

**Guidelines for Deriving
Numerical National Water Quality Criteria
for the Protection Of Aquatic Organisms
and Their Uses**

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Special Note

This December 2010 electronic version of the 1985 Guidelines serves to meet the requirements of Section 508 of the Rehabilitation Act. While converting the 1985 Guidelines to a 508-compliant version, EPA updated the taxonomic nomenclature in the tables of Appendix 1 to reflect changes that occurred since the table were originally produced in 1985. The numbers included for Phylum, Class and Family represent those currently in use from the Integrated Taxonomic Information System, or ITIS, and reflect what is referred to in ITIS as Taxonomic Serial Numbers. ITIS replaced the National Oceanographic Data Center (NODC) taxonomic coding system which was used to create the original taxonomic tables included in the 1985 Guidelines document (NODC, Third Addition - see Introduction). For more information on the NODC taxonomic codes, see <http://www.nodc.noaa.gov/General/CDR-detdesc/taxonomic-v8.html>.

The code numbers included in the reference column of the tables have not been updated from the 1985 version. These code numbers are associated with the old NODC taxonomic referencing system and are simply replicated here for historical purposes. Footnotes may or may not still apply.

EPA is working on a more comprehensive update to the 1985 Guidelines, including new taxonomic tables which better reflect the large number of aquatic animal species known to be propagating in U.S. waters.

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Executive Summary

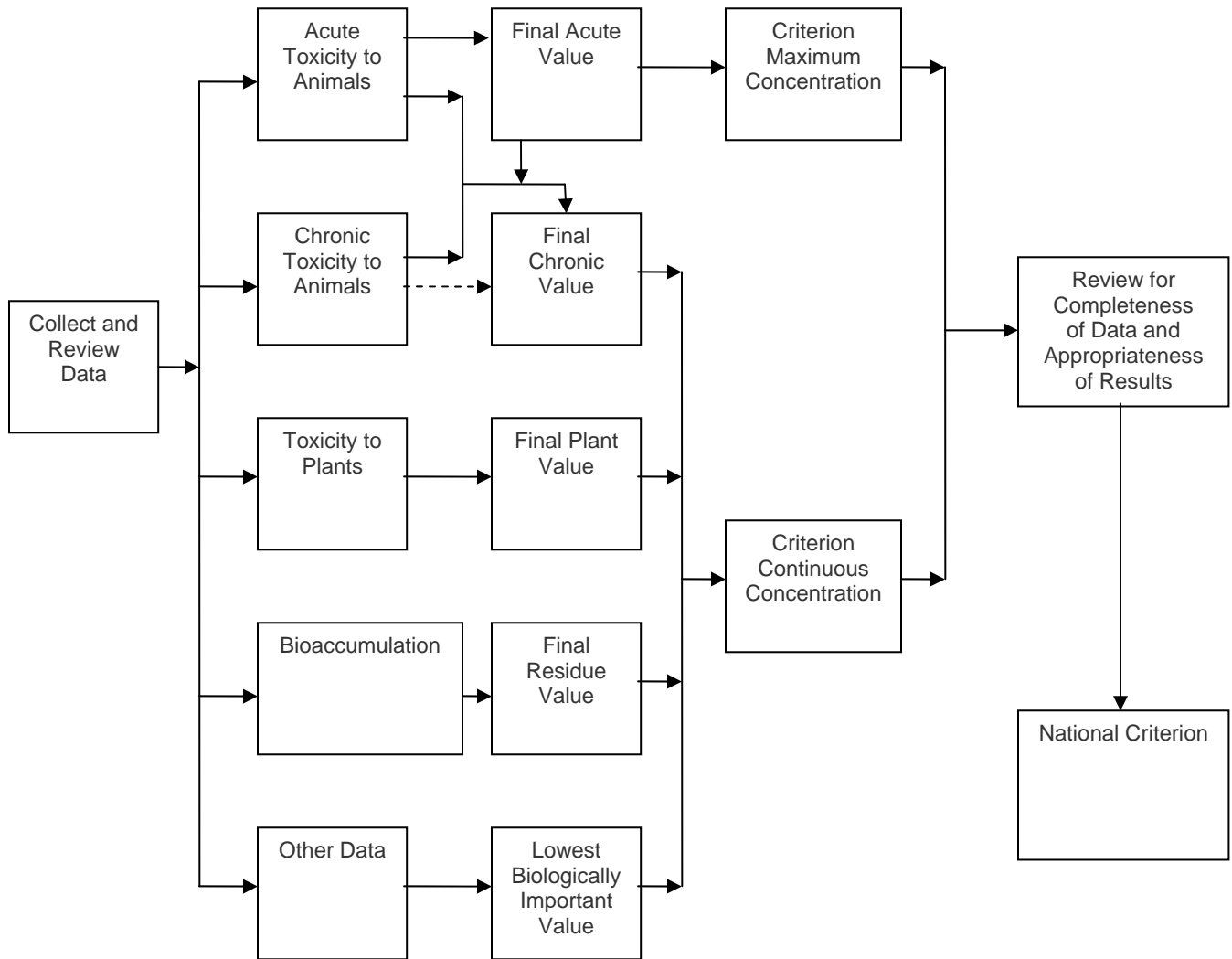
Derivation of numerical national water quality criteria for the protection of aquatic organism and their uses is a complex process (Figure 1) that uses information from many areas of aquatic toxicology. After a decision is made that a national criterion is needed for a particular material, all available information concerning toxicity to, and bioaccumulation by, aquatic organisms is collected, reviewed for acceptability, and sorted. If enough acceptable data on acute toxicity to aquatic animals are available, they are used to estimate the highest one-hour average concentration that should not result in unacceptable effects on aquatic organisms and their uses. If justified, this concentration is made a function of a water quality characteristic such as pH, salinity, or hardness. Similarly, data on the chronic toxicity of the material to aquatic animals are used to estimate the highest four-daily average concentration that should not cause unacceptable toxicity during a long-term exposure. If appropriate, this concentration is also related to a water quality characteristic.

Data on toxicity to aquatic plants are examined to determine whether plants are likely to be unacceptably affected by concentrations that should not cause unacceptable effects on animals. Data on bioaccumulation by aquatic organisms are used to determine if residues might subject edible species to restrictions by the U.S. Food and Drug Administration or if such residues might harm some wildlife consumers of aquatic life. All other available data are examined for adverse effects that might be biologically important.

If a thorough review of the pertinent information indicates that enough acceptable data are available, numerical national water quality criteria are derived for fresh water or salt water or both to protect aquatic organisms and their uses from unacceptable effects due to exposures to high concentrations for short periods of time, lower concentrations for longer periods of time, and combinations of the two.

Figure 1

Derivation of Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses



Introduction

Of the several possible forms of criteria, the numerical form is the most common, but the narrative (e.g., pollutants must not be present in harmful concentrations) and operational (e.g., concentrations of pollutants must not exceed one-tenth of the 96-hr LC50) forms can be used if numerical criteria are not possible or desirable. If it were feasible, a freshwater (or saltwater) numerical aquatic life national criterion* for a material should be determined by conducting field tests on a wide variety of unpolluted bodies of fresh (or salt) water. It would be necessary to add various amounts of the material to each body of water in order to determine the highest concentration that would not cause any unacceptable long-term or short-term effect on the aquatic organisms or their uses. The lowest of these highest concentrations would become the freshwater (or saltwater) national aquatic life water quality criterion for that material, unless one or more of the lowest concentrations were judged to be outliers. Because it is not feasible to determine national criteria by conducting such field tests, these Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses (hereafter referred to as the National Guidelines) describe an objective, internally consistent, appropriate, and feasible way of deriving national criteria, which are intended to provide the same level of protection as the infeasible field testing approach described above.

Because aquatic ecosystems can tolerate some stress and occasional adverse effects, protection of all species at all times and places is not deemed necessary. If acceptable data are available for a large number of appropriate taxa from an appropriate variety of taxonomic and functional groups, a reasonable level of protection will probably be provided if all except a small fraction of the taxa are protected, unless a commercially or recreationally important species is very sensitive. The small fraction is set at 0.05 because other fractions resulted in criteria that seemed too high or too low in comparison with the sets of data from which they were calculated. Use of 0.05 to calculate a Final Acute Value does not imply that this percentage of adversely affected taxa should be used to decide in a field situation whether a criterion is too high or too low or just right.

Determining the validity of a criterion derived for a particular body of water, possibly by modification of a national criterion to reflect local conditions^{1, 2, 3}, should be based on an operation definition of "protection of aquatic organisms and their uses" that takes into account the practicalities of field monitoring programs and the concerns of the public. Monitoring programs should contain sampling points at enough times and places that all unacceptable changes, whether caused directly or indirectly, will be detected. The programs should adequately monitor the kinds of species of concern to the public, i.e., fish in fresh water and fish and macroinvertebrates in salt water. If the kinds of species of concern cannot be adequately monitored at a reasonable cost, appropriate surrogate species should be monitored. The kinds of species most likely to be good surrogates are those that either (a) are a major food of the desired kinds of species or (b) utilize the same food as the desired species or (c) both. Even if a major adverse effect on appropriate surrogate species does not directly result in an unacceptable effect on the kinds of species of concern to the public, it indicates a high probability that such an effect will occur.

* The term "national criteria" is used herein because it is more descriptive than the synonymous term "section 304(a) criteria", which is used in the Water Quality Standards Regulation [1].

To be acceptable to the public and useful in field situations, protection of aquatic organisms and their uses should be defined as prevention of unacceptable long-term short-term effects on (1) commercially, recreationally, and other important species and (2) (a) fish and benthic invertebrate assemblages in rivers and streams, and (b) fish, benthic invertebrate, and zooplankton assemblages in lakes, reservoirs, estuaries, and oceans. Monitoring programs intended to be able to detect unacceptable effects should be tailored to the body of water of concern so that necessary samples are obtained at enough times and places to provide adequate data on the populations of the important species, as well as data directly related to the reasons for their being considered important. For example, for substances that are residue limited, species that are consumed should be monitored for contaminants to ensure that wildlife predators are protected, FDA action levels are not exceeded, and flavor is not impaired. Monitoring programs should also provide data on the number of taxa and number of individuals in the above-named assemblages that can be sampled at reasonable cost. The amount of decrease in the number of taxa or number of individuals in an assemblage that should be considered unacceptable should take into account appropriate features of the body of water and its aquatic community. Because most monitoring programs can only detect decreases of more than 20 percent, any statistically significant decrease should usually be considered unacceptable. The insensitivity of most monitoring programs greatly limits their usefulness for studying the validity of criteria because unacceptable changes can occur and not be detected. Therefore, although limited field studies can sometimes demonstrate that criteria are underprotective, only high quality field studies can reliably demonstrate that criteria are not underprotective.

If the purpose of water quality criteria were to protect only commercially and recreationally important species, criteria specifically derived to protect such species and their uses from the direct adverse effects of a material would probably, in most situations, also protect those species from indirect adverse effects due to effects of the material on other species in the ecosystem. For example, in most situations either the food chain would be more resistant than the important species and their uses or the important species and their food chains would be adaptable enough to overcome effects of the material on portions of the food chains.

These National Guidelines have been developed on the theory that effects which occur on a species in appropriate laboratory tests will generally occur on the same species in comparable field situations. All North American bodies of water and resident aquatic species and their uses are meant to be taken into account, except for a few that may be too atypical, such as the Great Salt Lake, brine shrimp, and the siscowet subspecies of lake trout, which occurs in Lake Superior and contains up to 67% fat in the fillets⁴. Derivation of criteria specifically for the Great Salt Lake or Lake Superior might have to take brine shrimp and siscowet, respectively, into account.

Numerical aquatic life criteria derived using these National Guidelines are expressed as two numbers, rather than the traditional one number, so that the criteria more accurately reflect toxicological and practical realities. If properly derived and used, the combination of a maximum concentration and a continuous concentration should provide an appropriate degree of protection of aquatic organisms and their uses from acute and chronic toxicity to animals, toxicity to plants, and bioaccumulation by aquatic organisms, without being as restrictive as a one-number criterion would have to be in order to provide the same degree of protection.

Criteria produced by these Guidelines are intended to be useful for developing water quality standards, mixing zone standards, effluent limitations, etc. The development of such standards

and limitations, however, might have to take into account such additional factors as social, legal, economic, and hydrological considerations, the environmental and analytical chemistry of the material, the extrapolation from laboratory data to field situations, and relationships between species for which data are available and species in the body of water of concern. As an intermediate step in the development of standards, it might be desirable to derive site-specific criteria by modification of national criteria to reflect such local conditions as water quality, temperature, or ecologically important species^{1,2,3}. In addition, with appropriate modifications these National Guidelines can be used to derive criteria for any specific geographical area, body of water (such as the Great Salt Lake), or group of similar bodies of water, if adequate information is available concerning the effects of the material of concern on appropriate species and their uses.

Criteria should attempt to provide a reasonable and adequate amount of protection with only a small possibility of considerable overprotection or underprotection. It is not enough that a national criterion be the best estimate that can be obtained using available data; it is equally important that a criterion be derived only if adequate appropriate data are available to provide reasonable confidence that it is a good estimate. Therefore, these National Guidelines specify certain data that should be available if a numerical criterion is to be derived. If all the required data are not available, usually a criterion should not be derived. On the other hand, the availability of all required data does not ensure that a criterion can be derived.

