

Environmental Protection Agency

§ 799.2155

date of paragraph (c)(4)(ii)(A) of this section is May 28, 1993.

(2) The guidelines and other test methods cited in this rule are referenced as they exist on the effective date of the final rule.

[52 FR 21530, June 8, 1987, as amended at 52 FR 43762, Nov. 16, 1987; 54 FR 27357, June 29, 1989; 54 FR 33148, Aug. 11, 1989; 55 FR 12643, Apr. 5, 1990; 56 FR 23230, May 21, 1991; 57 FR 24960, June 12, 1992; 58 FR 30992, May 28, 1993; 58 FR 34205, June 23, 1993]

§ 799.2155 Commercial hexane.

(a) *Identification of test substance.* (1) "Commercial hexane," for purposes of this section, is a product obtained from crude oil, natural gas liquids, or petroleum refinery processing in accordance with the American Society for Testing and Materials Designation D 1836-83 (ASTM D 1836), consists primarily of six-carbon alkanes or cycloalkanes, and contains at least 40 liquid volume percent *n*-hexane (CAS No. 110-54-3) and at least 5 liquid volume percent methylcyclopentane (MCP; CAS No. 96-37-7). ASTM D 1836, formally entitled "Standard Specification for Commercial Hexanes," is published in *1986 Annual Book of ASTM Standards: Petroleum Products and Lubricants*, ASTM D 1836-83, pp. 966-967, 1986, is incorporated by reference, and is available for public inspection at the National Archives and Records Administration (NARA). For information on the availability of this material at NARA, call 202-741-6030, or go to: http://www.archives.gov/federal_register/code_of_federal_regulations/ibr_locations.html. This incorporation by reference was approved by the Director of the Office of the Federal Register in accordance with 5 U.S.C. 522(a) and 1 CFR part 51. 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 Non-Confidential Information Center (NCIC) (7407), Office of Pollution Prevention and Toxics, U.S. Environmental Protection Agency, Room B-607 NEM, 401 M St., SW., Washington, DC 20460, between the hours of 12 p.m. and 4 p.m. weekdays excluding legal holidays.

(2) The commercial hexane test substance, for purposes of this section, is a product which conforms to the specifications of ASTM D1836 and contains at least 40 liquid volume percent but no more than 55 liquid volume percent *n*-hexane and no less than 10 liquid volume percent MCP.

(b) *Persons required to submit study plans, conduct tests, and submit data.* All persons who manufacture (including import) or process or intend to manufacture or process commercial hexane, as defined in paragraph (a)(1) of this section and other than as an impurity, from the effective date of the final rule to the end of the reimbursement period shall submit letters of intent to conduct testing, submit study plans, conduct tests in accordance with part 792 of this chapter, and submit data, or submit exemption applications, as specified in this section, subpart A of this part, and part 790 of this chapter for single-phase rulemaking. Persons who manufacture commercial hexane as a byproduct are covered by the requirements of this section. Notwithstanding §790.50(a)(1) of this chapter, persons who notify EPA of their intent to conduct neurotoxicity testing in compliance with paragraph (c)(7) of this section may submit study plans for those tests less than 45 days before beginning testing provided that EPA receives the study plans before this testing begins.

(c) *Health effects testing*—(1) *Subchronic inhalation toxicity*—(i) *Required testing.* (A) A subchronic inhalation toxicity test shall be conducted with commercial hexane in accordance with §798.2450 of this chapter except for the provisions in paragraphs (d)(4)(ii) and (5) of §798.2450.

(B) For the purposes of this section, the following provisions also apply:

(1) *High dose level.* The highest concentration should result in toxic effects but neither produce an incidence of fatalities which would prevent a meaningful evaluation nor exceed the lower explosive limit of commercial hexane.

(2) *Exposure conditions.* Animals shall be dosed for 6 hours/day, 5 days/week for 90 days.

