child are considered to be interests of the employee and must be reported.

7. Revise paragraphs (1), (3) and (4) of Appendix A to Subpart C to read as follows:

Appendix A to Subpart C—Procedures for Filing Confidential Statement of Employment and Financial Interests

(1) *Submission*—Each employee required to submit a Confidential Statement of Employment and Financial Interests must submit the completed EPA Form 3120–1 within 30 days after entrance on duty or (where the position is not specifically listed in this Part) within 30 days after being notified of the requirement to file. Interests which are exempt from the prohibition of 18 U.S.C. 208(a) (except for interests in mutual funds) need not be reported. *See* § 3.301(b). The completed form is sent to the Deputy Ethics Official for the employee's organization. Headquarters employees in the Office of General Counsel and employees in the immediate Office of the Administrator submit their forms to the Designated Agency Ethics Official.

* * * * *

(3) *Supplemental Statements*—For purposes of annual review, employees who are required to submit EPA Form 3120–1 must submit a new statement each July by no later than July 31, even if no changes have taken place during the year.

Deputy Ethics Officials must notify such employees of this requirement and must complete review of the statements within 30 days after submission. By September 30 of each year, Deputy Ethics Officials must submit a statement to the Designated Agency Ethics Official containing the following information: (i) the number of employees in their organization at GM/GS 13–15; (ii) the number required to file; (iii) the number of other employees required to file under § 3.302(c) or 3.302(d); (iv) the number of remedial actions taken by type of action (i.e., recusals, waivers, divestitures, reassignments or blind trusts); (v) a certification that no employee who performs any "functions or duties" under the Surface Mining Control and Reclamation Act holds any prohibited interests (see § 3.305); and (6) a certification that all required reports have been received, reviewed and signed and that any necessary remedial actions have been taken.

In addition, whenever additions or changes have taken place, employees who are required to file must submit a supplemental statement by the end of the four month period in which the transactions occurred; that is, by November 30 and March 31. Deputy Ethics Officials must notify employees of this requirement during the months when these updates are due, and must review and sign the updates within 30 days after submission. However, they need not provide any report to the Designated Agency Ethics Official regarding the updates.

(4) *Confidentiality*—EPA Form 3120–1 is confidential. No information from this form may be disclosed other than to the Designated Agency Ethics Official and the Alternate Agency Ethics Official, immediate staff assistants whom the responsible Deputy Ethics Official has specifically designated in writing, the Office of Inspector General, committees or subcommittees of Congress on the written request of the chairman, or as the Director of the Office of Government Ethics or the Administrator may determine for good cause.

* * * * *

8. In Appendix B to Subpart C revise paragraph (2) under Coverage to read as follows:

Appendix B to Subpart C—Employees Subject to Special Requirements Under the Clean Air Act

* * * * *

(2)(i) In the Office of the Administrator: Administrator, Deputy Administrator, and the Director of the Science Advisory Board.

(ii) Regional Administrators.

(iii) In the Office of General Counsel: General Counsel, Deputy General Counsel, Associate General Counsel for Air and Radiation.

(iv) In the Office of Enforcement and Compliance Monitoring: Assistant Administrator for Enforcement and Compliance Monitoring, Senior Enforcement Counsel, Associate Enforcement Counsel for Air Enforcement.

(v) In the Office of Policy, Planning and Evaluation: Assistant Administrator for Policy, Planning and Evaluation, Deputy Assistant Administrator for Policy, Planning and Evaluation, Director of the Office of Policy Analysis, Director of the Office of Standards and Regulations.

(vi) In the Office of Air and Radiation: Assistant Administrator for Air and Radiation, Deputy Assistant Administrator for Air and Radiation, Director of the Office of Policy Analysis and Review, Director of the Office of Program Development, Director of the Office of Air Quality Planning and Standards, Director of the Office of Mobile Sources, and Directors of the following Divisions: Control Programs Development, Emission Standards and Engineering, Monitoring and Data Analysis, Stationary Source Compliance, Strategies and Air Standards, Certification, Emission Control Technology, Engineering Operations, Field Operations and Support and Manufacturers Operations.

* * * * *

9. In § 3.501 revise paragraph (a) to read as follows:

§ 3.501 Policy.

(a) Subject to the approval requirements of § 3.508, employees may engage in outside employment or other outside activity consistent with the standards of this Subpart.

* * * * *

10. In § 3.502 revise paragraph (d) to read as follows:

§ 3.502 Guidelines and Limitations.

* * * * *

(d) Involve work with any EPA contractor or subcontractor on an EPA project or work with any holder of an EPA assistance agreement or subagreement on an EPA project (unless the Designated Agency Ethics Official approves work on such acquisition or assistance agreement in writing) or would involve work for any person or organization in a position to gain advantage through the employee's exercise of official duties;

* * * * *

11. In § 3.507 revise paragraph (a) to read as follows:

§ 3.507 Special conditions applicable to publishing.

* * * * *

(a) No income is derived from publishing materials which EPA makes available to the general public or which are available to the employee because of his or her official duties but are not available to the general public; and

* * * * *

12. In § 3.508 revise paragraphs (d) and (e) to read as follows:

§ 3.508 Administrative approval.