A common belief is that national criteria are based on "worst case" assumptions and that local considerations will raise, but not lower, criteria. For example, it will usually be assumed that if the concentration of a material in a body of water is lower than the national criterion, no unacceptable effects will occur and no site-specific criterion needs to be derived. If, however, the concentration of a material in a body of water is higher than the national criterion, it will usually be assumed that a site-specific criterion should be derived. In order to prevent the assumption of the "worst case" nature of national criteria from resulting in the underprotection of too many bodies of water, national criteria must be intended to protect all or almost all bodies of water. Thus, if bodies of water and the aquatic communities in them do differ substantially in their sensitivities to a material, national criteria should be at least somewhat overprotective for a majority of the bodies of water. To do otherwise would either (a) require derivation of site-specific criteria even if the site-specific concentration were substantially below the national criterion or (b) cause the "worst case" assumption to result in the underprotection of numerous bodies of water. On the other hand, national criteria are probably underprotective of some bodies of water.

The two factors that will probably cause the most difference between national and site-specific criteria are the species that will be exposed and the characteristics of the water. In order to ensure that national criteria are appropriately protective, the required data for national criteria include some species that are sensitive to many materials and national criteria are specifically based on tests conducted in water relatively low in particulate matter and organic matter. Thus, the two factors that will usually be considered in the derivation of site-specific criteria from national criteria are used to help ensure that national criteria are appropriately protective.

On the other hand, some local conditions might require that site-specific criteria be lower than national criteria. Some untested locally important species might be very sensitive to the material of concern, and local water quality might not reduce the toxicity of the material. In addition,

aquatic organisms in field situations might be stressed by diseases, parasites, predators, other pollutants, contaminated or insufficient food, and fluctuating and extreme conditions of flow, water quality, and temperature. Further, some materials might degrade to more toxic materials, or some important community functions or species interactions might be adversely affected by concentrations lower than those that affect individual species.

Criteria must be used in a manner that is consistent with the way in which they were derived if the intended level of protection is to be provided in the real world. Although derivation of water quality criteria for aquatic life is constrained by the ways toxicity and bioconcentration tests are usually conducted, there are still many different ways that criteria can be derived, expressed, and used. The means used to derive and state criteria should relate, in the best possible way, the kinds of data that are available concerning toxicity and bioconcentration and the ways criteria can be used to protect aquatic organisms and their uses.

The major problem is to determine the best way that the statement of a criterion can bridge the gap between the nearly constant concentrations used in most toxicity and bioconcentration tests and the fluctuating concentrations that usually exist in the real world. A statement of a criterion as a number that is not to be exceeded any time or place is not acceptable because few, if any, people who use criteria would take it literally and few, if any, toxicologists would defend a literal interpretation. Rather than try to reinterpret a criterion that is neither useful nor valid, it is better to develop a more appropriate way of stating criteria.

Although some materials might not exhibit thresholds, many materials probably do. For any threshold material, continuous exposure to any combination of concentrations below the threshold will not cause an unacceptable effect (as defined on pages 1 and 2) on aquatic organisms and their uses, except that the concentration of a required trace nutrient might be too low. However, it is important to note that this is a threshold of unacceptable effect, not a threshold of adverse effect. Some adverse effect, possibly even a small reduction in the survival, growth, or reproduction of a commercially or recreationally important species, will probably occur at, and possibly even below, the threshold. The Criterion Continuous Concentration (CCC) is intended to be a good estimate of this threshold of unacceptable effect. If maintained continuously, any concentration above the CCC is expected to cause an unacceptable effect. On the other hand, the concentration of a pollutant in a body of water can be above the CCC without causing an unacceptable effect if (a) the magnitudes and durations of the excursions above the CCC are appropriately limited and (b) there are compensating periods of time during which the concentration is below the CCC. The higher the concentration is above the CCC, the shorter the period of time it can be tolerated. But it is unimportant whether there is any upper limit on concentrations that can be tolerated instantaneously or even for one minute because concentrations outside mixing zones rarely change substantially in such short periods of time.

An elegant, general approach to the problem of defining conditions (a) and (b) would be to integrate the concentration over time, taking into account uptake and depuration rates, transport within the organism to a critical site, etc. Because such an approach is not currently feasible, an approximate approach is to require that the average concentration not exceed the CCC. The average concentration should probably be calculated as the arithmetic average rather than the geometric mean⁵. If a suitable averaging period is selected, the magnitudes and durations of concentrations above the CCC will be appropriately limited, and suitable compensating periods below the CCC will be required.

In the elegant approach mentioned above, the uptake and depuration rates would determine the effective averaging period, but these rates are likely to vary from species to species for any particular material. Thus the elegant approach might not provide a definitive answer to the problem of selecting an appropriate averaging period. An alternative is to consider that the purpose of the averaging period is to allow the concentration to be above the CCC only if the allowed fluctuating concentrations do not cause more adverse effect than would be caused by a continuous exposure to the CCC. For example, if the CCC caused a 10% reduction in growth of rainbow trout, or a 13% reduction in survival of oysters, or a 7% reduction in reproduction of smallmouth bass, it is the purpose of the averaging period to allow concentrations above the CCC only if the total exposure will not cause any more adverse effect than continuous exposure to the CCC would cause.

Even though only a few tests have compared the effects of a constant concentration with the effects of the same average concentration resulting from a fluctuating concentration, nearly all the available comparisons have shown that substantial fluctuations result in increased adverse effects^{5,6}. Thus if the averaging period is not to allow increased adverse effects, it must not allow substantial fluctuations. Life-cycle tests with species such as mysids and daphnids and early life-stage tests with warmwater fishes usually last for 20 to 30 days. An averaging period that is equal to the length of the test will obviously allow the worst possible fluctuations and would very likely allow increased adverse effects.

An averaging period of four days seems appropriate for use with the CCC for two reasons. First, it is substantially shorter than the 20 to 30 days that is obviously unacceptable. Second, for some species it appears that the results of chronic tests are due to the existence of a sensitive life stage at some time during the test⁷, rather than being caused by either long-term stress or long-term accumulation of the test material in the organism. The existence of a sensitive life stage is probably the cause of acute-chronic ratios that are not much greater than 1, and is also possible when the ratio is substantially greater than 1. In addition, some experimentally determined acute-chronic ratios are somewhat less than 1, possibly because prior exposure during the chronic test increased the resistance of the sensitive life stage⁸. A four-day averaging period will probably prevent increased adverse effects on sensitive life stages by limiting the durations and magnitudes of exceedences* of the CCC.

The considerations applied to interpretation of the CCC also apply to the CMC. For the CMC the averaging period should again be substantially less than the lengths of the tests it is based on, i.e., substantially less than

48 to 96 hours. One hour is probably an appropriate averaging period because high concentrations of some materials can cause death in one to three hours. Even when organisms do not die within the first hour or so, it is not known how many might have died due to delayed effects of this short of an exposure. Thus it is not appropriate to allow concentrations above the CMC to exist for as long as one hour.

The durations of the averaging periods in national criteria have been made short enough to restrict allowable fluctuations in the concentration of the pollutant in the receiving water and to restrict the length of time that the concentration in the receiving water can be continuously above

* Although "exceedence" has not been found in any dictionary, it is used here because it is not appropriate to use "violation" in conjunction with criteria, no other word seems appropriate, and all appropriate phrases are awkward.

a criterion concentrations. The statement of a criterion could specify that the four-day average should never exceed the CCC and that the one-hour average should never exceed the CMC. However, one of the most important uses of criteria is for designing waste treatment facilities. Such facilities are designed based on probabilities and it is not possible to design for a zero probability. Thus, one of the important design parameters is the probability that the four-day average or the one-hour-average will be exceeded, or, in other words, the frequency with which exceedences will be allowed.

The frequency of allowed exceedences should be based on the ability of aquatic ecosystems to recover from the exceedences, which will depend in part on the magnitudes and durations of the exceedences. It is important to realize that high concentrations caused by spills and similar major events are not what is meant by an "exceedence", because spills and other accidents are not part of the design of the normal operation of waste treatment facilities. Rather, exceedences are extreme values in the distribution of ambient concentrations and this distribution is the result of the usual variations in the flows of both the effluent and the receiving water and the usual variations in the concentrations of the material of concern in both the effluent and in the upstream receiving water. Because exceedences are the result of usual variation, most of the exceedences will be small and exceedences as large as a factor of two will be rare. In addition, because these exceedences are due to random variation, they will not be evenly spaced. In fact, because many receiving waters have both one-year and multi-year cycles and many treatment facilities have daily, weekly, and yearly cycles, exceedences will often be grouped, rather than being evenly spaced or randomly distributed. If the flow of the receiving water is usually much greater than the flow of the effluent, normal variation and the flow cycles will result in the ambient concentration usually being below the CCC, occasionally being near the CCC, and rarely being above the CCC. In addition, exceedences that do occur will be grouped. On the other hand, if the flow of the effluent is much greater than the flow of the receiving water, the concentration might be close to the CCC much of the time and rarely above the CCC, with exceedences being randomly distributed.

The abilities of ecosystems to recover differ greatly, and depend on the pollutant, the magnitude and duration of the exceedence, and the physical and biological features of the ecosystem. Documented studies of recoveries are few, but some systems recover from small stresses in six weeks whereas other systems take more than ten years to recover from severe stress³. Although most exceedences are expected to be very small, larger exceedences will occur occasionally. Most aquatic ecosystems can probably recover from most exceedences in about three years. Therefore, it does not seem reasonable to purposely design for stress above that caused by the CCC to occur more than once every three years on the average, just as it does not seem reasonable to require that these kinds of stresses only occur once every five or ten years on the average.

If the body of water is not subject to anthropogenic stress other than the exceedences of concern and if exceedences as large as a factor of two are rare, it seems reasonable that most bodies of water could tolerate exceedences once every three years on the average. In situations in which exceedences are grouped, several exceedences might occur in one or two years, but then there will be, for example, 10 to 20 years during which no exceedences will occur and the concentration will be substantially below the CCC most of the time. In situations in which the concentration is often close to the CCC and exceedences are randomly distributed, some adverse effect will occur regularly, and small additional, unacceptable effects will occur about every

third year. The relative long-term ecological consequences of evenly spaced and grouped exceedences are unknown, but because most exceedences will probably be small, the long-term consequences should be about equal over long periods of time.

The above considerations lead to a statement of a criterion in the frequency-intensity-duration format that is often used to describe rain and snow fall and stream flow, e.g., how often, on the average, does more than ten inches of rain fall in a week? The numerical values chosen for frequency (or average recurrence interval), intensity (i.e., concentration), and duration (of averaging period) are those appropriate for national criteria. Whenever adequately justified, a national criterion may be replaced by a site-specific criterion ¹, which may include not only site-specific criterion concentrations ², but also site-specific durations of averaging periods and site-specific frequencies of allowed exceedences ³.

The concentrations, durations, and frequencies specified in criteria are based on biological, ecological, and toxicological data, and are designed to protect aquatic organisms and their uses from unacceptable effects. Use of criteria for designing waste treatment facilities requires selection of an appropriate wasteload allocation model. Dynamic models are preferred for the application of water quality criteria, but a steady-state model might have to be used instead of a dynamic model in some situations. Regardless of the model that is used, the durations of the averaging periods and the frequencies of allowed exceedences must be applied correctly if the intended level of protection is to be provided. For example, in the criterion statement frequency refers to the average frequency, over a long period of time, of rare events (i.e., exceedences). However, in some disciplines, frequency is often thought of in terms of the average frequency, over a long period of time, of the years in which rare events occur, without any consideration of how many rare events occur within each of those eventful years. The distinction between the frequency of events and the frequency of years is important for all those situations in which the rare events, e.g., exceedences, tend to occur in groups within the eventful years. The two ways of calculating frequency produce the same results in situations in which each rare event occurs in a different year because then the frequency of events is the same as the frequency of eventful years.

Because fresh water and salt water have basically different chemical compositions and because freshwater and saltwater (i.e., estuarine and true marine) species rarely inhabit the same water simultaneously, these National Guidelines provide for the derivation of separate criteria for these two kinds of water. For some materials sufficient data might not be available to allow derivation of criteria for one or both kinds of water. Even though absolute toxicities might be different in fresh and salt waters, such relative data as acute-chronic ratios and bioconcentration factors often appear to be similar in the two waters. When data are available to indicate that these ratios and factors are probably similar, they are used interchangeably.

The material for which a criterion is desired is usually defined in terms of a particular chemical compound or ion, or a group of closely related compounds or ions, but it might possibly be defined in terms of an effluent. These National Guidelines might also be useful for deriving criteria for temperature, dissolved oxygen, suspended solids, pH, etc., if the kinds of data on which the Guidelines are based are available.

