Acrylonitrile
Aldrin
Aluminum
Anthracene
Antimony
Arsenic
Asbestos
1,2-Benzanthracene; benz[a]anthracene
Benzene
Benzidine
Benzo[a]pyrene; 3,4-benzopyrene
3,4-Benzofluoranthene; benzo[b]fluoranthene
11,12-Benzofluoranthene; benzo[k]fluoranthene
1,12-Benzoperylene; benzo[ghi]perylene
Beryllium
Bis(2-chloroethoxy) methane
Bis(2-chloroethyl) ether
Bis(2-chloroisopropyl) ether
Bromoform; tribomomethane
4-Bromophenyl phenyl ether
Butyl benzyl phthalate
Cadmium
Carbon tetrachloride; tetrachloromethane
Chlorobenzene
p-Chloro-m-cresol; 4-chloro-3-methylphenol
Chlorodibromomethane
Chlorethane
2-Chloroethyl vinyl ether
Chloroform; trichloromethane
2-Chloronaphthalene
2-Chlorophenol
4-Chlorophenyl phenyl ether
Chlorpyrifos
Chromium
Chrysene
Copper
Cyanide
2,4-D; 2,4-Dichlorophenoxyacetic acid
DEHP; di(2-ethylhexyl) phthalate
Diazinon
1,2:5,6-Dibenzanthracene; dibenz[a,h]anthracene
Dibutyl phthalate; di-n-butyl phthalate
1,2-Dichlorobenzene
1,3-Dichlorobenzene
1,4-Dichlorobenzene
3,3′-Dichlorobenzidine
Dichlorobromomethane; bromodichloromethane
1,1-Dichloroethane
1,2-Dichloroethane
1,1-Dichloroethylene; vinylidene chloride
1,2-trans-Dichloroethylene
2,4-Dichlorophenol
1,2-Dichloropropane
1,3-Dichloropropene; 1,3-dichloropropylene
Diethyl phthalate
2,4-Dimethylphenol; 2,4-xylenol
Dimethyl phthalate
4,6-Dinitro-o-cresol; 2-methyl-4,6-dinitrophenol
2,4-Dinitrophenol
2,4-Dinitrotoluene
2,6-Dinitrotoluene
Dioctyl phthalate; di-n-octyl phthalate
1,2-Diphenylhydrazine
Endosulfan; thiodan
alpha-Endosulfan
beta-Endosulfan
Endosulfan sulfate
Endrin
Endrin aldehyde
Ethylbenzene
Fluoranthene
Fluorene; 9H-fluorene
Fluoride
Guthion
Heptachlor
Heptachlor epoxide
Hexachlorocyclopentadiene
Hexachloroethane
Indeno[1,2,3-cd]pyrene; 2,3-o-phenylene pyrene
Isophorone
Lead
Malathion
Methoxychlor
Methyl bromide; bromomethane
Methyl chloride; chloromethane
Methylene chloride; dichloromethane
Napthalene
Nickel
Nitrobenzene
2-Nitrophenol
4-Nitrophenol
N-Nitrosodimethylamine
N-Nitrosodiphenylamine
N-Nitrosodipropylamine; N-nitrosodi-n-propylamine
Parathion
Pentachlorophenol
Phenanthrene
Phenol
Iron
Pyrene
Selenium
Silver
1,1,2,2-Tetrachloroethane
Tetrachloroethylene
Thallium
Toluene; methylbenzene
1,2,4-Trichlorobenzene
1,1,1-Trichloroethane
1,1,2-Trichloroethane
Trichloroethylene; trichloroethene
2,4,6-Trichlorophenol
Vinyl chloride; chloroethylene; chloroethene
Zinc

Appendix A to Part 132—Great Lakes Water Quality Initiative Methodologies for Development of Aquatic Life Criteria and Values

Methodology for Deriving Aquatic Life Criteria: Tier I

Great Lakes States and Tribes shall adopt provisions consistent with (as protective as) this appendix.

I. Definitions

A. *Material of Concern*. When defining the material of concern the following should be considered:

1. Each separate chemical that does not ionize substantially in most natural bodies of water should usually be considered a separate material, except possibly for structurally similar organic compounds that only exist in large quantities as commercial mixtures of the various compounds and apparently have similar biological, chemical, physical, and toxicological properties.

2. For chemicals that ionize substantially in most natural bodies of water (e.g., some phenols and organic acids, some salts of phenols and organic acids, and most inorganic salts and coordination complexes of metals and metalloid), all forms that would be in chemical equilibrium should usually be considered one material. Each different oxidation state of a metal and each different nonionizable covalently bonded organometallic compound should usually be considered a separate material.

3. The definition of the material of concern should include an operational analytical component. Identification of a material simply as ''sodium,'' for example, implies ''total sodium,'' but leaves room for doubt. If ''total'' is meant, it must be explicitly stated. Even ''total'' has different operational definitions, some of which do not necessarily measure ''all that is there'' in all samples. Thus, it is also necessary to reference or describe the analytical method that is intended. The selection of the operational analytical component should take into account the analytical and environmental chemistry of the material and various practical considerations, such as labor and equipment requirements, and whether the method would require measurement in the field or would allow measurement after samples are transported to a laboratory.

a. The primary requirements of the operational analytical component are that it be appropriate for use on samples of receiving water, that it be compatible with the available toxicity and bioaccumulation data without making extrapolations that are too hypothetical, and that it rarely result in underprotection or overprotection of aquatic organisms and their uses. Toxicity is the property of a material, or combination of materials, to adversely affect organisms.

b. Because an ideal analytical measurement will rarely be available, an appropriate compromise measurement will usually have to be used. This compromise measurement must fit with the general approach that if an ambient concentration is lower than the criterion, unacceptable effects will probably not occur, i.e., the compromise measure must not err on the side of underprotection when measurements are made on a surface water. What is an appropriate measurement in one situation might not be appropriate for another. For example, because the chemical and physical properties of an effluent are usually quite different from those of the receiving water, an analytical method that is appropriate for analyzing an effluent might not be appropriate for expressing a criterion, and vice versa. A criterion should be based on an appropriate analytical measurement, but the criterion is not rendered useless if an ideal measurement either is not available or is not feasible.