* * * * *

(d) Outside employment or other outside activity involving an EPA contractor or subcontractor or holder of an EPA assistance agreement or subagreement; and

(e) Employment by a firm which is regulated by the EPA program Office or Regional Office in which the employee serves.

* * * * *

13. In Appendix A to Subpart E revise the introductory text of paragraph 1 (Form and content of request) to read as follows:

Appendix A to Subpart E—Procedures for Permission To Engage in Outside Employment or Other Outside Activity

1. *Form and content of request*—A written request for administrative approval of outside employment must be addressed to the appropriate Deputy Ethics Official, or, in the immediate Office of the Administrator or Deputy Administrator, to the Designated Agency Ethics Official. The request must be sent through the employee's supervisors and must indicate:

* * * * *

§ 3.603 [Amended]

14. In § 3.603(a), change "Statements of employment and financial interest" to "Statements of Employment and Financial Interests."

[FR Doc. 85–22420 Filed 9–26–85; 8:45 am]

BILLING CODE 6560-50-M

federal register

Friday
September 27, 1985

Part X

Environmental Protection Agency

Intent To List Chloroform as a Hazardous Air Pollutant; Notice

ENVIRONMENTAL PROTECTION AGENCY

[AD-FRL-2861-5]

Intent To List Chloroform as a Hazardous Air Pollutant

AGENCY: Environmental Protection Agency.

ACTION: Intent to List Chloroform Under section 112 of the Clean Air Act and Solicitation of information.

SUMMARY: This notice announces the results of EPA's preliminary assessment of chloroform as a potentially toxic air pollutant. Based on the health and preliminary risk assessment described in today's notice, EPA now intends to add chloroform to the list of hazardous air pollutants for which it intends to establish emission standards under section 112(b)(1)(A) of the Clean Air Act (CAA). The EPA will decide whether to add chloroform to the list only after studying possible techniques that might be used to control emissions of chloroform and further assessing the public health risks. The EPA will add chloroform to the list if emission standards are warranted.

This notice has no effect on the regulation of chloroform as a volatile organic compound in order to attain and maintain the national ambient air quality standards (NAAQS) for ozone. In addition, this notice does not preclude any State or local air pollution control agency from specifically regulating emission sources of chloroform.

DATES: Written comments pertaining to this notice must be received on or before November 26, 1985.

ADDRESSES: Submit comments (duplicate copies are preferred) to: Central Docket Section (A-130), Environmental Protection Agency, ATTN: Docket No. A-85-12, 401 M Street SW, Washington, DC. Docket A-85-12, which contains information relevant to this proposed decision and is located in the Central Docket Section of the U.S. Environmental Protection Agency, West Tower Lobby Gallery I, 401 M Street SW, Washington, DC. The docket may be inspected between 8:00 a.m. and 4:30 p.m. on weekdays, and a reasonable fee may be charged for copying.

Availability of Related Informtion

The Health Assessment Document (HAD) for chloroform is available through the U.S. Department of Commerce, National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161. Information on the availability of the HAD is available from ORD Publications, CERI-FR, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268 (Telephone: 513-684-7562 commercial/684-7562 FTS).

FOR FURTHER INFORMATION CONTACT: Robert M. Schell, Pollutant Assessment Branch (MD-12), Strategies and Air Standards Division, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711 (Telephone: 919-541-5645 commercial/629-5645 FTS).

SUPPLEMENTARY INFORMATION: .

Introduction

Chloroform is a volatile organic compound (VOC) which is primarily used for the production of refrigerants (i.e., fluorocarbon 22; 90% of 1982 U.S. production). In the past, chloroform was extensively used as an anaesthetic agent for surgical procedures. This use continued for many years before chloroform was substituted with safer anaesthetics.

The HAD, which was reviewed by EPA's independent Science Advisory Board (SAB) in a public meeting on December 20, 1984, summarizes a number of studies which suggest that chloroform is the causative agent of liver and kidney tumors in experimental animals. Furthermore, the HAD concludes that chloroform is probably carcinogenic to humans. Non-cancer health effects have also been reported as a result of chloroform exposure. The SAB generally agreed wth the content and conclusions of the HAD.

Sources and Emissions

The sources of chloroform emissions can be divided into three major categories:

(1) *Primary Sources:* Those processes which produce or use chloroform and that emit chloroform from process vents, storage tanks and fugitive leaks.

(2) *Secondary Sources:* Processes which inadvertently form and release chloroform through volatilization.

(3) *Miscellaneous Sources:* Other sources assumed to exist based on nationwide material balance i.e., chloroform production estimates minus known uses and exports of chloroform; all of this difference is assumed to be emitted to the air.

The primary sources emit a relatively small amount of chloroform individually or collectively. Secondary sources (e.g., water treatment, pulp and paper production), account for the majority of chloroform emissions. Chloroform is formed by secondary sources via the reaction of free chlorine and dissolved organic material in aqueous solutions. The chloroform created in this way will spontaneously volatilize to the atmosphere. A significant amount of the chloroform estimated to be emitted nationwide results from the miscellaneous uses category which has not been adequately defined. A complete summary of estimated chloroform emissions is presented in Table 1.