Because they are meant to be applied only after a decision has been made that a national water quality criterion for aquatic organisms is needed for a material, these National Guidelines do not

address the rationale for making that decision. If the potential for adverse effects on aquatic organisms and their uses is part of the basis for deciding whether an aquatic life criterion is needed for a material, these Guidelines will probably be helpful in the collection and interpretation of relevant data. Such properties as volatility might affect the fate of a material in the aquatic environment and might be important when determining whether a criterion is needed for a material; for example, aquatic life criteria might not be needed for materials that are highly volatile or highly degradable in water. Although such properties can affect how much of the material will get from the point of discharge through any allowed mixing zone to some portion of the ambient water and can also affect the size of the zone of influence in the ambient water, such properties do not affect how much of the material aquatic organisms can tolerate in the zone of influence.

This version of the National Guidelines provides clarifications, additional details, and technical and editorial changes from the previous version⁹. These modifications are the result of comments on the previous version and subsequent drafts¹⁰, experience gained during the U.S. EPA's use of previous versions and drafts, and advances in aquatic toxicology and related fields. Future versions will incorporate new concepts and data as their usefulness is demonstrated. The major technical changes incorporated into this version of the National Guidelines are:

1. The requirement for acute data for freshwater animals has been changed to include more tests with invertebrate species. The taxonomic, functional, and probably the toxicological, diversities among invertebrate species are greater than those among vertebrate species and this should be reflected in the required data.
2. When available, 96-hr EC50s based on the percentage of fish immobilized plus the percentage of fish killed are used instead of 96-hr LC50s for fish; comparable EC50s are used instead of LC50s for other species. Such appropriately defined EC50s better reflect the total severe acute adverse impact of the test material on the test species than do LC50s or narrowly defined EC50s. Acute EC50s that are based on effects that are not severe, such as reduction in shell deposition and reduction in growth, are not used in calculating the Final Acute Value.
3. The Final Acute Value is now defined in terms of Genus Mean Acute Values rather than Species Mean Acute Values. A Genus Mean Acute Value is the geometric mean of all the Species Mean Acute Values available for species in the genus. On the average, species within a genus are toxicologically much more similar than species in different genera, and so the use of Genus Mean Acute Values will prevent data sets from being biased by an overabundance of species in one or a few genera.
4. The Final Acute Value is now calculated using a method¹¹ that is not subject to the bias and anomalous behavior that the previous method was. The new method is also less influenced by one very low value because it always gives equal weight to the four values that provide the most information about the cumulative probability of 0.05. Although the four values receive the most weight, the other values do have a substantial effect on the Final Acute Value (see examples in Appendix 2).
5. The requirements for using the results of tests with aquatic plants have been made more stringent.

6. Instead of being equal to the Final Acute Value, the Criterion Maximum Concentration is now equal to one-half the Final Acute Value. The Criterion Maximum Concentration is intended to protect 95 percent of a group of diverse genera, unless a commercially or recreationally important species is very sensitive. However, a concentration that would severely harm 50 percent of the fifth percentile or 50 percent of a sensitive important species cannot be considered to be protective of that percentile or that species. Dividing the Final Acute Value by 2 is intended to result in a concentration that will not severely adversely affect too many of the organisms.
7. The lower of the two numbers in the criterion is now called the Criterion Continuous Concentration, rather than the Criterion Average Concentration, to more accurately reflect the nature of the toxicological data on which it is based.
8. The statement of a criterion has been changed (a) to include durations of averaging periods and frequencies of allowed exceedences that are based on what aquatic organisms and their uses can tolerate, and (b) to identify a specific situation in which site-specific criteria^{1,2,3} are probably desirable.

In addition, Appendix 1 was added to aid in determining whether a species should be considered resident in North America and its taxonomic classification. Appendix 2 explains the calculation of the Final Acute Value.

The amount of guidance in these National Guidelines has been increased, but much of the guidance is necessarily qualitative rather than quantitative; much judgment will usually be required to derive a water quality criterion for aquatic organisms and their uses. In addition, although this version of the National Guidelines attempts to cover all major questions that have arisen during use of previous versions and drafts, it undoubtedly does not cover all situations that might occur in the future. All necessary decisions should be based on a thorough knowledge of aquatic toxicology and an understanding of these Guidelines and should be consistent with the spirit of these Guidelines, i.e., to make best use of the available data to derive the most appropriate criteria. These National Guidelines should be modified whenever sound scientific evidence indicates that a national criterion produced using these Guidelines would probably be substantially overprotective or underprotective of the aquatic organisms and their uses on a national basis. Derivation of numerical national water quality criteria for aquatic organisms and their uses is a complex process and requires knowledge in many areas of aquatic toxicology; any deviation from these Guidelines should be carefully considered to ensure that it is consistent with other parts of these Guidelines.

I. Definition of Material of Concern

- A. 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 various compounds and apparently have similar biological, chemical, physical, and toxicological properties.
- B. For chemicals that do 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), 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.

- C. The definition of the material should include an operational analytical component. Identification of a material simply, for example, as "sodium" obviously implies "total sodium", but leaves room for doubt. If "total" is meant, it should 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 operational analytical component should take into account the analytical and environmental chemistry of the material, the desirability of using the same analytical method on samples from laboratory tests, ambient water, and aqueous effluents, 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.

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. Because an ideal analytical measurement will rarely be available, a 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 national criterion, unacceptable effects will probably not occur, i.e., the compromise measurement must not err on the side of underprotection when measurements are made on a surface water. Because the chemical and physical properties of an effluent are usually quite different from those of the receiving water, an analytical method that is acceptable for analyzing an effluent might not be appropriate for analyzing a receiving water, and vice versa. If the ambient concentration *calculated* from a measured concentration in an effluent is higher than the national criterion, an additional option is to *measure* the concentration after dilution of the effluent with receiving water to determine if the measured concentration is lowered by such phenomena as complexation or sorption. A further option, of course, is to derive a site-specific criterion^{1,2,3}. Thus, the 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 should 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, not to underprotect aquatic life.

II. Collection of Data

- A. Collect all available data on the material concerning (a) toxicity to, and bioaccumulation by, aquatic animals and plants, (b) FDA action levels ¹², and (c) chronic feeding studies and long-term field studies with wildlife species that regularly consume aquatic organisms.
- B. All data that are used should be available in typed, dated, and signed hard copy (publication, manuscript, letter, memorandum, etc.) with enough supporting information to indicate that acceptable test procedures were used and that the results are probably reliable. In some cases it may be appropriate to obtain additional written information from the investigator, if possible. Information that is confidential or privileged or otherwise not available for distribution should not be used.
- C. Questionable data, whether published or unpublished, should not be used. For example, data should usually 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 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 of concern should not be used.
- E. For some highly volatile, hydrolyzable, or degradable materials it is probably appropriate to use only results of flow-through tests in which the concentrations of test material in the test solutions were measured often enough using acceptable analytical methods.
- F. Data should be rejected if they were 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 (see Appendix 1).
 - 3. Organisms that were previously exposed to substantial concentrations of the test material or other contaminants.
- G. Questionable data, data on formulated mixtures and emulsifiable concentrates, and data obtained with non-resident species in North America or previously exposed organisms may be used to provide auxiliary information but should not be used in the derivation of criteria.

III. Required data

- A. Certain data should be available to help ensure that each of the four major kinds of possible adverse effects receives adequate consideration. 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 required because procedures for conducting tests with plants and interpreting the results of such tests are not as well developed. Data concerning bioaccumulation by aquatic organisms are only required if relevant data are available concerning the significance of residues in aquatic organisms.

- B. To derive a criterion for freshwater aquatic organisms and their uses, the following should be available:
1. Results of acceptable acute tests (see Section IV) 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. a second family in the class Osteichthyes, preferably a commercially or recreationally important warmwater species (e.g., bluegill, channel catfish, etc.)
 - c. a third family in the phylum Chordata (may be in the class Osteichthyes or may be an amphibian, etc.)
 - d. a planktonic crustacean (e.g., cladoceran, copepod, etc.)
 - e. a benthic crustacean (e.g., ostracod, isopod, amphipod, crayfish, etc.)
 - f. an insect (e.g., mayfly, dragonfly, damselfly, stonefly, caddisfly, mosquito, midge, etc.)
 - g. a family in a phylum other than Arthropoda or Chordata (e.g., Rotifera, Annelida, Mollusca, etc.)
 - h. a family in any order of insect or any phylum not already represented.
 2. Acute-chronic ratios (see Section VI) with species of aquatic animals in at least three different families provided that one of the three species:
 - at least one is a fish
 - at least one is an invertebrate
 - at least one is an acutely sensitive freshwater species (the other two may be saltwater species).
 3. Results of at least one acceptable test with a freshwater alga or vascular plant (see Section VIII). If plants are among the aquatic organisms that are most sensitive to the material, results of a test with a plant in another phylum (division) should also be available.

4. At least one acceptable bioconcentration factor determined with an appropriate freshwater species, if a maximum permissible tissue concentration is available (see Section IX).
- C. To derive a criterion for saltwater aquatic organisms and their uses, the following should be available:
1. Results of acceptable acute tests (see Section IV) with at least one species of saltwater animal in at least eight different families such that all of the following are included:
 - a. two families in the phylum Chordata
 - b. a family in a phylum other than Arthropoda or Chordata
 - c. either the Mysidae or Penaeidae family
 - d. three other families not in the phylum Chordata (may include Mysidae or Penaeidae, whichever was not used above)
 - e. any other family.
 2. Acute-chronic ratios (see Section VI) with species of aquatic animals in at least three different families provided that of the three species:
 - at least one is a fish
 - at least one is an invertebrate
 - at least one is an acutely sensitive saltwater species (the other two may be freshwater species).
 3. Results of at least one acceptable test with a saltwater alga or vascular plant (see Section VIII). 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.
 4. At least one acceptable bioconcentration factor determined with an appropriate saltwater species, if a maximum permissible tissue concentration is available (see Section IX).
- D. If all the required data are available, a numerical criterion can usually be derived, except in special cases. For example, derivation of a criterion might not be possible if the available acute-chronic ratios 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), more data will be necessary.

Similarly, if all required data are not available, a numerical criterion should not be derived except in special cases. For example, even if not enough acute and chronic data are available, it might be possible to derive a criterion if the available data

clearly indicate that the Final Residue Value should be much lower than either the Final Chronic Value or Final Plant Value.

- E. Confidence in a criterion usually increases as the amount of available pertinent data 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. The Final Acute Value is an estimate of the concentration of the material corresponding to a cumulative probability of 0.05 in the acute toxicity values for the genera with which acceptable acute tests have been conducted on the material. However, in some cases, if the Species Mean Acute Value of a commercially or recreationally important species is lower than the calculated Final Acute Value, then that Species Mean Acute Value replaces the calculated Final Acute Value in order to provide protection for that important species.
- B. Acute toxicity tests should have been conducted using acceptable procedures ¹³.
- C. Except for test 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.
- D. Results of acute tests conducted in unusual dilution water, e.g., dilution water in which total organic carbon or particulate matter exceeded 5 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, particulate matter, etc., do not affect toxicity.
- E. Acute values should be based on endpoints which reflect the total severe acute 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 should be used:
 - 1. Tests with daphnids and other cladocerans should be started with organisms less than 24 hours old and tests with midges should be started with second- or third-instar larvae. The result should be the 48-hr EC50 based on percentage of organisms immobilized plus percentage of organisms killed. If such an EC50 is not available from a test, the 48-hr LC50 should be used in place of the desired 48-hr EC50. An EC50 or LC50 of longer than 48 hr can be used as long as the animals were not fed and the control animals were acceptable at the end of the test.
 - 2. The result of a test with embryos and larvae of barnacles, bivalve molluscs (clams, mussels, oysters, and scallops), sea urchins, lobsters, crabs, shrimp, and abalones, should be the 96-hr 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, the lower of the 96-hr EC50 based on the percentage of organisms with incompletely developed shells and the 96-hr LC50

should be used in place of the desired 96-hr EC50. If the duration of the test was between 48 and 96 hr, the EC50 or LC50 at the end of the test should be used.

3. The acute values from tests with all other freshwater and saltwater animal species and older life stages of barnacles, bivalve molluscs, sea urchins, lobsters, crabs, shrimps, and abalones should be the 96-hr EC50 based on the percentage of organisms exhibiting loss of equilibrium plus the percentage of organisms immobilized plus the percentage of organisms killed. If such an EC50 is not available from a test, the 96-hr LC50 should be used in place of the desired 96-hr EC50.
 4. Tests with single-celled organisms 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 unnecessarily lower the Final Acute Value by eliminating acute values for resistant species.
- F. If the acute toxicity of the material to aquatic animals apparently has been shown to be related to a water quality characteristic such as hardness or particulate matter for freshwater animals or salinity or particulate matter for saltwater animals, a Final Acute Equation should be derived based on that water quality characteristic. Go to Section V.
- G. 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 should not be used in the calculation of the Species Mean Acute Value because a species can only be considered protected from acute toxicity if all life stages are protected.
- H. The agreement of the data within and between species should 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 probably should not be used in calculation of a Species Mean Acute Value. For example, if the acute values available for a species or genus differ by more than a factor of 10, some or all of the values probably should not be used in calculations.
- I. For each species for which at least one acute value is available, the Species Mean Acute Value (SMAV) should be calculated as the geometric mean of the results of all flow-through tests in which the concentrations of test material were measured. For a species for which no such result is available, the SMAV should be calculated as the geometric mean of all available acute values, 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.