(ii) *Reporting requirements.* (A) The subchronic inhalation toxicity test

shall be completed and the final report submitted to EPA within 15 months of the effective date of the final rule.

(B) Interim progress reports shall be submitted to EPA for the subchronic inhalation toxicity test at 6-month intervals beginning 6 months after the effective date of the final rule, until the final report is submitted to EPA.

(2) *Oncogenicity*—(i) *Required testing.*

(A) An oncogenicity test shall be conducted with commercial hexane in accordance with §798.3300 of this chapter except for the provisions in paragraphs (b)(3)(ii) and (6) of §798.3300.

(B) For the purposes of this section, the following provisions also apply:

(1) *High dose level.* The high dose level should elicit signs of minimal toxicity without substantially altering the normal life span and should not exceed the lower explosive limit of commercial hexane.

(2) *Administration of test substance.* Animals shall be exposed to commercial hexane by inhalation.

(ii) *Reporting requirements.* (A) The oncogenicity test shall be completed and the final report submitted to EPA within 53 months of the effective date of the final rule. The mouse portion of the oncogenicity study shall be submitted by June 5, 1993.

(B) Interim progress reports shall be submitted to EPA for the oncogenicity test at 6-month intervals beginning 6 months after the effective date of the final rule, until the final report is submitted to EPA.

(3) *Reproduction and fertility effects*—

(i) *Required testing.* (A) A reproduction and fertility effects test shall be conducted with commercial hexane in accordance with §798.4700 of this chapter except for the provisions in paragraphs (c)(3)(ii) and (5) of §798.4700.

(B) For the purposes of this section, the following provisions also apply:

(1) *High dose level.* The highest dose level should induce toxicity but not high levels of mortality in the parental (P) animals. In addition, the highest dose level should not exceed the lower explosive limit of commercial hexane.

(2) *Administration of test substance.* Animals shall be exposed to commercial hexane by inhalation.

(ii) *Reporting requirements.* (A) The reproduction and fertility effects test

shall be completed and the final report submitted to EPA within 29 months of the effective date of the final rule.

(B) Interim progress reports shall be submitted to EPA for the reproduction and fertility effects test at 6-month intervals beginning 6 months after the effective date of the final rule, until the final report is submitted to EPA.

(4) *Inhalation developmental toxicity*—

(i) *Required testing.* (A) An inhalation developmental toxicity test shall be conducted with commercial hexane in accordance with §795.4350 of this chapter except for the provisions in paragraph (e)(3)(iv) of §795.4350.

(B) For the purposes of this section, the following provisions also apply:

(1) *High dose level.* Unless limited by the physical/chemical nature or biological properties of the test substance, the highest concentration level shall induce some overt maternal toxicity such as reduced body weight or body weight gain, but not more than 10 percent maternal deaths. In addition, the highest dose level should not exceed the lower explosive limit of commercial hexane.

(2) [Reserved]

(ii) *Reporting requirements.* (A) The inhalation developmental toxicity test shall be completed and the final report submitted to EPA within 12 months of the effective date of the final rule.

(B) Interim progress reports shall be submitted to EPA for the inhalation developmental toxicity test at 6-month intervals beginning 6 months after the effective date of the final rule, until the final report is submitted to EPA.

(5) *Mutagenic effects—gene mutations*—

(i) *Required testing.* (A)(I) A *Salmonella typhimurium* reverse mutation assay shall be conducted with commercial hexane in accordance with §798.5265 of this chapter except for the provisions in paragraphs (d)(4) and (e) of §798.5265.

(2) For the purposes of this section, the following provisions also apply:

(i) *Metabolic activation.* Bacteria shall be exposed to commercial hexane both in the presence and absence of an appropriate metabolic activation system.