TABLE 1.—SUMMARY OF ESTIMATED ANNUAL EMISSIONS OF CHLOROFORM

	Number of sources/States	Chloroform used (mg/yr)	Chloroform emitted (mg/yr)
Primary sources:			
Pharmaceutical production	2/2	2,000	1,000
Chloroform production	6/5		458
Ethylene dichloride (EDC) production	18/2		173
Hypalon® production	1/1	N.A.	60
Fluorocarbon 22 production	6/5	144,000	50
Oxybisphenoxarsine (OBPA) production	1/1	N.A.	50
Grain fumigation	Many	28	28
Secondary sources:			
Pulp and paper production	129/33		3,890
Drinking water treatment	Many		1,900
Waste-water treatment	Many		424
Cooling water treatment	Many		263
Trichloroethylene (TCE) photodegradation			420
Miscellaneous uses*	Many	6,000	6,000
Totals		152,000	14,736

N.A.—Not Available.

*Miscellaneous uses are unidentified. Emission estimate is based on the assumption that all chloroform estimated by material balance is emitted to the air.

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Source: GCA 1984.

There is uncertainty in many of these emission estimates. An example of this uncertainty can be found in the quantification of chloroform emissions from the miscellaneous uses category. The emission estimate developed for this category was based on a mass balance of production and use data and assumes that all chloroform in this category is released to the air. Similarly, there is uncertainty in the estimation of chloroform emissions from secondary sources. Emissions from drinking water treatment facilities were estimated based on the average chloroform concentration in finished drinking water from a subset of all drinking water treatment facilities. This estimate, as well as the emission estimates for the other secondary source categories, is uncertain because it is based on a generalized data base and there is a potential for considerable variability in individual sources.

Many of the primary source categories already control emissions of chloroform through equipment installed to reduce VOC emissions. The secondary source categories, however, with the exception of drinking water treatment, do not control chloroform emissions. Although drinking water treatment facilities are currently regulated under the Safe Drinking Water Act (44 FR 68624–68707), this regulation has little impact on chloroform air emissions.

Public Exposure

The chloroform exposure assessment is based on the Human Exposure Model (HEM) for estimating exposure around point sources, and the Hanna-Gifford algorithm for estimating exposure from area source emissions (e.g., miscellaneous uses, drinking water treatment). Chloroform sources and emissions are distributed widely throughout the nation, and chloroform is relatively persistent in the atmosphere. The entire population, therefore, is exposed to chloroform at some concentration. Ambient air quality data indicate that annual average urban and non-urban chloroform concentrations are 0.66 $\mu g/m^3$ (0.00014 ppm) and 0.1 $\mu g/m^3$ (0.00002 ppm), respectively.

An additional analysis was conducted to evaluate the potential for short-term exposures around major emission points to result in adverse health effects. This analysis, which used worst case meteorological conditions in a conservative screening model, indicated that the maximum chloroform concentrations predicted to occur nearby the facility associated with the highest annual emissions and highest individual cancer risk are 9.7 ppm averaged over 3 minutes, and 3.8 ppm averaged over 10 hours. Although the actual level of exposure associated with the onset of non-cancer health effects in humans is unknown, the National Institute of Occupational Safety and Health (NIOSH) and the Occupational Safety and Health Administration (OSHA) have established exposure limits to protect individuals occupationally exposed to chloroform from non-cancer health effects. NIOSH recommends an exposure limit of 10 ppm (averaged over 10 hours) and OSHA recommends a ceiling limit of 50 ppm, not be exceeded at any time in workplace air. The results of this analysis (shown above) indicate that modeled ambient chloroform concentrations resulting from continuous routine emissions from chloroform sources do not exceed the occupational exposure limits established by NIOSH and OSHA. Furthermore, the maximum monitored concentration at 0.07 ppm (24-hour average) is well below the exposure limits established by NIOSH and OSHA.

In the atmosphere, chloroform photochemically degrades relatively slowly with an estimated half life of 11.5 weeks (i.e., the time required to decrease the original concentration by 50%). Assuming an average wind speed of 4 meters/second, chloroform can be expected to travel great distances from its point of emission. Chloroform, however, will not accumulate in the atmosphere.

The end products of chloroform atmospheric reactions are carbon dioxide and hydrochloric acid, with an important intermediate product being phosgene. There are no data to suggest that any of the products or intermediates of chlorofrom atmospheric reactions significantly impact public health.

Health Effects

The HAD concludes that there is sufficient evidence to conclude that chloroform is carcinogenic in experimental animals. Confirming evidence from human studies, however, is limited to epidemiological studies of chlorinated drinking water ingestion (chlorinated drinking water contains chloroform as well as other trihalomethanes). Although the results of these studies suggest increased incidences of cancer in humans, chloroform could not be implicated as the sole causative agent; therefore, the available direct evidence for chloroform carcinogenicity in humans is inadequate to assess its carcinogenic potential. Applying the International Agency for Research on Cancer (IARC) criteria, the HAD concludes that chloroform is a Category 2B carcinogen (i.e., probably carcinogenic to humans). The HAD also concludes that the evidence for carcinogenicity would place chloroform into group B2, according to the proposed EPA classification scheme (November 23, 1984, FR 46294).