NOTE: Data reported by original investigators should not be rounded off. Results of all intermediate calculations should be rounded ¹⁴ to four significant digits.

NOTE: 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 logarithm used.

NOTE: 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 acute-chronic ratios and bioconcentration factors 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 corresponding 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 Genus Mean Acute Value (GMAV) should 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, arbitrarily 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 less than 59 GMAVs, these will always be the four lowest GMAVs).
- O. Using the selected GMAVs and Ps, calculate

$$S^2 = \frac{\sum ((\ln GMAV)^2) - ((\sum \ln GMAV))^2 / 4}{\sum (F) - ((\sum (\sqrt{P}))^2 / 4)}$$

$$L = (\sum (\ln GMAV) - S(\sum (\sqrt{P}))) / 4$$

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

$$FAV = e^A$$

(See ¹¹ for development of the calculation procedure and Appendix 2 for an example calculations and computer program.)

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 the geometric mean of the acute values from ~~the~~ flow-through tests in which the concentrations of test material were measured is lower than the calculated Final Acute Value, then that geometric mean should be used as the Final Acute Value instead of the calculated Final Acute Value.
- Q. Go to Section VI.

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 should be taken into account as described in Sections B-G below or using analysis of covariance^{15, 16}. 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 should 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% 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, or salinity, 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 is useful, 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 probably 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 is probably appropriate. 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., using the results of tests

conducted under conditions and in waters similar to those commonly used for toxicity tests with the species.

- D. Individually for each species calculate the geometric mean of the available acute values and then divide each of the acute values for a species by the 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.
- F. Individually for each species perform a least squares regression of the normalized acute toxicity values on the corresponding normalized values of the water quality characteristic. The resulting slopes and 95% confidence limits will be identical to those obtained in Section B above. 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.
- G. Treat all the normalized data as if they were all for the same species and perform a least squares regression of all the normalized acute values on the corresponding normalized values of the water quality characteristic to obtain the pooled acute slope, V , and its 95% confidence limits. If all 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 steps D and E above.)
- 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 Z using the equation: $\text{SMAV} = e^Y$.
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 Final Acute Value at Z by using the procedure described in Section IV.J-O.
- L. If the SMAV at Z of a commercially or recreationally important species is lower than the calculated Final Acute Value at Z , then that SMAV should be used as the Final Acute Value at Z instead of the calculated Final Acute Value.
- M. The Final Acute Equation is written as: $\text{Final Acute Value} = e^{(V[\ln(\text{water quality characteristic}) + \ln A - V[\ln Z])}$, where V = pooled acute slope and A = Final Acute Value at Z . Because

V, A, and Z are known, the Final Acute Value 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 might be calculated in the same manner as the Final Acute Value or by dividing the Final Acute Value by the Final Acute-Chronic Ratio. In some cases it may not be possible to calculate a Final Chronic Value.

NOTE: As the name implies, the acute-chronic ration (ARC) is a way of relating acute and chronic toxicities. The acute-chronic ratio is basically the inverse of the application factor, but this new name is better because it is more descriptive and should help prevent confusion between "application factors" and "safety factors". Acute-chronic ratios and application factors are ways of relating the acute and chronic toxicities of a material to aquatic organisms. Safety factors are used to provide an extra margin of safety beyond the known or estimated sensitivities of aquatic organisms. Another advantage of the acute-chronic ratio is that it will usually be greater than one; this should avoid the confusion as to whether a large application factor is one that is close to unity or one that has a denominator that is much greater than the numerator.

- B. Chronic values should 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.
- C. Results of chronic tests in which survival, growth, or reproduction in the control treatment was unacceptably low should 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 5 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 should be based on endpoints and lengths of exposure appropriate to the species. Therefore, only results of the following kinds of chronic toxicity tests should 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. Tests with mysids should begin with young less than 24 hours old and continue until 7 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 consisting of exposures of each of two or 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. Exposure to the test material should begin with immature juveniles at least 2 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 should not be used because results of such tests are possibly not good predictions of the results 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. 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. 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., 3%) might be

considered acceptable even if it is statistically significantly different from the control, whereas a large percent reduction (e.g., 30%) might be considered unacceptable even if it is not statistically significant.

- G. If the chronic toxicity of the material to aquatic animals apparently has been shown to be related to a water quality characteristic such as hardness or particulate matter for freshwater animals or salinity or particulate matter for saltwater animals, a Final Chronic Equation should be derived based on that water quality characteristic. Go to Section VII.
- H. If chronic values are available for species in eight families as described in Sections III.B.1 or III.C.1, a Species Mean Chronic Value (SMCV) should be calculated for each species for which at least one chronic value is available by calculating the geometric mean of all chronic values available for the species, and appropriate Genus Mean Chronic Values should be calculated. The Final Chronic Value should then be obtained using the procedure described in Section IV.J-O. Then go to Section VI.M.
- I. For each chronic value for which at least one corresponding appropriate acute value is available, calculate an acute-chronic ratio, using for the numerator the geometric mean of the results of all acceptable flow-through (except static is acceptable for daphnids) acute tests in the same dilution water and in which the concentrations were measured. For fish, the acute test(s) should have been conducted with juveniles. The acute test(s) should have been part of the same study as the chronic test. If acute tests were not conducted as part of the same study, acute tests conducted in the same laboratory and dilution water, but in a different study, 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 acute-chronic ratio should not be calculated.
- J. For each species, calculate the species mean acute-chronic ratio as the geometric mean of all acute-chronic ratios available for that species.
- K. For some materials the acute-chronic ratio seems to be the same for all species, but for other materials the ratio seems to increase or decrease as the Species Mean Acute Value (SMAV) increases. Thus the Final Acute-Chronic Ratio can be obtained in four ways, depending on the data available:
 - 1. If the species mean acute-chronic ratios seems to increase or decrease as the SMAV increases, the Final Acute-Chronic Ratio should be calculated as the geometric mean of the acute-chronic ratios for species whose SMAVs are close to the Final Acute Value.
 - 2. If no major trend is apparent and the acute-chronic ratios for a number of species are within a factor of ten, the Final Acute-Chronic Ratio should be calculated as the geometric mean of all the species mean acute-chronic ratios available for both freshwater and saltwater species.
 - 3. For acute tests conducted on metals and possibly other substances with embryos and larvae of barnacles, bivalve molluscs, sea urchins, lobsters,

crabs, shrimp, and abalones (see Section IV.E.2), it is probably appropriate to assume that the acute-chronic ratio is 2. Chronic tests are very difficult to conduct with most such species, but it is likely that the sensitivities of embryos and larvae would determine the results of life-cycle tests. Thus, if the lowest available SMAVs were determined with embryos and larvae of such species, the Final Acute-Chronic Ratio should probably be assumed to be 2, so that the Final Chronic Value is equal to the Criterion Maximum Concentration (see Section XI.B).

4. If the most appropriate species mean acute-chronic ratios are less than 2.0, and especially if they are less than 1.0, acclimation has probably occurred during the chronic test. Because continuous exposure and acclimation cannot be assured to provide adequate protection in field situations, the Final Acute-Chronic Ratio should be assumed to be 2, so that the Final Chronic Value is equal to the Criterion Maximum Concentration (see Section XI.B).

If the available species mean acute-chronic ratios do not fit one of these cases, a Final Acute-Chronic Ratio probably cannot be obtained, and a Final Chronic Value probably cannot be calculated.

- L. Calculate the Final Chronic Value by dividing the Final Acute Value by the Final Acute-Chronic Ratio. If there was a Final Acute Equation rather than a Final Acute Value, see also Section VII.A.
- M. If the Species Mean Chronic Value of a commercially or recreationally important species is lower than the calculated Final Chronic Value, then that Species Mean Chronic Value should be used as the Final Chronic Value instead of the calculated Final Chronic Value.
- N. Go to Section VIII.

VII. Final Chronic Equation

- A. A Final Chronic Equation can be derived in two ways. The procedure described here in Section A will result in the chronic slope being the same as the acute slope. The procedure described in Sections B-N will usually result in the chronic slope being different from the actual slope.
 1. If acute-chronic ratios are available for enough species at enough values of the water quality characteristic to indicate that the acute-chronic ratio is probably the same for all species and is probably independent of the water quality characteristic, calculate the Final Acute-Chronic Ratio as the geometric mean of the available species mean acute-chronic ratios.
 2. Calculate the Final Chronic Value at the selected value Z of the water quality characteristic by dividing the Final Acute Value at Z (see Section V.M.) by the Final Acute-Chronic Ratio.

3. Use V = pooled acute slope (see section V.M.) as L = pooled chronic slope.
 4. Go to Section VII.M.
- 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 B-G below or using analysis of covariance^{15,16}. 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 more factors affect toxicity, multiple regression analysis should 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% 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, or salinity, 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.

- D. Decide whether the data for each species is useful, 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, 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 probably 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 probably 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 acute-chronic ratio is independent of the water quality characteristic. Alternatively, return to Section VI.H, 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% confidence limits will be identical to those obtained in Section B above. 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 the normalized data as if they were all for the same species and perform a least squares regression of all the normalized chronic values on the corresponding normalized values of the water quality characteristic to obtain the pooled chronic slope, L, and its 95% confidence limits. If all the 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 were calculated in steps E and F above.)
- J. For each species calculate the logarithm, Q, of the Species Mean Chronic Value 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 will usually be best to use the same value of the water quality characteristic here as was used in Section V.I.

- K. For each species calculate a Species Mean Chronic Value at Z using the equation: $SMCV = e^Q$.

NOTE: Alternatively, the Species Mean Chronic Values at Z can be obtained by skipping step J above, using the equations in steps J and K to adjust each acute 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 Final Chronic Value at Z by using the procedure described in Section IV.J-O.
- M. If the Species Mean Chronic Value at Z of a commercially or recreationally important species is lower than the calculated Final Chronic Value at Z, then that Species Mean

- N. The Final Chronic Equation is written as: Final Chronic Value = $e^{(L[\ln(\text{water quality characteristic})] + \ln S - L [\ln Z])}$, where L = pooled chronic slope and S = Final Chronic Value at Z. Because L, S and Z are known, the Final Chronic Value can be calculated for any selected value of the water quality characteristic.

VIII. Final Plant Value

- A. 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 probably also protect aquatic plants and their uses.
- B. A plant value is the result of a 96-hr 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 usually should 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 about 200 µg/L should probably be considered excessive.

- C. The Final Plant Value should be obtained by selecting the lowest result from a test with an important aquatic plant species in which the concentrations of test material were measured and the endpoint was biologically important.

IX. Final Residue Value

- A. The Final Residue Value is intended to (a) prevent concentrations in commercially or recreationally important aquatic species from affecting marketability because of exceedance of applicable FDA action levels and (b) protect wildlife, including fishes and birds, that consume aquatic organisms from demonstrated unacceptable effects. The Final Residue Value is the lowest of the residue values that are obtained by dividing maximum permissible tissue concentrations by appropriate bioconcentration or bioaccumulation factors. A maximum permissible tissue concentration is either (a) an FDA action level¹² for fish oil or for the edible portion of fish or shellfish, or (b) a maximum acceptable dietary intake based on observations on survival, growth, or reproduction in a chronic wildlife feeding study or a long-term wildlife field study. If no maximum permissible tissue concentration is available, go to Section X because no Final Residue Value can be derived.
- B. Bioconcentration factors (BCFs) and bioaccumulation factors (BAFs) are quotients of the concentration of a material in one or more tissues of an aquatic organism divided by the average concentration in the solution in which the organism had been living. A BCF is intended to account only for net uptake directly from water, and thus almost

has to be measured in a laboratory test. Some uptake during the bioconcentration test might not be directly from water if the food sorbs some of the test material before it is eaten by the test organisms. A BAF is intended to account for the net uptake from both food and water in a real-world situation. A BAF almost has to be measured in a field situation in which predators accumulate the material directly from water and by consuming prey that itself could have accumulated the material from both food and water. The BCF and BAF are probably similar for a material with a low BCF, but the BAF is probably higher than the BCF for materials with high BCFs. Although BCFs are not too difficult to determine, very few BAFs have been measured acceptably because it is necessary to make enough measurements of the concentration of the material in water to show that it was reasonably constant for a long enough period of time over the range of territory inhabited by the organisms. Because so few acceptable BAFs are available, only BCFs will be discussed further. However, if an acceptable BAF is available for a material, it should be used instead of any available BCFs.

- C. If a maximum permissible tissue concentration is available for a substance (e.g., parent material, parent material plus metabolites, etc.), the tissue concentration used in the calculation of the BCF should be for the same substance. Otherwise, the tissue concentration used in the calculation of the BCF should be that of the material and its metabolites which are structurally similar and are not much more soluble in water than the parent material.
- D.
1. A BCF should be used only if the test was flow-through, the BCF was calculated based on measured concentrations of the test material in tissue and in the test solution, and the exposure continued at least until either apparent steady-state or 28 days was reached. Steady-state is reached when the BCF does not change significantly over a period of time, such as two days or 16 percent of the length of the exposure, whichever is longer. The BCF used from a test should be the highest of (a) the apparent steady-state BCF, if apparent steady-state was reached, (b) the highest BCF obtained, if apparent steady-state was not reached, and (c) the projected steady-state BCF, if calculated.
 2. Whenever a BCF is determined for a lipophilic material, the percent lipids should also be determined in the tissue(s) for which the BCF was calculated.
 3. A BCF obtained from an exposure that adversely affected the test organisms may be used only if it is similar to a BCF obtained with unaffected organisms of the same species at lower concentrations that did not cause adverse effects.
 4. Because maximum permissible tissue concentrations are almost never based on dry weights, a BCF calculated using dry tissue weights must be converted to a wet tissue weight basis. If no conversion factor is reported with the BCF, multiply the dry weight BCF by 0.1 for plankton and by 0.2 for individual species of fishes and invertebrates ¹⁷.