NOTE: The analytical chemistry of the material might have to be taken into account when defining the material or when judging the acceptability of some toxicity tests, but a criterion must not be based on the sensitivity of an analytical method. When aquatic organisms are more sensitive than routine analytical methods, the proper solution is to develop better analytical methods.

4. It is now the policy of EPA that the use of dissolved metal to set and measure compliance with water quality standards is the recommended approach, because dissolved metal more closely approximates the bioavailable fraction of metal in the water column that does total recoverable metal. One reason is that a primary mechanism for water column toxicity is adsorption at the gill surface which requires metals to be in the dissolved form. Reasons for the consideration of total recoverable metals criteria include risk management considerations not covered by evaluation of water column toxicity. A risk manager may consider sediments and food chain effects and may decide to take a conservative approach for metals, considering that metals are very persistent chemicals. This approach could include the use of total recoverable metal in water quality standards. A range of different risk management decisions can be justified. EPA recommends that State water quality standards be based on dissolved metal. EPA will also approve a State risk management decision to adopt standards based on total recoverable metal, if those standards are otherwise approvable under this program.

B. *Acute Toxicity*. Concurrent and delayed adverse effect(s) that results from an acute exposure and occurs within any short observation period which begins when the exposure begins, may extend beyond the exposure period, and usually does not constitute a substantial portion of the life span of the organism. (Concurrent toxicity is an adverse effect to an organism that results from, and occurs during, its exposure to one or more test materials.) Exposure constitutes contact with a chemical or physical agent. Acute exposure, however, is exposure of an organism for any short period which usually does not constitute a substantial portion of its life span.

C. *Chronic Toxicity*. Concurrent and delayed adverse effect(s) that occurs only as a result of a chronic exposure. Chronic exposure is exposure of an organism for any long period or for a substantial portion of its life span.

II. Collection of Data

A. Collect all data available on the material concerning toxicity to aquatic animals and plants.

B. All data that are used should be available in typed, dated, and signed hard copy (e.g., publication, manuscript, letter, memorandum, etc.) with enough supporting information to indicate that acceptable test procedures were used and that the results are reliable. In some cases, it might be appropriate to obtain written information from the investigator, if possible. Information that is not available for distribution shall not be used.

C. Questionable data, whether published or unpublished, must not be used. For example, data must be rejected if they are from tests that did not contain a control treatment, tests in which too many organisms in the control treatment died or showed signs of stress or disease, and tests in which distilled or deionized water was used as the dilution water without the addition of appropriate salts.

D. Data on technical grade materials may be used if appropriate, but data on formulated mixtures and emulsifiable concentrates of the material must not be used.

E. For some highly volatile, hydrolyzable, or degradable materials, it might be appropriate to use only results of flow-through tests in which the concentrations of test material in test solutions were measured using acceptable analytical methods. A flow-through test is a test with aquatic organisms in which test solutions flow into constant-volume test chambers either intermittently (e.g., every few minutes) or continuously, with the excess flowing out.

F. Data must be rejected if obtained using:

1. Brine shrimp, because they usually only occur naturally in water with salinity greater than 35 g/kg.

2. Species that do not have reproducing wild populations in North America.

3. Organisms that were previously exposed to substantial concentrations of the test material or other contaminants.

4. Saltwater species except for use in deriving acute-chronic ratios. An ACR is a standard measure of the acute toxicity of a material divided by an appropriate measure of the chronic toxicity of the same material under comparable conditions.

G. Questionable data, data on formulated mixtures and emulsifiable concentrates, and data obtained with species non-resident to North America or previously exposed organisms may be used to provide auxiliary information but must not be used in the derivation of criteria.

III. Required Data

A. Certain data should be available to help ensure that each of the major kinds of possible adverse effects receives adequate consideration. An adverse effect is a change in an organism that is harmful to the organism. Exposure means contact with a chemical or physical agent. Results of acute and chronic toxicity tests with representative species of aquatic animals are necessary so that data available for tested species can be considered a useful indication of the sensitivities of appropriate untested species. Fewer data concerning toxicity to aquatic plants are usually available because procedures for conducting tests with plants and interpreting the results of such tests are not as well developed.

B. To derive a Great Lakes Tier I criterion for aquatic organisms and their uses, the following must be available:

1. Results of acceptable acute (or chronic) tests (see section IV or VI of this appendix) with at least one species of freshwater animal in at least eight different families such that all of the following are included:

a. The family Salmonidae in the class Osteichthyes;

b. One other family (preferably a commercially or recreationally important, warmwater species) in the class Osteichthyes (e.g., bluegill, channel catfish);

c. A third family in the phylum Chordata (e.g., fish, amphibian);

d. A planktonic crustacean (e.g., a cladoceran, copepod);

e. A benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish);

f. An insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge);

g. A family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca);

h. A family in any order of insect or any phylum not already represented.

2. Acute-chronic ratios (see section VI of this appendix) with at least one species of aquatic animal in at least three different families provided that of the three species:

a. At least one is a fish;

b. At least one is an invertebrate; and

c. At least one species is an acutely sensitive freshwater species (the other two may be saltwater species).

3. Results of at least one acceptable test with a freshwater algae or vascular plant is desirable but not required for criterion derivation (see section VIII of this appendix). If plants are among the aquatic organisms most sensitive to the material, results of a test with a plant in another phylum (division) should also be available.

C. If all required data are available, a numerical criterion can usually be derived except in special cases. For example, derivation of a chronic criterion might not be possible if the available ACRs vary by more than a factor of ten with no apparent pattern. Also, if a criterion is to be related to a water quality characteristic (see sections V and VII of this appendix), more data will be required.

D. Confidence in a criterion usually increases as the amount of available pertinent information increases. Thus, additional data are usually desirable.

IV. Final Acute Value

A. Appropriate measures of the acute (short-term) toxicity of the material to a variety of species of aquatic animals are used to calculate the Final Acute Value (FAV). The calculated Final Acute Value is a calculated estimate of the concentration of a test material such that 95 percent of the genera (with which acceptable acute toxicity tests have been conducted on the material) have higher Genus Mean Acute Values (GMAVs). An acute test is a comparative study in which organisms, that are subjected to different treatments, are observed for a short period usually not constituting a substantial portion of their life span. However, in some cases, the Species Mean Acute Value (SMAV) of a commercially or recreationally important species of the Great Lakes System is lower than the calculated FAV, then the SMAV replaces the calculated FAV in order to provide protection for that important species.