Birth defects (teratogenicity and embryotoxicity) have been observed in experimental animals in association with chloroform exposure. These effects, however, were only observed at very high dose levels (adverse effects on the conceptus were only observed in association with maternal toxicity). At present, there is no accepted method of extrapolating animal studies reporting birth defects to low dose levels generally encountered by the public in ambient air. For this reason, the relevance of these studies to the current decision is unclear.

Several studies have been conducted to attempt to assess the mutagenic properties of chloroform. In analyzing the literature concerning mutagenicity, the HAD cited problems with the experimental protocol of many of these studies. The HAD concludes that, with the data currently available, no definitive conclusions can be reached concerning the mutagenicity of chloroform.

Other non-cancer health effects associated with chloroform exposure are central nervous system (CNS) depression (e.g., anaesthesia), liver and kidney damage, and cardiovascular irregularities. Prolonged exposure to high doses can lead to respiratory failure and death.

Risks to Public Health

The quantitative estimation of public health risks centers around the risk of cancer. The estimate of cancer risk to humans is based on data from oral exposure animal bioassays. In order to assess the risk of cancer to public health, the EPA's Carcinogen Assessment Group (CAG) used a linearized multistage extrapolation model, together with assumptions and conversion factors to adapt data from ingestion studies for inhalation use, to derive the unit risk estimate for chloroform. The unit risk estimate, at 2.3 x 10^{-5} $(\mu g/m^3)^{-1}$, is the upper bound probability of an individual contracting cancer if that individual were exposed to 1 microgram of chloroform per cubic meter of inspired air throughout his or her lifetime (assumed to be 70 years). This upper bound unit risk factor, combined with exposure information, was used to quantitatively estimate the risk chloroform poses to public health. The risk to public health is further quantified by two estimates: the maximum individual risk and the annual aggregate incidence. The maximum individual risk is the added lifetime probability of the most exposed individual contracting cancer as a result of exposure to chloroform in the ambient air. The annual aggregate incidence is an estimate of the excess cancer cases every year (nationwide) attributable to exposure from ambient air concentratiosn of chloroform. A complete summary of the risk estimates for each source category is presented in Table 2.

TABLE 2.—QUANTITATIVE RISK ASSESSMENT

Source category	Maximum individual risk	Aggregate incidence cancer year
Drinking water treatment *	6.5×10^{-6}	2.02
Waste-water treatment *	2.0×10^{-6}	0.46
Total of water treatment *	8.5×10^{-6}	2.48
Pulp and paper production	1.1×10^{-3}	0.76
Pharmaceutical production *	1.1×10^{-3}	0.02
Chloroform production	2.5×10^{-3}	0.09
TCE photodegration *	$<1.9 \times 10^{-6}$	0.46
Cooling water treatment *	1.2×10^{-5}	0.28

TABLE 2.—QUANTITATIVE RISK ASSESSMENT—Continued

Source category	Maximum individual risk	Aggregate incidence cancer year
EDC production	3.9×10^{-5}	0.02
Hypalon[c] production	1.2 x 10-6	<0.01
Fluorocarbon 22 production	4.4×10^{-4}	0.02
Grain fumigation[a]	1.3 x 10-6	0.02
OBPA production	7.1×10^{-4}	0.02
Miscellaneous uses[a]	2.8 x 10-7	6.42
Total		13.06

[a] Chloroform emissions were modeled as an area source.

[b] Exposure and risk estimated for only one plant (largest vitamin C producer). The aggregate incidence estimated for the entire source category is underestimated.

[c] Registered trademark.

There are a number of assumptions underlying these estimates that can yield either over or underestimates of the risk posed by chloroform. Further study and assessment will not likely narrow the uncertainties associated with some of the inputs to the risk assessment or yield an improvement in some of these assumptions (e.g., the carcinogenic potency of a chemical estimated through the use of a mathematical model for extrapolating high-dose animal studies to the much lower concentrations present in the ambient air). There are other inputs to the risk estimates which are very preliminary at the current stage of assessment and which will be substantially refined through further study. The primary example of this is the source information: number and types of sources, their locations, emission rates, stack parameters, variability of emissions, etc. Current source information is based on engineering estimates, data obtained under section 114 of the CAA, and other readily available information in the literature. This information, in many cases, will be improved through plant visits and source tests. The Agency has concluded that the preliminary risk estimates presented here are sufficient to warrant further study for possible regulation. The Agency will improve these estimates, particularly with respect to emissions and exposure, before making a final decision on whether to add chloroform to the list under section 112.

Statement of Intent

Section 112(b)(1)(A) of the Clean Air Act provides that the Administrator shall maintain "* * * a list which includes each hazardous air pollutant for which he intends to establish an emission standard under this section." In deciding whether to establish such an emission standard for carcinogens, EPA considers both public health risks and the feasibility and reasonableness of control techniques (e.g., 49 FR 23522; 23498 (June 6, 1984) (emission standards for benzene)].