5. If more than one acceptable BCF is available for a species, the geometric mean of the available values should be used, except that if the BCFs are from different lengths of exposure and the BCF increases with length of exposure, the BCF for the longest exposure should be used.
- E. If enough pertinent data exist, several residue values can be calculated by dividing maximum permissible tissue concentrations by appropriate BCFs:
1. For each available maximum acceptable dietary intake derived from a chronic feeding study or a long-term field study with wildlife, including birds and aquatic organisms, the appropriate BCF is based on the whole body of aquatic species which constitute or represent a major portion of the diet of the tested wildlife species.
 2. For an FDA action level for fish or shellfish, the appropriate BCF is the highest geometric mean species BCF for the edible portion (muscle for decapods, muscle with or without skin for fishes, adductor muscle for scallops, and total soft tissue for other bivalve molluscs) of a consumed species. The highest species BCF is used because FDA action levels are applied on a species-by-species basis.
- F. For lipophilic materials, it might be possible to calculate additional residue values. Because the steady-state BCF for a lipophilic material seems to be proportional to percent lipids from one tissue to another and from one species to another^{18, 19, 20}, extrapolations can be made from tested tissues or species to untested tissues or species on the basis of percent lipids.
1. For each BCF for which the percent lipids is known for the same tissue for which the BCF was measured, normalize the BCF to a one percent lipid basis by dividing the BCF by the percent lipids. This adjustment to a one percent lipid basis is intended to make all the measured BCFs for a material comparable regardless of the species or tissue with which the BCF was measured.
 2. Calculate the geometric mean normalized BCF. Data for both saltwater and freshwater species should be used to determine the mean normalized BCF, unless the data show that the normalized BCFs are probably not similar.
 3. Calculate all possible residue values by dividing the available maximum permissible tissue concentrations by the mean normalized BCF and by the percent lipids values appropriate to the maximum permissible tissue concentrations, i.e.,

$$\text{Residue Value} = \frac{(\text{maximum permissible tissue concentration})}{(\text{mean normalized BCF}) (\text{appropriate percent lipids})}$$

- a. For an FDA action level for fish oil, the appropriate percent lipids value is 100.

- b. For an FDA action level for fish, the appropriate percent lipids value is 11 for freshwater criteria and 10 for saltwater criteria because FDA action levels are applied on a species-by-species basis to commonly consumed species. The highest lipid contents in the edible portions of important consumed species are about 11 percent for both the freshwater chinook salmon and lake trout and about 10 percent for the saltwater Atlantic herring ²¹.
 - c. For a maximum acceptable dietary intake derived from a chronic feeding study or a long-term field study with wildlife, the appropriate percent lipids is that of an aquatic species or group of aquatic species which constitute a major portion of the diet of the wildlife species.
- G. The Final Residue Value is obtained by selecting the lowest of the available residue values.

NOTE: In some cases the Final Residue Value will not be low enough. For example, a residue value calculated from an FDA action level will probably result in an average concentration in the edible portion of a fatty species that is at the action level. Some individual organisms, and possibly some species, will have residue concentrations higher than the mean value but no mechanism has been devised to provide appropriate additional protection. Also, some chronic feeding studies and long-term field studies with wildlife identify concentrations that cause adverse effects but do not identify concentrations which do not cause adverse effects; again no mechanism has been devised to provide appropriate additional protection. These are some of the species and uses that are not protected at all times in all places.

X. Other Data

Pertinent information that could not be used in earlier sections might be available concerning adverse effects on aquatic organisms and their uses. The most important of these are data on cumulative and delayed toxicity, flavor impairment, reduction in survival, growth, or reproduction, or any other adverse effect that has been shown to be biologically important. 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 IV.D and VI.D), from chronic tests in which the concentrations were not measured (see VI.B), from tests with previously exposed organisms (see II.F), and from tests on formulated mixtures or emulsifiable concentrates (see II.D). 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.

XI. Criterion

- A. A criterion consists of two concentrations: the Criterion Maximum Concentration and the Criterion Continuous Concentration.

- B. The Criterion Maximum Concentration (CMC) is equal to one-half the Final Acute Value.
- C. The Criterion Continuous Concentration (CCC) is equal to the lowest of the Final Chronic Value, the Final Plant Value, and the Final Residue Value, unless other data (see Section X) show that a lower value should be used. If toxicity is related to a water quality characteristic, the CCC is obtained from the Final Chronic Equation, the Final Plant Value, and the Final Residue Value by selecting the one, or the combination, that results in the lowest concentrations in the usual range of the water quality characteristic, unless other data (see Section X) show that a lower value should be used.
- D. Round ¹⁴ both the CMC and the CCC to two significant digits.
- E. The criterion is stated as:

The procedures described in the "Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses" indicate that, except possibly where a locally important species is very sensitive, (1) aquatic organisms and their uses should not be affected unacceptably if the four-day average concentration of (2) does not exceed (3) µg/L more than once every three years on the average and if the one-hour average concentration does not exceed (4) µg/L more than once every three years on the average.

where (1) = insert "freshwater" or "saltwater"

(2) = insert name of material

(3) = insert the Criterion Continuous Concentration

(4) = insert the Criterion Maximum Concentration.

XII. Final Review

- A. The derivation of the criterion should be carefully reviewed by rechecking each step of the Guidelines. 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 Species Mean Acute Values for any genus greater than a factor of 10?
 - 5. Is there more than a factor of ten difference between the four lowest Genus Mean Acute Values?
 - 6. Are any of the four lowest Genus Mean Acute Values questionable?

7. Is the Final Acute Value reasonable in comparison with the Species Mean Acute Values and Genus Mean Acute Values?
 8. For any commercially or recreationally important species, is the geometric mean of the acute values from flow-through tests in which the concentrations of test material were measured lower than the Final Acute Value?
 9. Are any of the chronic values questionable?
 10. Are chronic values available for acutely sensitive species?
 11. Is the range of acute-chronic ratios greater than a factor of 10?
 12. Is the Final Chronic Value 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 below the Final Chronic Value?
 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 Guidelines? 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, should be derived using appropriate modifications of these Guidelines.

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- ⁹ U.S. EPA. 1980. Water Quality Criteria Documents; Availability. Federal Register 45: 79318-79379. November 28.
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- ¹² U.S. Food and Drug Administration. 1981. Compliance Policy Guide. Compliance Guidelines Branch, Washington, DC.
- ¹³ For good examples of acceptable procedures, see:
 - ASTM Standard E 729, Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians.
 - ASTM Standard E 724, Practice for Conducting Static Acute Toxicity Tests with Larvae of Four Species of Bivalve Molluscs.
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Appendix 1. Resident North American Species of Aquatic Animals Used in Toxicity and Bioconcentration Tests

Introduction

These lists identify species of aquatic animals which have reproducing wild populations in North America and have been used in toxicity or bioconcentration tests. "North America" includes only the 48 contiguous states, Canada, and Alaska; Hawaii and Puerto Rico are not included. Saltwater (i.e., estuarine and true marine) species are considered resident in North America if they inhabit or regularly enter shore waters on or above the continental shelf to a depth of 200 meters. Species do not have to be native to be resident. Unlisted species should be considered resident North American species if they can be similarly confirmed or if the test organisms were obtained from a wild population in North America.

The sequence for fishes is taken from A List of Common and Scientific Names of Fishes from the United States and Canada. For other species, the sequence of phyla, classes, and families is taken from the NODC Taxonomic Code, Third Edition, National Oceanographic Data Center, NOAA, Washington, DC 20235, July, 1981, and the numbers given are from that source to facilitate verification. Within a family, genera are in alphabetical order, as are species in a genus.

The references given are those used to confirm that the species is a resident North American species. (The NODC Taxonomic Code contains foreign as well as North American species.) If no such reference could be found, the species was judged to be nonresident. No reference is given for organisms not identified to species; these are considered resident only if obtained from wild North American populations. A few nonresident species are listed in brackets and noted as "nonresident" because they were mistakenly identified as resident in the past or to save other investigators from doing literature searches on the same species.

Special Note

This December 2010 electronic version of the 1985 Guidelines serves to meet the requirements of Section 508 of the Rehabilitation Act. While converting the 1985 Guidelines to a 508-compliant version, EPA updated the taxonomic nomenclature to reflect changes that occurred since the tables were originally produced in 1985. The numbers included for Phylum, Class and Family represent those currently in use from the Integrated Taxonomic Information System, or ITIS, and reflect what is referred to in ITIS as Taxonomic Serial Numbers. ITIS replaced the National Oceanographic Data Center (NODC) taxonomic coding system which was used to create the original taxonomic tables included in the 1985 Guidelines document (NODC, Third Addition - see Introduction). For more information on the NODC taxonomic codes, see <http://www.nodc.noaa.gov/General/CDR-detdesc/taxonomic-v8.html>.

The code numbers included in the reference column of the tables have not been updated from the 1985 version. These code numbers are associated with the old NODC taxonomic referencing system and are simply replicated here for historical purposes. Footnotes may or may not still apply.

EPA is working on a more comprehensive update to the 1985 Guidelines, including new taxonomic tables which better reflect the large number of aquatic animal species known to be propagating in U.S. waters.

Freshwater Species Table

Synonyms appear after the official Scientific Name and are marked with an asterisk (*).

Non-resident species are noted in the Reference column and are marked with a dagger (†)

Class	Family	Species		Reference
		Common Name	Scientific Name	
Phylum: Porifera (46861)				
Demospongiae 47528	Spongillidae 47691	Sponge	<i>Ephydatia fluviatilis</i>	P93
Phylum: Cnidaria (48738)				
Hydrozoa 48739	Hydridae 50844	Hydra	<i>Hydra oligactis</i>	E318, P112
		Hydra	<i>Hydra littoralis</i>	E321, P112
Phylum: Platyhelminthes (53963)				
Turbellaria 53964	Planariidae 54502	Planarian	<i>Dugesia dorotocephala</i>	D22
		Planarian	<i>Dugesia lugubris</i> <i>Dugesia polychroa</i> †	D24
		Planarian	<i>Planaria gonocephala</i>	¹
		Planarian	<i>Polycelis felina</i> §	nonresident
	Dendrocoelidae 54469	Planarian	<i>Procotyla fluviatilis</i> <i>Dendrocoelum lacteum</i> *	E334, P132, D63
Phylum: Gastrotricha (57597)				
Chaetonotida 57822	Chaetonotidae 57823	Gastrotrich	<i>Lepidodermella squamata</i> <i>Lepidodermella squamatum</i> *	E413
Phylum: Rotifera (58239)				
Eurotatoria (Formerly Bdelloidea) 654070	Philodinidae 58266	Rotifer	<i>Philodina acuticornis</i>	Y
		Rotifer	<i>Philodina roseola</i>	E487
Eurotatoria (Formerly Monogononta) 654070	Brachionidae 58344	Rotifer	<i>Keratella cochlearis</i>	E442, P188
		Rotifer	<i>Keratella sp.</i>	²
Phylum: Annelida (64357)				
Polychaeta (Formerly Archiannelida) 64358	Aeolosomatidae 68423	Worm	<i>Aeolosoma headleyi</i>	E528, P284
Clitellata (Formerly Oligochaeta) 568832	Lumbriculidae 68440	Worm	<i>Lumbriculus variegatus</i>	E533, P290
	Tubificidae 68585	Tubificid worm	<i>Branchiura sowerbyi</i>	E534, P289, GG
		Tubificid worm	<i>Limnodrilus hoffmeisteri</i>	E536, GG
		Tubificid worm	<i>Quistadrilus multisetosus</i> <i>Peloscolex multisetosus</i> *	E535, GG
		Tubificid worm	<i>Rhyacodrilus montanus</i>	GG
		Tubificid worm	<i>Spirosperma ferox</i> <i>Peloscolex ferox</i> *	GG
		Tubificid worm	<i>Spirosperma nikolskyi</i> <i>Peloscolex variegatus</i> *	E534, GG
		Tubificid worm	<i>Stylo-drilus heringianus</i>	GG