(ii) *Test performance.* The assay shall be performed using the desiccator method described as follows: The agar overlay plates shall be placed uncovered in a 9-liter desiccator. A volume of

the liquid test substance shall be added to the glass Petri dish suspended beneath the porcelain shelf of the desiccator. The highest exposure concentration should not result in a vapor concentration which exceeds the lower explosive limit of commercial hexane. A magnetic stirring bar to serve as a fan to assure rapid and even distribution of the vapor shall be placed on the bottom of the inside of the desiccator. The desiccator shall be placed on a magnetic stirrer within a 37° C room or chamber for 7 to 10 hours. The plates shall then be removed, their lids replaced, followed by incubation for an additional 40 hours at 37° C before counting. An appropriate selective medium with an adequate overlay agar shall be used. All plating should be done in at least triplicate.

(B)(1) A gene mutation test in mammalian cells shall be conducted with commercial hexane in accordance with § 798.5300 of this chapter except for the provisions in paragraphs (d)(3)(ii) and (4) of § 798.5300 if the results from the *Salmonella typhimurium* test conducted pursuant to paragraph (c)(5)(i)(A) of this section are negative.

(2) For the purposes of this section, the following provisions also apply:

(i) *Cell growth and maintenance.* Appropriate culture media and incubation conditions (culture vessels, CO₂ concentrations, temperature, and humidity) shall be used. The cell culture shall be directly dosed by pipetting liquid commercial hexane mixed with liquid DMSO into the culture medium. Cells shall be exposed to test substance both in the presence and absence of an appropriate metabolic activation system.

(ii) [Reserved]

(C)(1) A sex-linked recessive lethal test in *Drosophila melanogaster* shall be conducted with commercial hexane in accordance with § 798.5275 of this chapter except for the provisions in paragraphs (d)(5) (ii) and (iii) of § 798.5275, unless the results of both the *Salmonella typhimurium* test conducted pursuant to paragraph (c)(5)(i)(A) of this section and the mammalian cells in the culture gene mutation test conducted pursuant to paragraph (c)(5)(i)(B) of this section, if required, are negative.

(2) For the purposes of this section, the following provisions also apply:

(i) *Dose levels.* For the initial assessment of mutagenicity, it is sufficient to test a single dose of the test substance for screening purposes. This dose should be the maximum tolerated dose, or that which produces some indication of toxicity or shall be the highest dose attainable and should not exceed the lower explosive limit of commercial hexane. For dose-response purposes, at least three additional dose levels should be used.

(ii) *Route of administration.* The route of administration shall be by exposure to commercial hexane vapors.

(D)(1) Unless the results of the sex-linked recessive lethal test in *Drosophila melanogaster* conducted with commercial hexane pursuant to paragraph (c)(5)(i)(C) of this section are negative, EPA shall conduct a public program review of all of the mutagenicity data available for this substance. If, after this review, EPA decides that testing of commercial hexane for causing heritable gene mutations in mammals is necessary, it shall notify the test sponsor by certified letter or FEDERAL REGISTER notice that testing shall be initiated in either the mouse visible specific locus test or the mouse biochemical specific locus test. The mouse visible specific locus test, if conducted, shall be performed for commercial hexane in accordance with § 798.5200 of this chapter except for the provisions in paragraphs (d)(5)(ii) and (d)(5)(iii) of § 798.5200. The mouse biochemical specific locus test, if conducted, shall be performed for commercial hexane in accordance with § 798.5195 of this chapter except for the provisions in paragraphs (d)(5)(ii) and (d)(5)(iii) of § 798.5195.

(2) For the purposes of this section, the following provisions also apply:

(i) *Dose levels.* A minimum of two dose levels shall be tested. The highest dose tested shall be the highest dose tolerated without toxic effects, provided that any temporary sterility induced due to elimination of spermatogonia is of only moderate duration, as determined by a return of males to fertility within 80 days of treatment, or shall be the highest dose attainable below the lower explosive

limit concentration of commercial hexane. Exposure shall be for 6 hours a day. Duration of exposure shall be dependent upon the accumulated total dose desired for each group.