Based on the health and preliminary risk assessment described in today's notice, the EPA now intends to add chloroform to the section 112(b)(1)(A) list. The EPA will decide whether to add chloroform to the list only after studying techniques that might be used to control emissions of chloroform and further improving the assessment of the public health risks. The EPA will add chloroform to the list if national emission standards are warranted. The EPA will publish this decision in the **Federal Register.**

If standards are not warranted under section 112 of the Clean Air Act, the Agency will consider other options as described in EPA's report, "A Strategy to Reduce Public Health Risks from Air Toxics," June 1985. For example, in that strategy EPA described other approaches for dealing with routine releases of toxic air pollutants from stationary sources such as working with State and local air pollution control agencies to address problems that do not warrant federal regulatory action but which account for elevated risks in some areas.

Standards Development Process

The following discussion has been prepared to provide the reader with an explanation of the standards development process and the timing of the process. The standards development process involves two phases, each taking about two years. The first phase is the identification of the emission sources and the need and ability to control those sources. The second phase involves Agency decisionmaking and public review prior to a final action.

During the first phase, EPA identifies the industrial processes that are significant emitters of the pollutant and the specific emission points within each process, and then determines the quantities of pollution emitted, the alternative control systems available, and their cost and effectiveness in reducing emissions and associated public health risks. A set of alternative regulations is developed and the environmental, economic, and energy impacts, as well as public health risks are evaluated.

The first phase requires investigation of the many different ways in which a candidate pollutant can be emitted and controlled. Within a source category there is wide variation in design, size, and process. This variation affects the emission rates, the public health risks, and the cost and availability of controls for the pollutant. Assessment of source emissions and controls is further complicated by the fact that emissions are not always contained in stacks or ducts (i.e., some are fugitive emissions), and emission test programs are technically difficult and costly.

The decisionmaking and review phase involves a series of EPA internal and external activities. Prior to publication of proposed rules, the Agency reviews all of the technical, cost, and exposure/risk data and makes decisions on the level of standards. The data and conclusions are reviewed publicly by an independent technical advisory committee. The standard is proposed for public comment. The comment period is open a minimum of two months and a public hearing is held, if requested. Following the comment period, Agency technical staff reviews the comments and resolves technical issues, an activity that often requires obtaining and analyzing new data.

Call for Information

Information is requested on source and emissions data, and the potential health effects associated with chloroform. People with information to submit on a voluntary basis should either provide this information by November 26, 1985 or notify the Agency by November 26, 1985 that they will be providing this information. Information should be submitted in duplicate to the Central Docket Section (A-130), Environmental Protection Agency, Attn: Docket No. A-85-12, 401 M Street, SW, Washington, DC 20460.

Miscellaneous

Chloroform is currently listed as a hazardous substance under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) section 101(14). The statutory Reportable Quantity (RQ) for chloroform is listed as 5000 pounds. This RQ may soon change, however, because chloroform is currently under assessment for carcinogenic effects.

Pursuant to CERCLA section 103(a), any person in charge of a vessel or an offshore or an onshore facility shall, as soon as he has knowledge of any release (other than a federally-permitted release or normal application of a pesticide) of a hazardous substance from such vessel or facility in a quantity equal to or exceeding the RQ determined in any 24-hour period, immediately notify the National Response Center (NRC) (1-800-424-8802; in the Washington, D.C. metropolitan area 202-426-2675).

Since chloroform is already listed specifically by CERCLA authority as a hazardous substance which requires reporting of such releases equaling or exceeding an RQ to all media, this notice poses no additional burden on the regulated community, the government or the public. However, all parties are given notice here that such a requirement for reporting exists under the authority of CERCLA. For additional information on CERCLA hazardous substance reporting, refer to 50 FR, No. 65 P-13481 (April 4, 1985).

Under Executive Order 12291, EPA must judge whether this action is "major" and therefore subject to the requirement of a Regulatory Impact Analysis. This action is not major because it imposes no additional regulatory requirements on States or sources. This proposal was submitted to the Office of Management and Budget (OMB) for review. Any written comments from OMB and any EPA responses are available in the docket. Pursuant to 5 U.S.C. 605(6), I hereby certify that this action will not have a significant economic impact on a substantial number of small entities because it imposes no new requirements. This action does not contain any information collection requirements subject to OMB review under the Paperwork Reduction Act of 1980.

Dated: September 18, 1985.

Lee M. Thomas,

Administrator.

References

Mohin, Timothy J. (1985). Chloroform Exposure and Risk Assessment, Memorandum to Files, EPA, Office of Air Quality Planning and Standards, Research Triangle Park, N.C. 27711. January 31, 1985.

Rehm, Richard et al. (GCA Corp.) (1984). Assessment of Chloroform Source Categories. Final Report. EPA Contract No. 68-02-3510. Work Assignment No. 39. July 1984.

U.S. Environmental Protection Agency (1985). Health Assessment Document for Chloroform (Final Report). EPA-600/8-84-004F, Environmental Criteria and Assessment Office. Research Triangle Park, N.C. 27711. August, 1985.