B. Acute toxicity tests shall be conducted using acceptable procedures. For good examples of acceptable procedures see American Society for Testing and Materials (ASTM) Standard E 729, Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians.

C. Except for results with saltwater annelids and mysids, results of acute tests during which the test organisms were fed should not be used, unless data indicate that the food did not affect the toxicity of the test material. (NOTE: If the minimum acute-chronic ratio data requirements (as described in section III.B.2 of this appendix) are not met with freshwater data alone, saltwater data may be used.)

D. Results of acute tests conducted in unusual dilution water, e.g., dilution water in which total organic carbon or particulate matter exceeded five mg/L, should not be used, unless a relationship is developed between acute toxicity and organic carbon or particulate matter, or unless data show that organic carbon or particulate matter, etc., do not affect toxicity.

E. Acute values must be based upon endpoints which reflect the total severe adverse impact of the test material on the organisms used in the test. Therefore, only the following kinds of data on acute toxicity to aquatic animals shall be used:

1. Tests with daphnids and other cladocerans must be started with organisms less than 24 hours old and tests with midges must be started with second or third instar larvae. It is preferred that the results should be the 48-hour EC50 based on the total percentage of organisms killed and immobilized. If such an EC50 is not available for a test, the 48-hour LC50 should be used in place of the desired 48-hour EC50. An EC50 or LC50 of longer than 48 hours can be used as long as the animals were not fed and the control animals were acceptable at the end of the test. An EC50 is a statistically or graphically estimated concentration that is expected to cause one or more specified effects in 50% of a group of organisms under specified conditions. An LC50 is a statistically or graphically estimated concentration that is expected to be lethal to 50% of a group of organisms under specified conditions.

2. It is preferred that the results of a test with embryos and larvae of barnacles, bivalve molluscs (clams, mussels, oysters and scallops), sea urchins, lobsters, crabs, shrimp and abalones be the 96-hour EC50 based on the percentage of organisms with incompletely developed shells plus the percentage of organisms killed. If such an EC50 is not available from a test, of the values that are available from the test, the lowest of the following should be used in place of the desired 96-hour EC50: 48- to 96-hour EC50s based on percentage of organisms with incompletely developed shells plus percentage of organisms killed, 48- to 96-hour EC50s based upon percentage of organisms with incompletely developed shells, and 48-hour to 96-hour LC50s. (NOTE: If the minimum acute-chronic ratio data requirements (as described in section III.B.2 of this appendix) are not met with freshwater data alone, saltwater data may be used.)

3. It is preferred that the result of tests with all other aquatic animal species and older life stages of barnacles, bivalve molluscs (clams, mussels, oysters and scallops), sea urchins, lobsters, crabs, shrimp and abalones be the 96-hour EC50 based on percentage of organisms exhibiting loss of equilibrium plus percentage of organisms immobilized plus percentage of organisms killed. If such an EC50 is not available from a test, of the values that are available from a test the lower of the following should be used in place of the desired 96-hour EC50: the 96-hour EC50 based on percentage of organisms exhibiting loss of equilibrium plus percentage of organisms immobilized and the 96-hour LC50.

4. Tests whose results take into account the number of young produced, such as most tests with protozoans, are not considered

acute tests, even if the duration was 96 hours or less.

5. If the tests were conducted properly, acute values reported as ''greater than'' values and those which are above the solubility of the test material should be used, because rejection of such acute values would bias the Final Acute Value by eliminating acute values for resistant species.

F. If the acute toxicity of the material to aquatic animals has been shown to be related to a water quality characteristic such as hardness or particulate matter for freshwater animals, refer to section V of this appendix.

G. The agreement of the data within and between species must be considered. Acute values that appear to be questionable in comparison with other acute and chronic data for the same species and for other species in the same genus must not be used. For example, if the acute values available for a species or genus differ by more than a factor of 10, rejection of some or all of the values would be appropriate, absent countervailing circumstances.

H. If the available data indicate that one or more life stages are at least a factor of two more resistant than one or more other life stages of the same species, the data for the more resistant life stages must not be used in the calculation of the SMAV because a species cannot be considered protected from acute toxicity if all of the life stages are not protected.

I. For each species for which at least one acute value is available, the SMAV shall be calculated as the geometric mean of the results of all acceptable flow-through acute toxicity tests in which the concentrations of test material were measured with the most sensitive tested life stage of the species. For a species for which no such result is available, the SMAV shall be calculated as the geometric mean of all acceptable acute toxicity tests with the most sensitive tested life stage, i.e., results of flow-through tests in which the concentrations were not measured and results of static and renewal tests based on initial concentrations (nominal concentrations are acceptable for most test materials if measured concentrations are not available) of test material. A renewal test is a test with aquatic organisms in which either the test solution in a test chamber is removed and replaced at least once during the test or the test organisms are transferred into a new test solution of the same composition at least once during the test. A static test is a test with aquatic organisms in which the solution and organisms that are in a test chamber at the beginning of the test remain in the chamber until the end of the test, except for removal of dead test organisms.

NOTE 1: Data reported by original investigators must not be rounded off. Results of all intermediate calculations must not be rounded off to fewer than four significant digits.

NOTE 2: The geometric mean of N numbers is the Nth root of the product of the N numbers. Alternatively, the geometric mean can be calculated by adding the logarithms of the N numbers, dividing the sum by N, and taking the antilog of the quotient. The geometric mean of two numbers is the square root of the product of the two numbers, and the geometric mean of one number is that number. Either natural (base e) or common (base 10) logarithms can be used to calculate geometric means as long as they are used consistently within each set of data, i.e., the antilog used must match the logarithms used.

NOTE 3: Geometric means, rather than arithmetic means, are used here because the distributions of sensitivities of individual organisms in toxicity tests on most materials and the distributions of sensitivities of species within a genus are more likely to be lognormal than normal. Similarly, geometric means are used for ACRs because quotients are likely to be closer to lognormal than normal distributions. In addition, division of the geometric mean of a set of numerators by the geometric mean of the set of denominators will result in the geometric mean of the set of corresponding quotients.