(ii) *Route of administration.* Animals shall be exposed to commercial hexane by inhalation.

(ii) *Reporting requirements.* (A) The gene mutation tests shall be completed and final reports submitted to EPA as follows:

(1) The *Salmonella typhimurium* reverse mutation assay within 8 months of the effective date of the final rule.

(2) The gene mutation in mammalian cells assay within 17 months of the effective date of the final rule.

(3) The sex-linked recessive-lethal test in *Drosophila melanogaster* within 24 months of the effective date of the final rule.

(4) The mouse visible specific locus test or the mouse biochemical specific locus test shall be completed and a final report shall be submitted to EPA within 51 months of the date on which the test sponsor is notified by EPA by certified letter or FEDERAL REGISTER notice that testing shall be initiated.

(B) Interim progress reports for each test shall be submitted to EPA for the gene mutation in mammalian cells assay and *Drosophila* sex-linked recessive lethal test at 6-month intervals beginning 6 months after the effective date of the final rule, until the applicable final report is submitted to EPA.

(C) Interim progress reports for either the mouse visible specific locus test or the mouse biochemical specific locus test shall be submitted to EPA at 6-month intervals, beginning 6 months after EPA's notification of the test sponsor that testing should be initiated, until the applicable final report is submitted to EPA.

(6) *Mutagenic effects—chromosomal aberrations—(i) Required testing.* (A)(1) An *in vitro* cytogenetics test shall be conducted with commercial hexane in accordance with §798.5375 of this chapter except for the provisions in paragraph (e)(3) of §798.5375.

(2) For the purposes of this section, the following provisions also apply:

(i) *Treatment with test substance.* The test substance shall be added in liquid

form mixed with DMSO to the treatment vessels.

(ii) [Reserved]

(B)(1) An *in vivo* cytogenetics test shall be conducted with commercial hexane in accordance with §798.5385 of this chapter except for the provisions in paragraphs (d)(5) (ii), (iii) and (iv) of §798.5385, if the *in vitro* test conducted pursuant to paragraph (c)(6)(i)(A) of this section is negative.

(2) For the purposes of this section, the following provisions also apply:

(i) *Dose levels.* For an initial assessment, one dose level of the test substance may be used, the dose being the maximum tolerated dose (to a maximum of 5,000 mg/kg), or that producing some indication of cytotoxicity (e.g., partial inhibition of mitosis), or shall be the highest dose attainable (to a maximum of 5,000 mg/kg) and should not exceed the lower explosive limit of commercial hexane. Additional dose levels may be used. For determination of dose-response, at least three dose levels should be used.

(ii) *Route of administration.* Animals shall be exposed to commercial hexane by inhalation.

(iii) *Treatment schedule.* The duration of exposure shall be for 6 hours per day for 5 consecutive days.

(C)(1) A dominant lethal assay shall be conducted with commercial hexane in accordance with §798.5450 of this chapter except for the provisions in paragraphs (d)(5) (ii) and (iii) of §798.5450, unless both the *in vitro* and *in vivo* cytogenetics tests conducted pursuant to paragraphs (c)(6)(i) (A) and (B) of this section are negative.

(2) For the purposes of this section, the following provisions also apply:

(i) *Dose levels.* Normally, three dose levels shall be used. The highest dose shall produce signs of toxicity (e.g., slightly reduced fertility and slightly reduced body weight). The highest dose should not exceed the lower explosive limit of commercial hexane. However, in an initial assessment of dominant lethality, a single high dose may be sufficient. Nontoxic substances shall be tested at 5 g/kg or, if this is not practicable, then at the highest dose attainable.

(ii) *Route of administration.* Animals shall be exposed to commercial hexane by inhalation.

(iii) *Treatment schedule.* The duration of exposure shall be for 6 hours per day for 5 consecutive days.