[FR Doc. 85-23116 Filed 9-26-85; 8:45 am]

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federal register

Friday
September 27, 1985

Part XI

Environmental Protection Agency

Assessment of Chloroprene as a Potentially Toxic Air Pollutant; Notice

ENVIRONMENTAL PROTECTION AGENCY

[ADL-FRL-2859-3]

Assessment of Chloroprene as a Potentially Toxic Air Pollutant

AGENCY: Environmental Protection Agency (EPA).

ACTION: Notice of intent not to regulate and solicitation of information.

SUMMARY: This notice describes the results of EPA's assessment of chloroprene as a potentially toxic air pollutant. The EPA is announcing its intent not to specifically regulate chloroprene as an air pollutant under any section of the Clean Air Act (CAA). Given that there are uncertainties in the health and exposure information incorporated in this notice and that there has been limited opportunity for public review, the Agency is also soliciting comment on this notice. A further notice will be published, however, only if the public comment indicates a need to reconsider the conclusions presented here. This determination has no effect on the regulation of chloroprene as a volatile organic compound in order to attain the national ambient air quality standards (NAAQS) for ozone. In addition, this determination does not preclude any State or local air pollution control agency from specifically regulating emission sources of chloroprene.

DATES: Written comments pertaining to this notice must be received on or before November 26, 1985.

ADDRESSES: Submit written materials (duplicate copies are preferred) to: Central Docket Section (A-130), Environmental Protection Agency, Attn: Docket No. A-85-11, 401 M Street, SW, Washington, DC. Docket A-85-11, which contains information relevant to this decision, is located in the Central Docket Section of the U.S. Environmental Protection Agency, West Tower Lobby Gallery I, 401 M Street, SW, Washington, DC. The docket may be inspected between 8:00 a.m. and 4:30 p.m. on weekdays, and a reasonable fee may be charged for copying.

FOR FURTHER INFORMATION CONTACT: Robert M. Schell, Pollutant Assessment Branch (MD-12), Strategies and Air Standards Division, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711 (Telephone: 919-541-5645 commercial/629-5645 FTS).

SUPPLEMENTARY INFORMATION: The EPA initiated this assessment because of the potential for human exposure to chloroprene as a result of emissions from industrial sources, because of reports of adverse health effects, and because of its chemical structural similarity to vinyl chloride, which is a carcinogen. As a first step in this process, a Health Assessment Summary (HAS) for chloroprene was drafted, summarizing properties, sources, emissions, and health effects. Because of the lack of available data, a full health assessment document was not written nor was the HAS reviewed by the Science Advisory Board (SAB), a group of independent scientists. The HAS was reviewed both internally and externally for technical quality and conclusions.

Other Federal Activities

Other EPA review activities involving gathering information on chloroprene are being conducted under section 4 of the Toxic Substances Control Act (TSCA). The EPA is committed to further analysis of new data as it becomes available.

Introduction

Chloroprene (2-chloro-1,3-butadiene), a monomer used in the manufacture of synthetic rubber, is a volatile and highly reactive chemical with an estimated residence time in the atmosphere of 4.8 hours. Chloroprene's only known use is in the manufacture of polychloroprene synthetic rubber. Solid polychloroprene, also known as neoprene synthetic rubber, is used in the automotive industry for tubing, belts, and gaskets, in the construction industry, and in the manufacture of wire and cable jackets, and consumer goods. Liquid or latex polychloroprene is used in adhesives and as fabric coatings.

Health Effects

Carcinogenicity/Mutagenicity—Epidemiological studies conducted in the Soviet Union have reported conflicting results (IARC, 1979; HAS). Results from studies of chloroprene workers in this country are suggestive of a slightly increased cancer risk (Infante, 1977; Pell, 1978; HAS). Serious limitations in data regarding the likelihood of chloroprene being carcinogenic for humans preclude the development of conclusions (Infante, 1977; IARC, 1979, HAS).

Tumorigenic effects of chloroprene have been studied in mice following skin application and in rats by oral, subcutaneous, and intratracheal administration. No conclusive tumorigenic effects were found. However, the compound was reported to increase the rate of tumor growth of transplanted tumor cells possibly due to immunosuppression. None of these studies are adequate for evaluating the carcinogenicity of chloroprene in experimental animals, as they lacked adequate durations of exposure and the experimental details reported are not adequate (HAS). Based on both the International Agency for Research on Cancer (IARC, 1979) and EPA's proposed weight-of-evidence criteria (FR 49 46294-46301, November 23, 1984), the available data are inadequate to evaluate the carcinogenic potential for chloroprene (IARC Group 3, EPA Group D).

Chloroprene has been reported to be mutagenic in bacteria. Poor quality Russian studies have reported mutagenesis in other systems (HAS).

Acute Toxicity (less than 24 hours)—The HAS reports human exposure to chloroprene at 970 parts per million (ppm) for less than 15 minutes causes giddiness and nausea (Nystrom, 1948; HAS). The threshold limit value (TLV), recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) is 10 ppm averaged over 8 hours.