‡ Synonym

§ Non-resident species

Class	Family	Species		Reference	
		Common Name	Scientific Name		
		Tubificid worm	<i>Tubifex tubifex</i>		E536, P289, GG
		Tubificid worm	<i>Varichaeta pacifica</i>	GG	
	Naididae 68854	Worm	<i>Nais sp.</i>	2	
		Worm	<i>Paranais sp.</i>	2	
		Worm	<i>Pristina sp.</i>	2	
Clitellata (Formerly Hirudinea) 568832	Erpobdellidea 69438	Leech	<i>Erpobdella octoculata</i>	Formerly nonresident (BB16)	
Phylum: Mollusca (69458)					
Gastropoda 69459	Viviparidae 70304	Snail	<i>Campeloma decisum</i>	P731, M216	
	Bithyniidae (Amnicolidae) (Bulimidae) (Hydrobiidae) 70745	Snail	<i>Amnicola sp.</i>	2	
	Pleuroceridae 71541	Snail	<i>Goniobasis livescens</i>	P732	
		Snail	<i>Elimia virginica</i> <i>Goniobasis virginica</i>	E1137	
		Snail	<i>Leptoxis carinata</i> <i>Nitocris carinata</i> <i>Mudalia carinata</i>	X, E1137	
		Snail	<i>Nitocris sp.</i>	2	
	Lymnaeidae 76483	Snail	<i>Lymnaea acuminata</i> [†]	nonresident	
		Snail	<i>Lymnaea catascopium</i> <i>Lymnaea emerginata</i> <i>Stagnicola emerginata</i>	M328	
		Snail	<i>Lymnaea elodes</i> <i>Lymnaea palustris</i>	E1127, M351	
		Snail	<i>Lymnaea luteola</i> [†]	nonresident M266	
		Snail	<i>Lymnaea stagnalis</i>	E1127, P728, M296	
		Snail	<i>Lymnaea sp.</i>	2	
	Planorbidae 76591	Snail	<i>Biomphalaria glabrata</i>	Formerly nonresident (M390)	
		Snail	<i>Gyraulus circumstriatus</i>	P729, M397	
		Snail	<i>Helisoma campanulatum</i>	M445	
		Snail	<i>Helisoma trivolvis</i>	P729, M452	
	Physidae 76676	Snail	<i>Aplexa hypnorum</i>	E1126, P727, M373	
		Snail	<i>Physa fontinalis</i> [†]	nonresident M373	
		Snail	<i>Physa gyrina</i>	E1126, P727, M373	
		Snail	<i>Physa heterostropha</i>	M378	
		Snail	<i>Physa integra</i>	P727	
		Snail	<i>Physa sp.</i>	2	
	Bivalvia (Pelecypoda) 79118	Margaritiferidae 79914	Mussel	<i>Margaritifera margaritifera</i>	E1138, P748, J11
		Unionidae (Formerly Amblemidae) 79913	Mussel	<i>Amblema plicata</i>	AA122
		Unionidae	Mussel	<i>Anodonta imbecillis</i>	J72, AA122

Class	Family	Species		Reference	
		Common Name	Scientific Name		
	79913	Mussel	<i>Carunculina parva</i> <i>Toxolasma texasensis</i> [*]	J19, AA122	
		Mussel	<i>Cyrtonaias tampicoensis</i>	P759, AA122	
		Mussel	<i>Elliptio complanata</i>	J13	
	Corbiculidae 81381	Asiatic clam	<i>Corbicula fluminea</i>	E1159	
		Asiatic clam	<i>Corbicula manilensis</i>	P749	
	Pisidiidae Sphaeriidae 81388	Fingernail clam	<i>Eupera cubensis</i> <i>Eupera singleyi</i> [†]	E1158, P763, G9	
		Fingernail clam	<i>Musculium transversum</i> <i>Sphaerium transversum</i> [*]	M160, G11	
		Fingernail clam	<i>Sphaerium corneum</i>	G12	
	Phylum: Arthropoda (82696)				
	Branchiopoda (Formerly Crustacea) 83687	Lynceidae 83769	Conchostracan	<i>Lynceus brachyurus</i>	E580, P344
Sididae 83834		Cladoceran	<i>Diaphanosoma sp.</i>	2	
Daphniidae 83872		Cladoceran	<i>Ceriodaphnia acanthina</i>	E618	
		Cladoceran	<i>Ceriodaphnia reticulata</i>	E618, P368	
		Cladoceran	<i>Daphnia ambigua</i>	E607, P369	
		Cladoceran	<i>Daphnia carinata</i>	³	
		Cladoceran	<i>Daphnia cucullata</i> [†]	nonresident	
		Cladoceran	<i>Daphnia galeata mendotae</i>	E610, P370	
		Cladoceran	<i>Daphnia hyalina</i>	⁴	
		Cladoceran	<i>Daphnia longispina</i>	⁵	
		Cladoceran	<i>Daphnia magna</i>	E605, P367	
		Cladoceran	<i>Daphnia parvula</i>	E611	
		Cladoceran	<i>Daphnia pulex</i>	E613, P367	
		Cladoceran	<i>Daphnia pulicaria</i>	A	
		Cladoceran	<i>Daphnia similis</i>	E606, P367	
Cladoceran		<i>Simocephalus serrulatus</i>	E617, P370		
Cladoceran		<i>Simocephalus vetulus</i>	E617, P370		
Moinidae (Formerly Daphnidae) 84162		Cladoceran	<i>Moina macrocopa</i>	E622, P372	
		Cladoceran	<i>Moina rectoris</i>	E623	
Bosminidae 83935	Cladoceran	<i>Bosmina longirostris</i>	E624, P373		
Polyphemidae 83959	Cladoceran	<i>Polyphemus pediculus</i>	E599, P385		
Ostracoda (Formerly Crustacea) 84195	Cyprididae Cypridae 84462	Ostracod	<i>Cyprretta kawatai</i> [†]	nonresident U	
		Ostracod	<i>Cypridopsis vidua</i>	E770, P430	
Maxillopoda (Formerly Crustacea) 621145	Diaptomidae 85779	Copepod	<i>Eudiaptomus padanus</i> [†]	nonresident	
	Temoridae 85855	Copepod	<i>Epischura lacustris</i>	E751, P407	
	Cyclopidae 88634	Copepod	<i>Cyclops abyssorum</i> [†]	nonresident	
		Copepod	<i>Cyclops bicuspidatus</i>	E807, P405	
		Copepod	<i>Cyclops vernalis</i>	E804, P405	
		Copepod	<i>Cyclops viridis</i> <i>Acanthocyclops viridis</i> [*]	E803, P397	

Class	Family	Species		Reference
		Common Name	Scientific Name	
Malacostraca (Formerly Crustacea) 89787		Copepod	<i>Acanthocyclops sp.</i>	2
		Copepod	<i>Diacyclops sp.</i>	2
		Copepod	<i>Eucyclops agilis</i>	P403
		Copepod	<i>Mesocyclops leuckarti</i>	E812, P403
	Asellidae 92657	Isopod	<i>Asellus aquaticus</i> [†]	nonresident (I2)
		Isopod	<i>Caecidotea bicrenata</i> (Formerly <i>Asellus bicrenata</i>)	HH (I1,2)
		Isopod	<i>Asellus brevicaudus</i>	E875, P447, I
		Isopod	<i>Asellus communis</i>	E875, P448, I
		Isopod	<i>Asellus intermedius</i>	E875, P448, I
		Isopod	<i>Asellus meridionalis</i> [‡] <i>Asellus meridianus</i> [‡]	nonresident
		Isopod	<i>Asellus racovitzai</i>	P449, I
		Isopod	<i>Lirceus alabamae</i>	P875, I
	Crangonyctidae (Formerly Gammaridae) 95080	Amphipod	<i>Crangonyx pseudogracilis</i>	P459, T68, FF23
	Gammaridae 93745	Amphipod	<i>Gammarus fasciatus</i>	E877, P458, T53
		Amphipod	<i>Gammarus lacustris</i>	E877, P458, FF23
		Amphipod	<i>Gammarus pseudolimnaeus</i>	E877, P458, T48
		Amphipod	<i>Gammarus pulex</i> [‡]	nonresident
		Amphipod	<i>Gammarus tigrinus</i>	L51, FF17
		Amphipod	<i>Gammarus sp.</i>	2
Hyalellidae (Talitridae) 94022	Amphipod	<i>Hyalella azteca</i> <i>Hyalella knickerbockeri</i> [†]	E876, P457, T154	
Palaemonidae 96213	Prawn	<i>Macrobrachium lamarrei</i> [†]	nonresident	
	Prawn	<i>Macrobrachium rosenbergii</i>	⁶	
	Prawn	<i>Palaemonetes kadiakensis</i>	E881, P484	
Cambaridae (Formerly Astacidae) 97336	Crayfish	<i>Cambarus latimanus</i>	E897	
	Crayfish	<i>Faxonella clypeata</i>	E890	
	Crayfish	<i>Orconectes immunis</i>	E894, P482	
	Crayfish	<i>Orconectes limosus</i>	E893, P482	
	Crayfish	<i>Orconectes propinquus</i>	E894, P482	
	Crayfish	<i>Orconectes nais</i>	E894	
	Crayfish	<i>Orconectes rusticus</i>	E893, P482	
	Crayfish	<i>Orconectes virilis</i>	E894, P483	
	Crayfish	<i>Pacifastacus trowbridgii</i>	E883	
	Crayfish	<i>Procambarus acutus</i>	P482	
	Crayfish	<i>Procambarus clarki</i> <i>Procambarus clarkii</i> [†]	E885, P482	
	Crayfish	<i>Procambarus simulans</i>	E888, P482	
	Crayfish	<i>Procambarus sp.</i>	2	
	Insecta 99208	Heptageniidae 100504	Mayfly	<i>Maccaffertium ithaca</i> <i>Stenonema ithaca</i>
Mayfly			<i>Maccaffertium modestum</i> <i>Stenonema rubrum</i>	S178, O205
Baetidea		Mayfly	<i>Callibaetis skokianus</i>	S116, N9

Class	Family	Species		Reference
		Common Name	Scientific Name	
	100755	Mayfly	<i>Callibaetis sp.</i>	2
		Mayfly	<i>Cloeon dipterum</i>	O173
	Leptophlebiidae 101095	Mayfly	<i>Paraleptophlebia praepedita</i>	S89, O233
	Ephemereillidae 101232	Mayfly	<i>Drunella doddsii</i> <i>Ephemerella doddsi</i>	O245
		Mayfly	<i>Drunella grandis</i> <i>Ephemerella grandis</i>	O245
		Mayfly	<i>Ephemerella subvaria</i>	N9, O248, S71
		Mayfly	<i>Ephemerella sp.</i>	2
	Caenidea 101467	Mayfly	<i>Caenis diminuta</i>	S51, O268
	Ephemeridae 101525	Mayfly	<i>Ephemera simulans</i>	S36, N9, O283
		Mayfly	<i>Hexagenia bilineata</i>	N9, S39, O290
		Mayfly	<i>Hexagenia rigida</i>	O290, S41, N9
		Mayfly	<i>Hexagenia sp.</i>	2
	Libellulidae 101797	Dragonfly	<i>Pantala hymenaea</i> <i>Pantala hymenea</i>	N15, V603
	Coenagrionidae Agrionidae Coenagriidae 102077	Damselfly	<i>Enallagma aspersum</i>	DD
		Damselfly	<i>Ischnura elegans</i>	nonresident
		Damselfly	<i>Ischnura verticalis</i>	N15, E918
		Damselfly	<i>Ischnura sp.</i>	2
	Pteronarcyidae (Formerly Pteronarcidae) Pteronarcyidae 102470	Stonefly	<i>Pteronarcella badia</i>	L172
		Stonefly	<i>Pteronarcys californica</i>	L173
		Stonefly	<i>Pteronarcys dorsata</i>	E947
		Stonefly	<i>Pteronarcys sp.</i>	2
	Nemouridae 102517	Stonefly	<i>Nemoura cinerea</i>	nonresident
	Perlidae 102914	Stonefly	<i>Acroneuria lycorias</i>	N4, E953
		Stonefly	<i>Acroneuria pacifica</i>	E953, L180
		Stonefly	<i>Claassenia sabulosa</i>	E953
		Stonefly	<i>Agneta capitata</i> <i>Neophasganophora capitata</i> <i>Phasganophora capitata</i>	E953, CC407
	Perlodidae 102994	Stonefly	<i>Skwala americana</i> <i>Arcynopteryx parallela</i>	E954
	Nepidae 103747	Water Scorpion	<i>Ranatra elongate</i> (Species cannot be confirmed in ITIS)	nonresident
	Dytiscidae 111963	Beetle	-	2
	Elmidae Elminthidae 114093	Beetle	<i>Stenelmis sexlineata</i>	W21
	Hydropsychidae 115398	Caddisfly	<i>Arctopsyche grandis</i>	L251, I198
		Caddisfly	<i>Hydropsyche betteni</i>	N24
		Caddisfly	<i>Hydropsyche californica</i>	L253
		Caddisfly	<i>Hydropsyche sp.</i>	2
	Limnephilidae 115933	Caddisfly	<i>Clistoronia magnifica</i>	I1206
		Caddisfly	<i>Philarctus quaeris</i>	I1272