J. For each genus for which one or more SMAVs are available, the GMAV shall be calculated as the geometric mean of the SMAVs available for the genus.

K. Order the GMAVs from high to low.

L. Assign ranks, R, to the GMAVs from ''1'' for the lowest to ''N'' for the highest. If two or more GMAVs are identical, assign them successive ranks.

M. Calculate the cumulative probability, P, for each GMAV as R/(N+1).

N. Select the four GMAVs which have cumulative probabilities closest to 0.05 (if there are fewer than 59 GMAVs, these will always be the four lowest GMAVs).

O. Using the four selected GMAVs, and Ps, calculate

$$S^2 = \frac{\sum\left((\ln \mathrm{GMAV})^2\right) - \frac{\left(\sum(\ln \mathrm{GMAV})\right)^2}{4}}{\sum(\mathrm{P}) - \frac{\left(\sum\left(\sqrt{\mathrm{P}}\right)\right)^2}{4}}$$

$$L = \frac{\sum(\ln \mathrm{GMAV}) - S\left(\sum\left(\sqrt{\mathrm{P}}\right)\right)}{4}$$

$$A = S\left(\sqrt{0.05}\right) + L$$

$$FAV = e^{A}$$

NOTE: Natural logarithms (logarithms to base e, denoted as ln) are used herein merely because they are easier to use on some hand calculators and computers than common (base 10) logarithms. Consistent use of either will produce the same result.

P. If for a commercially or recreationally important species of the Great Lakes System the geometric mean of the acute values from flow-through tests in which the concentrations of test material were measured is lower than the calculated Final Acute Value (FAV), then that geometric mean must be used as the FAV instead of the calculated FAV.

Q. See section VI of this appendix.

V. Final Acute Equation

A. When enough data are available to show that acute toxicity to two or more species is similarly related to a water quality characteristic, the relationship shall be taken into account as described in sections V.B through V.G of this appendix or using analysis of covariance. The two methods are equivalent and produce identical results. The manual method described below provides an understanding of this application of covariance analysis, but computerized versions of covariance analysis are much more convenient for analyzing large data sets. If two or more factors affect toxicity, multiple regression analysis shall be used.

B. For each species for which comparable acute toxicity values are available at two or more different values of the water quality characteristic, perform a least squares regression of the acute toxicity values on the corresponding values of the water quality characteristic to obtain the slope and its 95 percent confidence limits for each species.

NOTE: Because the best documented relationship is that between hardness and acute toxicity of metals in fresh water and a log-log relationship fits these data, geometric means and natural logarithms of both toxicity and water quality are used in the rest of this section. For relationships based on other water quality characteristics, such as Ph, temperature, no transformation or a different transformation might fit the data better, and appropriate changes will be necessary throughout this section.

C. Decide whether the data for each species are relevant, taking into account the range and number of the tested values of the water quality characteristic and the degree of agreement within and between species. For example, a slope based on six data points might be of limited value if it is based only on data for a very narrow range of values of the water quality characteristic. A slope based on only two data points, however, might be useful if it is consistent with other information and if the two points cover a broad enough range of the water quality characteristic. In addition, acute values that appear to be questionable in comparison with other acute and chronic data available for the same species and for other species in the same genus should not be used. For example, if after adjustment for the water quality characteristic, the acute values available for a species or genus differ by more than a factor of 10, rejection of some or all of the values would be appropriate, absent countervailing justification. If useful slopes are not available for at least one fish and one invertebrate or if the available slopes are too dissimilar or if too few data are available to adequately define the relationship between acute toxicity and the

water quality characteristic, return to section IV.G of this appendix, using the results of tests conducted under conditions and in waters similar to those commonly used for toxicity tests with the species.

D. For each species, calculate the geometric mean of the available acute values and then divide each of the acute values for the species by the geometric mean for the species. This normalizes the acute values so that the geometric mean of the normalized values for each species individually and for any combination of species is 1.0.

E. Similarly normalize the values of the water quality characteristic for each species individually using the same procedure as above.

F. Individually for each species perform a least squares regression of the normalized acute values of the water quality characteristic. The resulting slopes and 95 percent confidence limits will be identical to those obtained in section V.B. of this appendix. If, however, the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

G. Treat all of the normalized data as if they were all for the same species and perform a least squares regression of all of the normalized acute values on the corresponding normalized values of the water quality characteristic to obtain the pooled acute slope, V, and its 95 percent confidence limits. If all of the normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

H. For each species calculate the geometric mean, W, of the acute toxicity values and the geometric mean, X, of the values of the water quality characteristic. (These were calculated in sections V.D and V.E of this appendix).

I. For each species, calculate the logarithm, Y, of the SMAV at a selected value, Z, of the water quality characteristic using the equation:

$$Y=\ln W-V(\ln X-\ln Z)$$

J. For each species calculate the SMAV at X using the equation:

$$SMAV=e^{Y}$$

NOTE: Alternatively, the SMAVs at Z can be obtained by skipping step H above, using the equations in steps I and J to adjust each acute value individually to Z, and then calculating the geometric mean of the adjusted values for each species individually. This alternative procedure allows an examination of the range of the adjusted acute values for each species.

K. Obtain the FAV at Z by using the procedure described in sections IV.J through IV.O of this appendix.

L. If, for a commercially or recreationally important species of the Great Lakes System the geometric mean of the acute values at Z from flow-through tests in which the concentrations of the test material were measured is lower than the FAV at Z, then the geometric mean must be used as the FAV instead of the FAV.

M. The Final Acute Equation is written as:

$$FAV=e^{(V[\ln(waterqualitycharacteristic)]=A-V[\ln Z])},$$

where:

V=pooled acute slope, and A=ln(FAV at Z).

Because V, A, and Z are known, the FAV can be calculated for any selected value of the water quality characteristic.