Based on various species and exposure paradigms Von Oettingen et al. (1936) concluded that several thousand ppm for several hours should be considered lethal, 280 ppm should be considered dangerous and 80 ppm may cause less severe toxic effects. Recent better controlled studies indicate lethal acute inhalation exposures of rats at 2280 ppm for 4 hours (Clary et al., 1978, HAS). A single inhalation exposure of 225 ppm for 4 hours has been reported to result in liver damage in rats. This effect was not seen after exposure to 150 ppm for 4 hours (Plugge & Jaeger, 1979).

Subchronic Toxicity (less than 3 months but greater than 24 hours)—Exposures of humans to 55 to 333 ppm for a minimum of one week have been reported to produce fatigue, pressure and chest pain (electrocardiograms showed no abnormalities), dermatitis and hair loss in a substantial number of workers (Nystrom, 1948; HAS).

Exposure of rats and hamsters to chloroprene (39 ppm for 6 hours per day, 5 days per week for 4 weeks) resulted in slight growth depression, behavioral effects, eye and skin irritation. At higher concentrations (625 and 160 ppm) tissue damage, especially to lung and livers, and mortality were observed (Clary et al., 1978; HAS). Exposure of both male and pregnant female rats to 25 ppm for 4 hours per day for 12 to 22 days resulted in no obvious toxic effects for parents or embryos, including teratogenic effects (Culik et al., 1978; HAS).

Chronic Toxicity (Noncarcinogenic)—There are no epidemiological studies with reported exposure levels. In one study, biochemical and hematological evaluation of workers exposed to chloroprene showed no significant differences compared to controls (Gooch and Hawn, 1981; HAS). However, another study suggested that exposure to chloroprene may contribute to liver function abnormalities (Ward et al., 1981; HAS). Exposure concentrations were not reported in either study.

Reproductive Toxicity—A number of papers, chiefly from the Soviet Union, have consistently reported reproductive toxicity in the range of 1–10 ppm. These studies are poorly reported and consequently are inadequate for risk assessment purposes. Attempts have been made to validate these studies but these attempts have largely been unsuccessful (NIOSH, 1977). One animal study performed in this country has not substantiated these effects (Culik et al., 1978; HAS). Based primarily on these Soviet studies and the lack of additional information, the National Institute of Occupational Safety and Health has recommended a 1 ppm, 15-minute ceiling for workplace chloroprene exposure.

Overall, the data available on potential reproductive hazard, carcinogenicity or other toxicity subsequent to chronic chloroprene exposure is inadequate to support a decision to regulate under the Clean Air Act.

Sources and Emissions

Limited data are available to determine the occurrence of chloroprene in the environment (SAI, 1982; Radian, 1985). Given the short predicted residence time in the atmosphere (4.8 hours), it is unlikely that detectable amounts would be observed distant from chloroprene emitting sources (Cupitt, 1980; HAS). Only four facilities manufacture or use chloroprene in the United States. Annual production is estimated to be 1.2×10^5 megagrams per year (49 FR 46938, November 29, 1984). Annual emissions are estimated to be 770 megagrams per year (Radian, 1985). Limited measurements have been reported for ambient chloroprene concentrations. Those made in Deer Park, TX were reported as 73.9 and 1111.1 ppt (2 hour sampling periods) (Pellizzari et al., 1979; HAS). Measurements made at an industrial waste treatment facility in Houston, TX ranged from less than 0.02 to 0.40 ppm with approximately 7 hour sampling periods (Timm, 1985).

Exposure Estimates and Risks To Public Health

A preliminary analysis was conducted to examine the potential for short-term concentrations of chloroprene in the ambient air surrounding industrial facilities to approach or exceed those concentrations at which noncarcinogenic health effects have been reported. This is a rough analysis, which uses worst case meteorological conditions in a conservative screening model. This analysis indicated that ambient concentrations resulting from continuous routine emissions would not be expected to approach levels at which systemic toxicity has been reported as a result of acute or subchronic exposures. Approximately 4.7 million people live within 50 kilometers of the four domestic chloroprene producing facilities. A 15-minute concentration of 4.5 ppm, a 4-hour concentration of 2.8 ppm, a 6-hour concentration of 2.6 ppm and an 8-hour concentration of 2.5 ppm were estimated using this short-term exposure model. Given the health effects data, it appears that the potential for systemic toxicity to occur in the general population subsequent to acute or subchronic chloroprene exposures is low (see Table 1) (Cote, 1985). As stated earlier, the health effects data for chronic exposure are inadequate to assess risk.

Discussion

Based on currently available data, EPA has determined that no regulation directed specifically at chloroprene is appropriate at this time under the CAA. Unless additional information becomes available during the public comment period, the effect of this notice is to remove chloroprene from EPA's list of potential air toxics currently under assessment.

In order to improve upon the health effects information base for chloroprene, the National Toxicology Program is testing chloroprene carcinogenicity and reproductive hazard in animal bioassays; however, results are not expected before 1987. Further assessment and review of chloroprene will be initiated upon completion of these studies.