(D)(1) A heritable translocation test shall be conducted with commercial hexane in accordance with § 798.5460 of this chapter except for the provisions in paragraphs (d)(5) (ii) and (iii) of § 798.5460, if the results of the dominant lethal assay conducted pursuant to paragraph (c)(6)(i)(C) of this section are positive and if, after a public program review, EPA issues a FEDERAL REGISTER notice or sends a certified letter to the test sponsor specifying that the testing shall be initiated.

(2) For the purposes of this section, the following provisions also apply:

(i) *Dose levels.* At least two dose levels shall be used. The highest dose level shall result in toxic effects (which shall not produce an incidence of fatalities which would prevent a meaningful evaluation) or shall be the highest dose attainable or 5 g/kg body weight and should not exceed the lower explosive limit of commercial hexane.

(ii) *Route of administration.* Animals shall be exposed to commercial hexane by inhalation.

(iii) *Reporting requirements.* (A) The chromosomal aberration tests shall be completed and the final reports submitted to EPA as follows:

(1) The *in vitro* cytogenetics test within 15 months of the effective date of the final rule.

(2) The *in vivo* cytogenetics test within 19 months of the effective date of the final rule.

(3) The dominant lethal assay within 28 months of the effective date of the final rule.

(4) The heritable translocation test within 25 months of the date of EPA's notification of the test sponsor by certified letter or FEDERAL REGISTER notice that testing shall be initiated.

(B) Interim progress reports for each test shall be submitted to EPA for the *in vivo* cytogenetics and the dominant lethal assays at 6-month intervals beginning 6 months after the effective date of the final rule, until the applicable final report is submitted to EPA.

(C) Interim progress reports shall be submitted to EPA for the heritable translocation assay at 6-month intervals beginning 6 months after the date of EPA's notification of the test sponsor that testing shall be initiated, until the final report is submitted to EPA.

(7) *Neurotoxicity*—(i) *Required testing.* (A)(1) A schedule-controlled operant behavior test shall be conducted with commercial hexane in accordance with § 798.6500 of this chapter except for the provisions in paragraphs (d)(5)(i), (6) and (7) of § 798.6500.

(2) For the purposes of this section, the following provisions also apply:

(i) *High dose level.* The highest dose shall produce clear behavioral effects or life-threatening toxicity. In addition, the highest dose should not exceed the lower explosive limit of commercial hexane.

(ii) *Duration and frequency of exposure.* Animals shall be dosed once for 4 to 6 hours.

(iii) *Route of administration.* Animals shall be exposed to commercial hexane by inhalation.

(B)(1) A functional observation battery shall be conducted with commercial hexane in accordance with § 798.6050 of this chapter except for the provisions in paragraphs (d)(4)(i), (5), and (6) of § 798.6050.

(2) For the purposes of this section, the following provisions also apply:

(i) *High dose level.* The highest dose shall produce clear behavioral effects or life-threatening toxicity. In addition, the highest dose should not exceed the lower explosive limit of commercial hexane.

(ii) *Duration and frequency of exposure.* Animals shall be dosed for 6 hours/day, 5 days/week for 90 days.

(iii) *Route of exposure.* Animals shall be exposed to commercial hexane by inhalation.

(C)(1) A motor activity test shall be conducted with commercial hexane in accordance with § 798.6200 of this chapter except for the provisions in paragraphs (d)(4)(i), (5), and (6) of § 798.6200.

(2) For the purposes of this section, the following provisions also apply:

(i) *High dose level.* The highest dose shall produce clear effects on motor activity of life-threatening toxicity. In addition, the highest dose should not

exceed the lower explosive limit of commercial hexane.

(ii) *Duration and frequency of exposure.* Animals shall be dosed for 6 hours/day, 5 days/week for 90 days.

(iii) *Route of exposure.* Animals shall be exposed to commercial hexane by inhalation.