VI. Final Chronic Value

A. Depending on the data that are available concerning chronic toxicity to aquatic animals, the Final Chronic Value (FCV) can be calculated in the same manner as the FAV or by dividing the FAV by the Final Acute-Chronic Ratio (FACR). In some cases, it might not be possible to calculate a FCV. The FCV is (a) a calculated estimate of the concentration of a test material such that 95 percent of the genera (with which acceptable chronic toxicity tests have been conducted on the material) have higher GMCVs, or (b) the quotient of an FAV divided by an appropriate ACR, or (c) the SMCV of an important and/or critical species, if the SMCV is lower than the calculated estimate or the quotient, whichever is applicable.

NOTE: As the name implies, the ACR is a way of relating acute and chronic toxicities.

B. Chronic values shall be based on results of flow-through (except renewal is acceptable for daphnids) chronic tests in which the concentrations of test material in the test solutions were properly measured at appropriate times during the test. A chronic test is a comparative study in which organisms, that are subjected to different treatments, are observed for a long period or a substantial portion of their life span.

C. Results of chronic tests in which survival, growth, or reproduction in the control treatment was unacceptably low shall not be used. The limits of acceptability will depend on the species.

D. Results of chronic tests conducted in unusual dilution water, e.g., dilution water in which total organic carbon or particulate matter exceeded five mg/L, should not be

used, unless a relationship is developed between chronic toxicity and organic carbon or particulate matter, or unless data show that organic carbon, particulate matter, etc., do not affect toxicity.

E. Chronic values must be based on endpoints and lengths of exposure appropriate to the species. Therefore, only results of the following kinds of chronic toxicity tests shall be used:

1. Life-cycle toxicity tests consisting of exposures of each of two or more groups of individuals of a species to a different concentration of the test material throughout a life cycle. To ensure that all life stages and life processes are exposed, tests with fish should begin with embryos or newly hatched young less than 48 hours old, continue through maturation and reproduction, and should end not less than 24 days (90 days for salmonids) after the hatching of the next generation. Tests with daphnids should begin with young less than 24 hours old and last for not less than 21 days, and for ceriodaphnids not less than seven days. For good examples of acceptable procedures see American Society for Testing and Materials (ASTM) Standard E 1193 Guide for conducting renewal life-cycle toxicity tests with *Daphnia magna* and ASTM Standard E 1295 Guide for conducting three-brood, renewal toxicity tests with *Ceriodaphnia dubia*. Tests with mysids should begin with young less than 24 hours old and continue until seven days past the median time of first brood release in the controls. For fish, data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability. For daphnids, data should be obtained and analyzed on survival and young per female. For mysids, data should be obtained and analyzed on survival, growth, and young per female.

2. Partial life-cycle toxicity tests consist of exposures of each of two more groups of individuals of a species of fish to a different concentration of the test material through most portions of a life cycle. Partial life-cycle tests are allowed with fish species that require more than a year to reach sexual maturity, so that all major life stages can be exposed to the test material in less than 15 months. A life-cycle test is a comparative study in which organisms, that are subjected to different treatments, are observed at least from a life stage in one generation to the same life-stage in the next generation. Exposure to the test material should begin with immature juveniles at least two months prior to active gonad development, continue through maturation and reproduction, and end not less than 24 days (90 days for salmonids) after the hatching of the next generation. Data should be obtained and analyzed on survival and growth of adults and young, maturation of males and females, eggs spawned per female, embryo viability (salmonids only), and hatchability.

3. Early life-stage toxicity tests consisting of 28- to 32-day (60 days post hatch for salmonids) exposures of the early life stages of a species of fish from shortly after fertilization through embryonic, larval, and early juvenile development. Data should be obtained and analyzed on survival and growth.

NOTE: Results of an early life-stage test are used as predictions of results of life-cycle and partial life-cycle tests with the same species. Therefore, when results of a life-cycle or partial life-cycle test are available, results of an early life-stage test with the same species should not be used. Also, results of early life-stage tests in which the incidence of mortalities or abnormalities increased substantially near the end of the test shall not be used because the results of such tests are possibly not good predictions of comparable life-cycle or partial life-cycle tests.

F. A chronic value may be obtained by calculating the geometric mean of the lower and upper chronic limits from a chronic test or by analyzing chronic data using regression analysis.

1. A lower chronic limit is the highest tested concentration:

a. In an acceptable chronic test;

b. Which did not cause an unacceptable amount of adverse effect on any of the specified biological measurements; and

c. Below which no tested concentration caused an unacceptable effect.

2. An upper chronic limit is the lowest tested concentration:

a. In an acceptable chronic test;

b. Which did cause an unacceptable amount of adverse effect on one or more of the specified biological measurements; and,

c. Above which all tested concentrations also caused such an effect.

NOTE: Because various authors have used a variety of terms and definitions to interpret and report results of chronic tests, reported results should be reviewed carefully. The amount of effect that is considered unacceptable is often based on a statistical hypothesis test, but might also be defined in terms of a specified percent reduction from the controls. A small percent reduction (e.g., three percent) might be considered acceptable even if it is statistically significantly different from the control, whereas a large percent reduction (e.g., 30 percent) might be considered unacceptable even if it is not statistically significant.

G. If the chronic toxicity of the material to aquatic animals has been shown to be related to a water quality characteristic such as hardness or particulate matter for freshwater animals, refer to section VII of this appendix.

H. If chronic values are available for species in eight families as described in section III.B.1 of this appendix, a SMCV shall be calculated for each species for which at least one chronic value is available by calculating the geometric mean of the results of all acceptable life-cycle and partial life-cycle toxicity tests with the species; for a species of fish for which no such result is available, the SMCV is the geometric mean of all acceptable early life-stage tests. Appropriate GMCVs shall also be calculated. A GMCV is the geometric mean of the SMCVs for the genus. The FCV shall be obtained using the procedure described in sections IV.J through IV.O of this appendix, substituting SMCV and GMCV for SMAV and GMAV respectively. See section VI.M of this appendix.

NOTE: Section VI.I through VI.L are for use when chronic values are not available for species in eight taxonomic families as described in section III.B.1 of this appendix.

I. For each chronic value for which at least one corresponding appropriate acute value is available, calculate an ACR, using for the numerator the geometric mean of the results of all acceptable flow-through (except static is acceptable for daphnids and midges) acute tests in the same dilution water in which the concentrations are measured. For fish, the acute test(s) should be conducted with juveniles. The acute test(s) should be part of the same study as the chronic test. If acute tests were not conducted as part of the same study, but were conducted as part of a different study in the same laboratory and dilution water, then they may be used. If no such acute tests are available, results of acute tests conducted in the same dilution water in a different laboratory may be used. If no such acute tests are available, an ACR shall not be calculated.