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Brachycentridae 116905	Caddisfly	<i>Brachycentrus sp.</i>	2
	Tipulidae 118840	Crane fly	<i>Tipula sp.</i>	2
	Ceratopogonidae 127076	Biting midge	-	2
	Culicidae 125930	Mosquito	<i>Aedes aegypti</i>	EE3
		Mosquito	<i>Culex pipiens</i>	EE3
	Chironomidae 127917	Midge	<i>Chironomus plumosus</i> <i>Tendipas plumosus</i>	L423
		Midge	<i>Chironomus tentans</i>	Q
		Midge	<i>Chironomus thummi</i>	nonresident
		Midge	<i>Chironomus sp.</i>	2
		Midge	<i>Paratanytarsus parthenogeneticus</i>	7
		Midge	<i>Paratanytarsus dissimilis</i> <i>Tanytarsus dissimilis</i>	R11
	Athericidae (Formerly Rhagionidae) Leptidae 130928	Snipe fly	<i>Atherix sp.</i>	2
Phylum: Ectoprocta (155470)				
Phylactolaemata 156688	Pectinatellidae (Formerly Pectinatelcidae) 156729	Bryozoan	<i>Pectinatella magnifica</i>	E502, P269
	Lophopodidae 156714	Bryozoan	<i>Lophopodella carteri</i>	E502, P2671
	Plumatellidae 156690	Bryozoan	<i>Plumatella emarginata</i>	E505, P272
Phylum: Chordata (158852)				
Agnatha 159693	Petromyzontidae 159697	Sea lamprey	<i>Petromyzon marinus</i>	F11
Actinopterygii (Formerly Osteichthyes) 161061	Anguillidae 161125 Salmonidae 161931	American eel	<i>Anguilla rostrata</i>	F15
		Pink salmon	<i>Oncorhynchus gorbuscha</i>	F18
		Coho salmon	<i>Oncorhynchus kisutch</i>	F18
		Sockeye salmon	<i>Oncorhynchus nerka</i>	F19
		Chinook Salmon	<i>Oncorhynchus tshawytscha</i>	F19
		Mountain whitefish	<i>Prosopium williamsoni</i>	F19
		Golden Trout	<i>Oncorhynchus aguabonita</i> (Formerly <i>Salmo aguabonita</i>)	F19
		Cutthroat trout	<i>Oncorhynchus clarki</i> (Formerly <i>Salmo clarki</i>)	F19
		Rainbow trout Steelhead trout	<i>Oncorhynchus mykiss</i> (Formerly <i>Salmo gairdneri</i>)	F19
		Atlantic salmon	<i>Salmo salar</i>	F19
		Brown trout	<i>Salmo trutta</i>	F19
		Brook trout	<i>Salvelinus fontinalis</i>	F19
Lake trout	<i>Salvelinus namaycush</i>	F19		

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Esocidae 162137	Northern pike	<i>Esox lucius</i>	F20
	Cyprinidae 163342	Chiselmouth	<i>Acrocheilus alutaceus</i>	F21
		Longfin dace	<i>Agosia chrysogaster</i>	F21
		Central stoneroller	<i>Campostoma anomalum</i>	F21
		Goldfish	<i>Carassius auratus</i>	F21
		Common carp	<i>Cyprinus carpio</i>	F21
		Zebra danio Zebrafish*	<i>Danio rerio</i> [†] <i>Brachydanio rerio</i> [†]	nonresident F96
		Silverjaw minnow	<i>Notropis buccatus</i> <i>Ericymba buccata</i>	F21
		Golden shiner	<i>Notemigonus crysoleucas</i>	F23
		Pugnose shiner	<i>Notropis anogenus</i>	F23
		Emerald shiner	<i>Notropis atherinoides</i>	F23
		Striped shiner	<i>Luxilus chrysocephalus</i> <i>Notropis chrysocephalus</i> *	F23
		Common shiner	<i>Luxilus cornutus</i> <i>Notropis cornutus</i> *	F23
		Pugnose minnow	<i>Opsopoeodus emiliae</i> <i>Notropis emiliae</i> *	F24
		Spottail shiner	<i>Notropis hudsonius</i>	F24
		Red shiner	<i>Cyprinella lutrensis</i> <i>Notropis lutrensis</i> *	F24
		Spotfin shiner	<i>Cyprinella spiloptera</i> <i>Notropis spilopterus</i>	F25
		Sand shiner	<i>Notropis stramineus</i>	F25
		Steelcolor shiner	<i>Cyprinella whipplei</i> <i>Notropis whipplei</i>	F25
		Northern redbelly dace	<i>Phoxinus eos</i>	F25
		Bluntnose minnow	<i>Pimephales notatus</i>	F25
		Fathead minnow	<i>Pimephales promelas</i>	F25
		Northern squawfish	<i>Ptychocheilus oregonensis</i>	F25
		Blacknose dace	<i>Rhinichthys atratulus</i>	F25
		Speckled dace	<i>Rhinichthys osculus</i>	F25
		Bitterling	<i>Rhodeus sericeus</i>	F26
		Rudd	<i>Scardinius erythrophthalmus</i>	F26
		Creek chub	<i>Semotilus atromaculatus</i>	F26
	Pearl dace	<i>Margariscus margarita</i> <i>Semotilus margarita</i>	F26	
	Tench	<i>Tinca tinca</i>	F26	
	Catostomidae 163892	White sucker	<i>Catostomus commersoni</i>	F26
		Mountain sucker	<i>Catostomus platyrhynchus</i>	F26
	Ictaluridae 163995	Black bullhead	<i>Ameiurus melas</i> <i>Ictalurus melas</i> *	F27
		Yellow bullhead	<i>Ameiurus natalis</i> <i>Ictalurus natalis</i>	F27
		Brown bullhead	<i>Ameiurus nebulosus</i> <i>Ictalurus nebulosus</i> *	F27
		Channel catfish	<i>Ictalurus punctatus</i>	F27

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Clariidae 164118	Walking catfish	<i>Clarias batrachus</i>	F28
	Adrianichthyidae (Formerly Oryziidae) 165623	Medaka	<i>Oryzias latipes</i>	nonresident F96
	Cyprinodontidae 165629	Banded killifish	<i>Fundulus diaphanus</i>	F33
		Flagfish	<i>Jordanella floridae</i>	F33
	Poeciliidae 165876	Mosquitofish	<i>Gambusia affinis</i>	F33
		Amazon molly	<i>Poecilia formosa</i>	F34
		Sailfin molly	<i>Poecilia latipinna</i>	F34
		Molly	<i>Poecilia sp.</i>	
		Guppy	<i>Poecilia reticulata</i> (<i>Lebistes reticulatus</i> , Obs.)	F34
	Gasterosteidae 166363	Southern platyfish	<i>Xiphophorus maculatus</i>	F34
		Brook stickleback	<i>Culaea inconstans</i>	F35
		Threespine stickleback	<i>Gasterosteus aculeatus</i>	F35
	Percichthyidae 170315	Ninespine stickleback	<i>Pungitius pungitius</i>	F35
		White perch	<i>Morone americana</i> (<i>Roccus americanus</i> , Obs.)	F36
		Striped bass	<i>Morone saxatilis</i> (<i>Roccus saxatilis</i> , Obs.)	F36
	Centrarchidae 168093	Rock bass	<i>Ambloplites rupestris</i>	F38
		Green sunfish	<i>Lepomis cyanellus</i>	F38
		Pumpkinseed	<i>Lepomis gibbosus</i>	F38
		Orangespotted sunfish	<i>Lepomis humilis</i>	F38
		Bluegill	<i>Lepomis macrochirus</i>	F38
		Longear sunfish	<i>Lepomis megalotis</i>	F38
		Redear sunfish	<i>Lepomis microlophus</i>	F38
		Smallmouth bass	<i>Micropterus dolomieu</i>	F39
		Largemouth bass	<i>Micropterus salmoides</i>	F39
		White crappie	<i>Pomoxis annularis</i>	F39
		Black crappie	<i>Pomoxis nigromaculatus</i>	F39
	Percidae 168356	Rainbow darter	<i>Etheostoma caeruleum</i>	F39
		Johnny darter	<i>Etheostoma nigrum</i>	F40
		Orangethroat darter	<i>Etheostoma spectabile</i>	F40
		Yellow perch	<i>Perca flavescens</i>	F41
		Walleye	<i>Sander vitreus</i> <i>Stizostedion vitreum vitreum</i>	F41
	Sciaenidae 169237	Freshwater drum	<i>Aplodinotus grunniens</i>	F45
	Cichlidae 169770	Oscar	<i>Astronotus ocellatus</i>	F47
		Blue tilapia	<i>Tilapia aurea</i>	F47
		Mozambique tilapia	<i>Oreochromis mossambicus</i> <i>Tilapia mossambica</i>	F47
	Cottidae 167196	Mottled sculpin	<i>Cottus bairdi</i>	F60
Amphibia 173420	Ranidae 173433	Bullfrog	<i>Rana catesbeiana</i>	B206
		Green frog	<i>Rana clamitans</i>	B206

Class	Family	Species		Reference
		Common Name	Scientific Name	
		Pig frog	<i>Lithobates grylio</i> <i>Rana grylio</i>	B206
		River frog	<i>Rana heckscheri</i>	B206
		Leopard frog	<i>Rana pipiens</i>	B205
		Wood frog	<i>Rana sylvatica</i>	B206
		Frog	<i>Rana temporaria</i> ²	nonresident
		Leopard frog	<i>Lithobates sphenoccephalus</i> <i>sphenocephalus</i> (Formerly <i>Rana spenocephala</i>)	JJ
	Microhylidae 173465	Eastern narrow-mouthed toad	<i>Gastrophryne carolinensis</i>	B192
	Bufonidae 173471	American toad	<i>Anaxyrus americanus americanus</i> <i>Bufo americanus</i>	B196
		Toad	<i>Bufo bufo</i> ²	nonresident
		Green toad	<i>Anaxyrus debilis debilis</i> <i>Bufo debilis</i>	B197
		Fowler's toad	<i>Anaxyrus fowleri</i> <i>Bufo fowleri</i> ²	B196
		Red-spotted toad	<i>Anaxyrus punctatus</i> <i>Bufo punctatus</i>	B198
		Woodhouse's toad	<i>Anaxyrus woodhousii woodhousii</i> <i>Bufo woodhousii</i>	B196
	Hylidae 173497	Northern cricket frog	<i>Acris crepitans</i>	B203
		Southern gray treefrog	<i>Hyla chrysoscelis</i>	B201
		Spring creeper	<i>Pseudacris crucifer</i> <i>Hyla crucifer</i> ²	B202
		Barking treefrog	<i>Hyla gratiosa</i>	B201
		Squirrel treefrog	<i>Hyla squirella</i>	B201
		Gray treefrog	<i>Hyla versicolor</i>	B200
		Northern chorus frog	<i>Pseudacris triseriata</i>	B202
	Pipidae 173547	African clawed frog	<i>Xenopus laevis</i>	Z16
	Ambystomatidae 173588	Spotted salamander	<i>Ambystoma maculatum</i>	B176
		Mexican axolotl	<i>Ambystoma mexicanum</i> ²	nonresident
		Marbled salamander	<i>Ambystoma opacum</i>	B176
	Salamandridae 173613	Newt	<i>Notophthalmus viridescens</i> <i>Triturus viridescens</i> ²	B179

Footnotes for Freshwater Species

- ¹ Apparently this is an outdated name (D19, 20). Organisms identified as such should only be used if they were obtained from North America.
- ² Apparently this is an outdated name (D19, 20). Organisms identified as such should only be used if they were obtained from North America.
- ³ If from North America, it is resident and should be called *D. similis* (C). If not from North America, it should be considered nonresident.
- ⁴ If from North America, it is resident and may be any one of a number of species such as *D. laevis*, *D. dubia*, or *D. galeate mendoca* (C). If not from North America, it should be considered nonresident.

⁵ If from North America, it is resident and may be any one of a number of species such as *D. ambigua*, *D. longiremis*, or *D. rosea* (C). If not from North America, it should be considered nonresident.

⁶ This species might be established in portions of the southern United States.

⁷ The taxonomy of this species and this and similar genera has not been clarified, but this species should be considered resident.

References for Freshwater Species

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Saltwater Species Table

Synonyms appear after the official Scientific Name and are marked with an asterisk (*).

Non-resident species are noted in the Reference column and are marked with a dagger (†).