(D)(1) A neuropathology test shall be conducted with commercial hexane in accordance with § 798.6400 of this chapter except for the provisions in paragraphs (d)(4)(i), (5), and (6) of § 798.6400.

(2) For the purposes of this section, the following provisions also apply:

(i) *High dose level.* The highest dose shall produce clear behavior effects or life-threatening toxicity. In addition, the highest dose should not exceed the lower explosive limit of commercial hexane.

(ii) *Duration and frequency of exposure.* Animals shall be dosed for 6 hours/day, 5 days/week for 90 days.

(iii) *Route of exposure.* Animals shall be exposed to commercial hexane by inhalation.

(ii) *Reporting requirements.* (A) The schedule-controlled operant behavior, functional observation battery, motor activity, and neuropathology tests shall be completed and the final reports submitted to EPA within 15 months of the effective date of the final rule.

(B) Interim progress reports for each test shall be submitted to EPA for the schedule-controlled operant behavior, functional observation battery, motor activity, and neuropathology tests at 6-month intervals beginning 6 months after the effective date of the applicable final rule, until the applicable final report is submitted to EPA.

(8) *Pharmacokinetics*—(i) *Required testing.* (A) Pharmacokinetics testing shall be conducted in rats in accordance with § 795.232 of this chapter, except for paragraph (c)(1)(ii) of § 795.232.

(B) For the purposes of this section, the following provisions also apply:

(1) *Test animals.* Adult male and female rats shall be used for testing. The rats shall be 9 to 11 weeks old and their weight range should be comparable from group to group. The animals shall be purchased from a reputable dealer and shall be permanently identified upon arrival. The animals shall be se-

lected at random for the testing groups, and any animal showing signs of ill health shall not be used.

(2) *Species and strain.* The rat strain used shall be the same as the strain used in the subchronic and chronic tests required under § 798.2450(d)(1)(i) and § 798.3300(b)(1)(i).

(ii) *Reporting requirements.* (A) The inhalation and dermal pharmacokinetics tests shall be completed and the final report submitted to EPA by August 21, 1992.

(B) Interim progress reports shall be submitted to EPA for the inhalation and dermal pharmacokinetics tests at 6-month intervals, beginning 6 months after the effective date specified in paragraph (d)(1) of this section, until the final report is submitted to EPA.

(d) *Effective date.* (1) The effective date of this final rule is November 17, 1988, except for the provisions of paragraphs (c)(2)(ii)(A), (c)(5)(i)(D), (c)(5)(ii)(A)(4), (c)(5)(ii)(C), (c)(8)(i) and (c)(8)(ii)(A) of this section. The effective date for paragraphs (c)(5)(i)(D), (c)(5)(ii)(A)(4) and (c)(5)(ii)(C) of this section is May 21, 1990. The effective date for paragraphs (c)(8)(i) and (c)(8)(ii)(A) of this section is June 12, 1992. The effective date of paragraph (c)(2)(ii)(A) is September 8, 1994.

(2) The guidelines and other test methods cited in this rule are referenced as they exist on the effective date of the final rule.

[53 FR 3392, Feb. 5, 1988, as amended at 53 FR 38953, Oct. 4, 1988; 55 FR 634, Jan. 8, 1990; 55 FR 7325, Mar. 1, 1990; 55 FR 12643, Apr. 5, 1990; 57 FR 24961, June 12, 1992; 58 FR 34205, June 23, 1993; 59 FR 46357, Sept. 8, 1994; 60 FR 34467, July 3, 1995; 69 FR 18803, Apr. 9, 2004]

§ 799.2325 Isopropanol.

(a) *Identification of test substance.* (1) Isopropanol (CAS No. 67-63-0) shall be tested in accordance with this section.

(2) Isopropanol of at least 99.8 percent purity shall be used as the test substance.

(b) *Persons required to submit study plans, conduct tests, and submit data.* All persons who manufacture (including import or byproduct manufacture) or intend to manufacture or process isopropanol, from the effective date of