J. For each species, calculate the SMACR as the geometric mean of all ACRs available for that species. If the minimum ACR data requirements (as described in section III.B.2 of this appendix) are not met with freshwater data alone, saltwater data may be used along with the freshwater data.

K. For some materials, the ACR seems to be the same for all species, but for other materials the ratio seems to increase or decrease as the SMAV increases. Thus the FACR can be obtained in three ways, depending on the data available:

1. If the species mean ACR seems to increase or decrease as the SMAVs increase, the FACR shall be calculated as the geometric mean of the ACRs for species whose SMAVs are close to the FAV.

2. If no major trend is apparent and the ACRs for all species are within a factor of ten, the FACR shall be calculated as the geometric mean of all of the SMACRs.

3. If the most appropriate SMACRs are less than 2.0, and especially if they are less than 1.0, acclimation has probably occurred during the chronic test. In this situation, because continuous exposure and acclimation cannot be assured to provide adequate protection in field situations, the FACR should be assumed to be two, so that the FCV is equal to the Criterion Maximum Concentration (CMC). (See section X.B of this appendix.)

If the available SMACRs do not fit one of these cases, a FACR may not be obtained and a Tier I FCV probably cannot be calculated.

L. Calculate the FCV by dividing the FAV by the FACR.

FCV=FAV÷FACR

If there is a Final Acute Equation rather than a FAV, see also section V of this appendix.

M. If the SMCV of a commercially or recreationally important species of the Great Lakes System is lower than the calculated FCV, then that SMCV must be used as the FCV instead of the calculated FCV.

N. See section VIII of this appendix.

VII. Final Chronic Equation

A. A Final Chronic Equation can be derived in two ways. The procedure described in section VII.A of this appendix will result in the chronic slope being the same as the acute slope. The procedure described in sections VII.B through N of this appendix will usually result in the chronic slope being different from the acute slope.

1. If ACRs are available for enough species at enough values of the water quality characteristic to indicate that the ACR appears to be the same for all species and appears to be independent of the water quality characteristic, calculate the FACR as the geometric mean of the available SMACRs.

2. Calculate the FCV at the selected value Z of the water quality characteristic by dividing the FAV at Z (see section V.M of this appendix) by the FACR.

3. Use V=pooled acute slope (see section V.M of this appendix), and

L=pooled chronic slope.

4. See section VII.M of this appendix.

B. When enough data are available to show that chronic toxicity to at least one species is related to a water quality characteristic, the relationship should be taken into account as described in sections C through G below or using analysis of covariance. The two methods are equivalent and produce identical results. The manual method described below provides an understanding of this application of covariance analysis, but computerized versions of covariance analysis are much more convenient for analyzing large data sets. If two or more factors affect toxicity, multiple regression analysis shall be used.

C. For each species for which comparable chronic toxicity values are available at two or more different values of the water quality

characteristic, perform a least squares regression of the chronic toxicity values on the corresponding values of the water quality characteristic to obtain the slope and its 95 percent confidence limits for each species.

NOTE: Because the best documented relationship is that between hardness and acute toxicity of metals in fresh water and a log-log relationship fits these data, geometric means and natural logarithms of both toxicity and water quality are used in the rest of this section. For relationships based on other water quality characteristics, such as Ph, temperature, no transformation or a different transformation might fit the data better, and appropriate changes will be necessary throughout this section. It is probably preferable, but not necessary, to use the same transformation that was used with the acute values in section V of this appendix.

D. Decide whether the data for each species are relevant, taking into account the range and number of the tested values of the water quality characteristic and the degree of agreement within and between species. For example, a slope based on six data points might be of limited value if it is based only on data for a very narrow range of values of the water quality characteristic. A slope based on only two data points, however, might be more useful if it is consistent with other information and if the two points cover a broad range of the water quality characteristic. In addition, chronic values that appear to be questionable in comparison with other acute and chronic data available for the same species and for other species in the same genus in most cases should not be used. For example, if after adjustment for the water quality characteristic, the chronic values available for a species or genus differ by more than a factor of 10, rejection of some or all of the values is, in most cases, absent countervailing circumstances, appropriate. If a useful chronic slope is not available for at least one species or if the available slopes are too dissimilar or if too few data are available to adequately define the relationship between chronic toxicity and the water quality characteristic, it might be appropriate to assume that the chronic slope is the same as the acute slope, which is equivalent to assuming that the ACR is independent of the water quality characteristic. Alternatively, return to section VI.H of this appendix, using the results of tests conducted under conditions and in waters similar to those commonly used for toxicity tests with the species.

E. Individually for each species, calculate the geometric mean of the available chronic values and then divide each chronic value for a species by the mean for the species. This normalizes the chronic values so that the geometric mean of the normalized values for each species individually, and for any combination of species, is 1.0.

F. Similarly, normalize the values of the water quality characteristic for each species individually.

G. Individually for each species, perform a least squares regression of the normalized chronic toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and the 95 percent confidence limits will be identical to those obtained in section VII.B of this appendix. Now, however, if the data are actually plotted, the line of best fit for each individual species will go through the point 1,1 in the center of the graph.

H. Treat all of the normalized data as if they were all the same species and perform a least squares regression of all of the normalized chronic values on the corresponding normalized values of the water quality characteristic to obtain the pooled chronic slope, L, and its 95 percent confidence limits.

If all normalized data are actually plotted, the line of best fit will go through the point 1,1 in the center of the graph.

I. For each species, calculate the geometric mean, M, of the toxicity values and the geometric mean, P, of the values of the water quality characteristic. (These are calculated in sections VII.E and F of this appendix.)

J. For each species, calculate the logarithm, Q, of the SMCV at a selected value, Z, of the water quality characteristic using the equation:

$$Q=\ln M—L(\ln P-\ln Z)$$

NOTE: Although it is not necessary, it is recommended that the same value of the water quality characteristic be used here as was used in section V of this appendix.