Due to uncertainties in assessing the risk of health effects, the EPA is soliciting health effects and exposure information on chloroprene as well as comments on the analysis and conclusions on which this notice is based. A further notice will be published, however, only if public comments indicate a need to reconsider these conclusions. In addition, if significant new information becomes available, the Agency will reexamine the need to regulate chloroprene.

This notice has no effect on the regulation of chloroprene as a volatile organic compound in order to attain the NAAQS for ozone. In addition, this notice does not preclude any State or local air pollution control agency from specifically regulating emission sources of chloroprene.

Dated: September 17, 1985.

Lee M. Thomas,

Administrator.

TABLE 1.—COMPARISON OF SYSTEMIC TOXICITY, MODELING AND MONITORING DATA

NOEL or TLV[1]	Lowest observed effect level	Modeling	Monitoring
Acute:			
10 ppm (8 hr TLV).	972 ppm × 15 min. (human).	4.5 ppm × 15 min.	1.1 ppb.
150 ppm × 4 hrs (rats).	225 ppm × 4 hrs (rat).	2.8 ppm × 4 hrs.	1.1 ppb.
Subchronic:			
10 ppm (8 hr TLV).	55 ppm × 8 hr (1 wk—humans).	2.5 ppm × 8 hrs[2].	1.1 ppb.
25 ppm × 4 hr (4 wks—rats).	39 ppm × 6 hr (4 wks—rats).	2.6 ppm × 6 hrs[2].	1.1 ppb.

[1] The American Conference of Governmental Industrial Hygienists' recommended threshold limit value (TLV) is presented for comparison in addition to the no observed effect level (NOEL) in animals.

[2] Subchronic exposure estimates were not modeled. The 8-hour and 6-hour acute exposure estimates are used for comparison and are shown here.

Information on health effects subsequent to chronic exposure is inadequate for risk assessment purposes.

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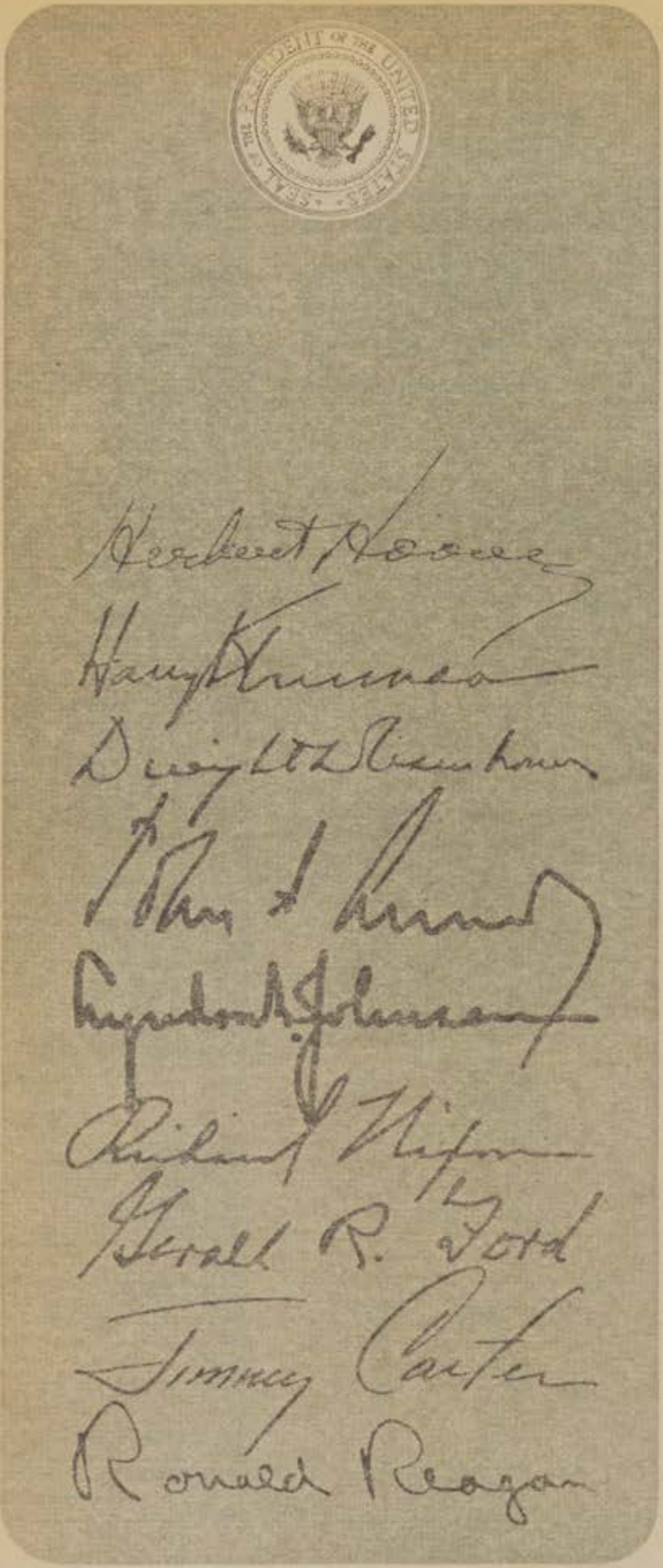

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