K. For each species, calculate a SMCV at Z using the equation:

$$SMCV=e^{Q}$$

NOTE: Alternatively, the SMCV at Z can be obtained by skipping section VII.J of this appendix, using the equations in sections VII.J and K of this appendix to adjust each chronic value individually to Z, and then calculating the geometric means of the adjusted values for each species individually. This alternative procedure allows an examination of the range of the adjusted chronic values for each species.

L. Obtain the FCV at Z by using the procedure described in sections IV.J through O of this appendix.

M. If the SMCV at Z of a commercially or recreationally important species of the Great Lakes System is lower than the calculated FCV at Z, then that SMCV shall be used as the FCV at Z instead of the calculated FCV.

N. The Final Chronic Equation is written as:

$$FCV=e^{(L\&[\ln(\text{waterqualitycharacteristic})]=\ln S-L[\ln Z])}$$

Where:

L=pooled chronic slope and S = FCV at Z.

Because L, S, and Z are known, the FCV can be calculated for any selected value of the water quality characteristic.

VIII. Final Plant Value

A. A Final Plant Value (FPV) is the lowest plant value that was obtained with an important aquatic plant species in an acceptable toxicity test for which the concentrations of the test material were measured and the adverse effect was biologically important. Appropriate measures of the toxicity of the material to aquatic plants are used to compare the relative sensitivities of aquatic plants and animals. Although procedures for conducting and interpreting the results of toxicity tests with plants are not well-developed, results of tests with plants usually indicate that criteria which adequately protect aquatic animals and their uses will, in most cases, also protect aquatic plants and their uses.

B. A plant value is the result of a 96-hour test conducted with an alga or a chronic test conducted with an aquatic vascular plant.

NOTE: A test of the toxicity of a metal to a plant shall not be used if the medium contained an excessive amount of a complexing agent, such as EDTA, that might affect the toxicity of the metal. Concentrations of EDTA above 200 µg/L should be considered excessive.

C. The FPV shall be obtained by selecting the lowest result from a test with an important aquatic plant species in which the concentrations of test material are measured and the endpoint is biologically important.

IX. Other Data

Pertinent information that could not be used in earlier sections might be available concerning adverse effects on aquatic organisms. The most important of these are data on cumulative and delayed toxicity, reduction in survival, growth, or reproduction, or any other adverse effect that has been shown to be biologically important. Delayed toxicity is an adverse effect to an organism that results from, and occurs after the end of, its exposure to one or more test materials. Especially important are data for species for which no other data are available. Data from behavioral, biochemical, physiological, microcosm, and field studies might also be available. Data might be available from tests conducted in unusual dilution water (see sections IV.D and VI.D of this appendix), from chronic tests in which the concentrations were not measured (see section VI.B of this appendix), from tests with previously exposed organisms (see section II.F.3 of this appendix), and from tests on formulated mixtures or emulsifiable concentrates (see section II.D of this appendix). Such data might affect a criterion if the data were obtained with an important species, the test concentrations were measured, and the endpoint was biologically important.

X. Criterion

A. A criterion consists of two concentrations: the CMC and the Criterion Continuous Concentration (CCC).

B. The CMC is equal to one-half the FAV. The CMC is an estimate of the highest concentration of a material in the water column to which an aquatic community can be exposed briefly without resulting in an unacceptable effect.

C. The CCC is equal to the lowest of the FCV or the FPV (if available) unless other data (see section IX of this appendix) show that a lower value should be used. The CCC is an estimate of the highest concentration of a material in the water column to which an aquatic community can be exposed indefinitely without resulting in an unacceptable effect. If toxicity is related to a water quality characteristic, the CCC is obtained from the Final Chronic Equation or FPV (if available) that results in the lowest concentrations in the usual range of the water quality characteristic, unless other data (see section IX) show that a lower value should be used.

D. Round both the CMC and the CCC to two significant digits.

E. The criterion is stated as:

The procedures described in the Tier I methodology indicate that, except possibly where a commercially or recreationally important species is very sensitive, aquatic organisms should not be affected unacceptably if the four-day average concentration of (1) does not exceed (2) µg/L more than once every three years on the average and if the one-hour average concentration does not exceed (3) µg/L more than once every three years on the average.

Where:

(1) = insert name of material
(2) = insert the CCC
(3) = insert the CMC

If the CMC averaging period of one hour or the CCC averaging period of four days is inappropriate for the pollutant, or if the once-in-three-year allowable excursion frequency is inappropriate for the pollutant or for the sites to which a criterion is applied, then the State may specify alternative averaging periods or frequencies. The choice of an alternative averaging period or frequency shall be justified by a scientifically defensible analysis demonstrating that the alternative values will protect the aquatic life uses of the water. Appropriate laboratory data and/or well-designed field biological surveys shall be submitted to EPA as justification for differing averaging periods and/or frequencies of exceedance.

XI. Final Review

A. The derivation of the criterion should be carefully reviewed by rechecking each step of the Guidance in this part. Items that should be especially checked are:

1. If unpublished data are used, are they well documented?
2. Are all required data available?
3. Is the range of acute values for any species greater than a factor of 10?
4. Is the range of SMAVs for any genus greater than a factor of 10?
5. Is there more than a factor of 10 difference between the four lowest GMAVs?
6. Are any of the lowest GMAVs questionable?
7. Is the FAV reasonable in comparison with the SMAVs and GMAVs?
8. For any commercially or recreationally important species of the Great Lakes System, is the geometric mean of the acute values from flow-through tests in which the concentrations of test material were measured lower than the FAV?
9. Are any of the chronic values used questionable?
10. Are any chronic values available for acutely sensitive species?
11. Is the range of acute-chronic ratios greater than a factor of 10?
12. Is the FCV reasonable in comparison with the available acute and chronic data?
13. Is the measured or predicted chronic value for any commercially or recreationally important species of the Great Lakes System below the FCV?
14. Are any of the other data important?
15. Do any data look like they might be outliers?
16. Are there any deviations from the Guidance in this part? Are they acceptable?

B. On the basis of all available pertinent laboratory and field information, determine if the criterion is consistent with sound scientific evidence. If it is not, another criterion, either higher or lower, shall be derived consistent with the Guidance in this part.

METHODOLOGY FOR DERIVING AQUATIC LIFE VALUES: TIER II

XII. Secondary Acute Value

If all eight minimum data requirements for calculating an FAV using Tier I are not met, a Secondary Acute Value (SAV) for the waters of the Great Lakes System shall be calculated for a chemical as follows:

To calculate a SAV, the lowest GMAV in the database is divided by the Secondary Acute Factor (SAF) (Table A–1 of this appendix) corresponding to the number of satisfied minimum data requirements listed in the Tier I methodology (section III.B.1 of this appendix). (Requirements for definitions, data collection and data review, contained in sections I, II, and IV shall be applied to calculation of a SAV.) If all eight minimum data requirements are satisfied, a Tier I criterion calculation may be possible. In order to calculate a SAV, the database must contain, at a minimum, a genus mean acute value (GMAV) for one of the following three genera in the family Daphnidae—*Ceriodaphnia sp.*, *Daphnia sp.*, or *Simocephalus sp.*

If appropriate, the SAV shall be made a function of a water quality characteristic in a manner similar to that described in Tier I.

XIII. Secondary Acute-Chronic Ratio

If three or more experimentally determined ACRs, meeting the data collection and review requirements of Section VI of this appendix, are available for the chemical, determine the FACR using the procedure described in Section VI. If fewer than three acceptable experimentally determined ACRs are available, use enough assumed ACRs of 18 so that the total number of ACRs equals three. Calculate the Secondary Acute-Chronic Ratio (SACR) as the geometric mean of the three ACRs. Thus, if no experimentally determined ACRs are available, the SACR is 18.

XIV. Secondary Chronic Value

Calculate the Secondary Chronic Value (SCV) using one of the following:

$$\text{A. } SCV = \frac{FAV}{SACR} \left(\text{use FAV from Tier I}\right)$$

$$\text{B. } SCV = \frac{SAV}{FACR}$$

$$\text{C. } SCV = \frac{SAV}{SACR}$$

If appropriate, the SCV will be made a function of a water quality characteristic in a manner similar to that described in Tier I.

XV. Commercially or Recreationally Important Species

If for a commercially or recreationally important species of the Great Lakes System the geometric mean of the acute values or chronic values from flow-through tests in which the concentrations of the test materials were measured is lower than the calculated SAV or SCV, then that geometric mean must be used as the SAV or SCV instead of the calculated SAV or SCV.

XVI. Tier II Value

A. A Tier II value shall consist of two concentrations: the Secondary Maximum Concentration (SMC) and the Secondary Continuous Concentration (SCC).

B. The SMC is equal to one-half of the SAV.

C. The SCC is equal to the lowest of the SCV or the Final Plant Value, if available, unless other data (see section IX of this appendix) show that a lower value should be used.

If toxicity is related to a water quality characteristic, the SCC is obtained from the Secondary Chronic Equation or FPV, if available, that results in the lowest concentrations in the usual range of the water quality characteristic, unless other data (See section IX of this appendix) show that a lower value should be used.

D. Round both the SMC and the SCC to two significant digits.

E. The Tier II value is stated as:

The procedures described in the Tier II methodology indicate that, except possibly where a locally important species is very sensitive, aquatic organisms should not be affected unacceptably if the four-day average concentration of (1) does not exceed (2) μg/L more than once every three years on the average and if the one-hour average concentration does not exceed (3) μg/L more than once every three years on the average.

Where:

(1) = insert name of material
(2) = insert the SCC
(3) = insert the SMC

As discussed above, States and Tribes have the discretion to specify alternative averaging periods or frequencies (see section X.E. of this appendix).

XVII. Appropriate Modifications

On the basis of all available pertinent laboratory and field information, determine if the Tier II value is consistent with sound scientific evidence. If it is not, another value, either higher or lower, shall be derived consistent with the Guidance in this part.

TABLE A–1—SECONDARY ACUTE FACTORS

Number of minimum data requirements satisfied	Adjustment factor
1	21.9
2	13.0
3	8.0
4	7.0
5	6.1
6	5.2
7	4.3

APPENDIX B TO PART 132—GREAT LAKES WATER QUALITY INITIATIVE

METHODOLOGY FOR DERIVING BIOACCUMULATION FACTORS

Great Lakes States and Tribes shall adopt provisions consistent with (as protective as) this appendix.

I. Introduction

A. The purpose of this methodology is to describe procedures for deriving bioaccumulation factors (BAFs) to be used in the calculation of Great Lakes Water Quality Guidance (Guidance) human health Tier I criteria and Tier II values and wildlife Tier I criteria. A subset of the human health BAFs are also used to identify the chemicals that are considered bioaccumulative chemicals of concern (BCCs).

B. Bioaccumulation reflects uptake of a substance by aquatic organisms exposed to the substance through all routes (i.e., ambient water and food), as would occur in nature. Bioconcentration reflects uptake of a substance by aquatic organisms exposed to the substance only through the ambient water. Both BAFs and bioconcentration factors (BCFs) are proportionality constants that describe the relationship between the concentration of a substance in aquatic organisms and its concentration in the ambient water. For the Guidance in this part, BAFs, rather than BCFs, are used to calculate Tier I criteria for human health and wildlife and Tier II values for human health because they better account for the total exposure of aquatic organisms to chemicals.

C. For organic chemicals, baseline BAFs can be derived using four methods. Measured baseline BAFs are derived from field-measured BAFs; predicted baseline BAFs are derived using biota-sediment accumulation factors (BSAFs) or are derived by multiplying a laboratory-measured or predicted BCF by a food-chain multiplier (FCM). The lipid content of the aquatic organisms is used to account for partitioning of organic chemicals within organisms so that data from different tissues and species can be integrated. In addition, the baseline BAF is based on the concentration of freely dissolved organic chemicals in the ambient water to facilitate extrapolation from one water to another.

D. For inorganic chemicals, baseline BAFs can be derived using two of the four methods. Baseline BAFs are derived using either field-measured BAFs or by multiplying laboratory-measured BCFs by a FCM. For inorganic chemicals, BAFs are assumed to equal BCFs (i.e., the FCM is 1.0), unless chemical-specific biomagnification data support using a FCM other than 1.0.

E. Because both humans and wildlife consume fish from both trophic levels 3 and 4,