Class	Family	Species		Reference
		Common Name	Scientific Name	
Phylum: Cnidaria (Coelenterata) (48738)				
Hydrozoa 48739	Campanulariidae 49470	Hydroid	<i>Campanularia flexiosa</i> ** <i>Campanularia flexuosa</i> **	B122, E81
		Hydroid	<i>Laomedea loveni</i> ^{††}	nonresident
		Hydromedusa	<i>Phialidium</i> sp.	[†] (E81)
	Campanulinidae 49756	Hydroid	<i>Eirene viridula</i> [†]	nonresident
Phylum: Ctenophora (53856)				
Tentaculata 53858	Pleurobrachiidae 53860	Ctenophore	<i>Pleurobrachia pileus</i>	B218, E162
	Mnemiidae 53915	Ctenophore	<i>Mnemiopsis mccradyi</i>	C39, I94
Phylum: Nemertea (Rhynchocoela) (57411)				
Heteronemertea 57438	Lineidae 57443	Nemertine worm	<i>Cerebratulus fuscus</i>	B252
Phylum: Rotifera (Rotatoria) (58239)				
Monogononta 58342	Brachionidae 58344	Rotifer	<i>Brachionus plicatilis</i>	B272
Phylum: Annelida (64357)				
Polychaeta 64358	Phyllodoceidae 65228	Polychaete worm	<i>Phyllodoce maculata</i> <i>Anaitides maculata</i> <i>Nereiphylla maculata</i> [*]	E334
	Nereididae (Nereidae) 65870	Polychaete worm	<i>Neanthes arenaceodentata</i> <i>Nereis arenaceodentata</i>	E377
		Polychaete worm	<i>Neanthes vaali</i> [†]	nonresident
		Polychaete worm	<i>Nereis diversicolor</i> <i>Neanthes diversicolor</i> [*]	E337, F527
		Sand worm	<i>Nereis virens</i> <i>Neanthes virens</i> [*]	B317, E337, C58
		Polychaete worm	<i>Nereis</i> sp.	
	Dorvilleidae 66478	Polychaete worm	<i>Ophryotrocha diadema</i>	P23
		Polychaete worm	<i>Ophryotrocha labronica</i> [†] <i>Ophryotrocha labrunica</i> [†]	nonresident
	Spionidae 66781	Polychaete worm	<i>Polydora websteri</i>	E338
	Cirratulidae 67116	Polychaete worm	<i>Cirriformia spirabranchia</i>	G253
	Ctenodrilidae 67217	Polychaete worm	<i>Ctenodrilus serratus</i>	G275
	Capitellidae 67413	Polychaete worm	<i>Capitella capitata</i>	B358, E337
	Arenicolidae 67500	Polychaete worm	<i>Arenicola marina</i>	B369, E337

** Synonym

†† Non-resident species

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Sabellidae 68076	Polychaete worm	<i>Eudistylia vancouveri</i>	DD
Oligochaeta 68422	Tubificidae 68585	Oligochaete worm	<i>Limnodriloides verrucosus</i>	Z
		Oligochaete worm	<i>Monopylephorus cuticulatus</i>	Z
		Oligochaete worm	<i>Peloscolex gabriellae</i> <i>Tubificoides gabriellae</i>	Z
Phylum: Mollusca (69458)				
Gastropoda 69459	Haliotididae 566897	Black abalone	<i>Haliotis cracherodii</i>	C88, D17
		Red abalone	<i>Haliotis rufescens</i>	D18
	Calyptraeidae 72611	Common Atlantic slippershell	<i>Crepidula fornicata</i>	C90, D141
	Muricidae 73236	Oyster drill	<i>Urosalpinx cinerea</i> <i>Urosalpinx cinereus</i>	B646, D179, E264
	Melongenidae (Neptuneidae) 74069	Channeled whelk	<i>Busycotypus canaliculatus</i> (Formerly <i>Busycon canaliculatum</i>)	B655, D223, E264
	Nassariidae (Nassidae) 74102	Mud snail	<i>Nassarius obsoletus</i> <i>Nassa obsoleta</i> <i>Icyanassa obsoleta</i>	B649, D226, E264
Bivalvia (Pelecypoda) 79118	Mytilidae 79451	Northern horse mussel	<i>Modiolus modiolus</i>	D434
		Blue mussel	<i>Mytilus edulis</i>	B566, C101, D428, E299
		Mediterranean mussel	<i>Mytilus galloprovincialis</i> ²	nonresident
	Pectinidae 79611	Bay scallop	<i>Argopecten irradians</i>	D447
	Ostreidae 79866	Pacific oyster	<i>Crassostrea gigas</i>	C102, D456, E300
		Eastern oyster	<i>Crassostrea virginica</i>	D456, E300
		Oyster	<i>Crassostrea sp.</i>	1
		Oyster	<i>Ostrea edulis</i>	E300
	Cardiidae 80865	Cockle	<i>Cerastoderma edule</i> ² <i>Cardium edule</i> ¹	nonresident
	Mactridae 80942	Clam	<i>Mulinia lateralis</i>	D491
		Common rangia	<i>Rangia cuneata</i>	D491, E301
		Surf clam	<i>Spisula solidissima</i>	B599, D489, E301
	Tellinidae 81032	Clam	<i>Macoma inquinata</i>	D507
		Bivalve	<i>Tellina tenuis</i> ²	nonresident
	Veneridae 81439	Quahog clam	<i>Mercenaria mercenaria</i>	D523, E301
		Common Pacific littleneck	<i>Protothaca staminea</i>	D526
		Japanese littleneck clam	<i>Tapes philippinarum</i>	D527
Myidae 81688	Soft-shell clam	<i>Mya arenaria</i>	B602, D536, E302	
Phylum: Arthropoda (82696)				
Merostomata 82698	Limulidae 82701	Horseshoe crab	<i>Limulus polyphemus</i>	B533, E403, H30
Branchiopoda (Formerly Crustacea) 83687	Artemiidae 83689	Brine shrimp	<i>Artemia salina</i> ²	² nonresident
Maxillopoda (Formerly Crustacea) 621145	Calanidae 85259	Copepod	<i>Calanus helgolandicus</i>	Q25
		Copepod	<i>Undinula vulgaris</i>	Q29
	Eucalanidae 85299	Copepod	<i>Eucalanus elongatus</i>	AA
		Copepod	<i>Subeucalanus pileatus</i> <i>Eucalanus pileatus</i>	AA
	Pseudocalanidae 85351	Copepod	<i>Pseudocalanus minutus</i>	E447, I155, Q43

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Euchaetidae 85524	Copepod	<i>Euchaeta marina</i>	Q63
	Metridinidae (Formerly Metridiidae) 593501	Copepod	<i>Metridia pacifica</i>	X179, Y
	Pseudodiaptomidae 85847	Copepod	<i>Pseudodiaptomus coronatus</i>	E447, I154, Q101
	Temoridae 85855	Copepod	<i>Eurytemora affinis</i>	E450, I155, Q111
	Pontellidae 86038	Copepod	<i>Labidocera scotti</i>	R157
	Acartiidae 86083	Copepod	<i>Acartia clausi</i>	E447
		Copepod	<i>Acartia tonsa</i>	E447, I154
	Harpacticidae 86329	Copepod	<i>Tigriopus californicus</i>	J78
		Copepod	<i>Tigriopus japonicus</i> [†]	nonresident
	Tisbidae 86444	Copepod	<i>Tisbe holothuriae</i>	BB
	Ameiridae (Formerly Canthocamptidae) 86999	Copepod	<i>Nitokra spinipes</i> <i>Nitocra spinipe</i>	Q240
	Archaeobalanidae (Formerly Balanidae) 89681	Barnacle	<i>Semibalanus balanoides</i> <i>Balanus balanoides</i>	B424, E457
	Balanidae 89599	Barnacle	<i>Balanus crenatus</i>	B426, E457
		Barnacle	<i>Balanus eburneus</i>	B424, E457
		Barnacle	<i>Balanus improvisus</i>	B426, E457
Malacostraca (Formerly Crustacea) 89787	Mysidae 89856	Mysid	<i>Heteromysis formosa</i>	E513, K720
		Mysid	<i>Americamysis bahia</i> <i>Mysidopsis bahia</i>	U173
		Mysid	<i>Americamysis bigelowi</i> <i>Mysidopsis bigelowi</i> [†]	E513, K720
		Mysid	<i>Neomysis sp.</i>	1
	Idoteidae 92564	Isopod	<i>Idotea balthica</i> <i>Idothea baltica</i>	B446, E483
		Isopod	<i>Idotea emarginata</i> [†]	nonresident
		Isopod	<i>Idotea neglecta</i> [†]	nonresident
	Janiridae 92810	Isopod	<i>Jaera albifrons</i> [†]	nonresident
		Isopod	<i>Jaera albifrons sensu</i> [†]	nonresident
		Isopod	<i>Jaera nordmanni</i> [†]	nonresident
	Ampeliscidae 93320	Amphipod	<i>Ampelisca abdita</i>	E488, L136
	Eusiridae (Pontogeneiidae) 93681	Amphipod	<i>Pontogeneia sp.</i>	1
	Gammaridae 93745	Amphipod	<i>Gammarus duebeni</i>	L56
		Amphipod	<i>Gammarus oceanicus</i>	E489, L50
		Amphipod	<i>Gammarus tigrinus</i>	L51
		Amphipod	<i>Gammarus zaddachi</i> [†]	nonresident
		Amphipod	<i>Marinogammarus obtusatus</i>	L58
	Uristidae (Formerly Lysianassidae) 621432	Amphipod	<i>Anonyx sp.</i>	1
	Euphausiidae (Thysanopodidae) 95500	Euphausiid	<i>Euphausia pacifica</i>	M15
	Penaeidae	Brown shrimp	<i>Penaeus aztecus</i>	E518, N17

Class	Family	Species		Reference
		Common Name	Scientific Name	
	95602	Pink shrimp	<i>Penaeus duorarum</i>	E518, N17
		White shrimp	<i>Penaeus setiferus</i>	E518, N17
		Blue Shrimp	<i>Penaeus stylirostris</i> [†]	nonresident
	Palaemonidae 96213	Shrimp	<i>Leander paucidens</i> [†]	nonresident
		Prawn	<i>Leander squilla</i> [†] <i>Palaemon elegans</i> [†]	nonresident
		Prawn	<i>Macrobrachium rosenbergii</i>	³
		Korean shrimp	<i>Palaemon macrodactylus</i>	T380
		Grass shrimp	<i>Palaemonetes pugio</i>	E521, N59
		Grass shrimp	<i>Palaemonetes vulgaris</i>	B500, E521, N56
	Hippolytidae 96746	Sargassum shrimp	<i>Latreutes fucorum</i>	N78
	Pandalidae 96965	Coon stripe shrimp	<i>Pandalus danae</i>	T306, W163
		Shrimp	<i>Pandalus goniurus</i>	W163
		Pink shrimp	<i>Pandalus montagui</i>	B494, E522, W163
	Crangonidae 97106	Sand shrimp	<i>Crangon crangon</i> [†]	nonresident
		Bay shrimp	<i>Crangon franciscorum</i> <i>Crango franciscorum</i> [†]	V176, W164
		Shrimp	<i>Crangon nigricauda</i>	V176, W164
		Sand shrimp	<i>Crangon septemspinosus</i>	B500, E522
	Nephropidae (Homaridae) 97307	American lobster	<i>Homarus americanus</i>	B502, E532
		European lobster	<i>Homarus gammarus</i> [†]	nonresident
	Paguridae 97774	Hermit crab	<i>Pagurus longicarpus</i>	B514, E537, N125
	Cancridae 98670	Rock crab	<i>Cancer irroratus</i>	B518, E543, N175
		Dungeness crab	<i>Cancer magister</i>	T166, V185, W177
	Portunidae 98689	Blue crab	<i>Callinectes sapidus</i>	B521, C80, E543, N168
		Green crab	<i>Carcinus maenas</i>	C80, E543
	Xanthidae (Pilumnidae) 98748	Mud crab	<i>Eurypanopeus depressus</i>	B522, E543, N195
		Crab	<i>Leptodius floridanus</i>	S80
		Mud crab	<i>Rhithropanopeus harrisi</i>	E543, N187
	Varunidae (formerly Grapsidae) 621521	Shore crab	<i>Hemigrapsus nudus</i>	CC
Shore crab		<i>Hemigrapsus oregonensis</i>	CC	
Sesarmidae (formerly Grapsidae) 621520	Drift line crab	<i>Armases cinereum</i> (<i>Sesarma cinereum</i>)	B526, E544, N222	
	Crab	<i>Sesarma haematocheir</i> [†]	nonresident	
Ocypodidae 99080	Fiddler crab	<i>Uca pugilator</i>	B526, E544, N232	
Phylum: Echinodermata (156857)				
Asteroidea 156862	Asteriidae 157212	Starfish	<i>Asterias forbesi</i>	B728, E578, O392
Ophiuroidea 157325	Ophiothricidae 157792	Brittle star	<i>Ophiothrix spiculata</i>	O672, T526
Echinoidea 157821	Arbaciidae 157904	Sea urchin	<i>Arbacia lixula</i> [†]	nonresident
		Sea urchin	<i>Arbacia punctulata</i>	B762, E572
	Toxopneustidae 157919	Sea urchin	<i>Lytechinus pictus</i>	T253
		Sea urchin	<i>Pseudocentrotus depressus</i> [†]	nonresident
	Echinidae 157940	[chinoderm]	<i>Paracentrotus lividus</i> [†]	nonresident
Echinometridae 157955		Coral reef echinoid	<i>Echinometra mathaei</i> [†]	nonresident [Hawaii only]

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Strongylocentrotidae 157965	Sea urchin	<i>Strongylocentrotus purpuratus</i>	O574, T202
	Dendrasteridae 158008	Sand dollar	<i>Dendraster excentricus</i>	O537, V363
Phylum: Chaetognatha (158650)				
Sagittoidea 158655	Sagittidae 158726	Arrow worm	<i>Ferosagitta hispida</i> <i>Sagitta hispida</i>	E218
Phylum: Chordata (158852)				
Chondrichthyes 159785	Rajidae 160845	Thornback ray	<i>Raja clavata</i> [†]	nonresident
Actinopterygii (Formerly Osteichthyes) 161061	Anguillidae 161125	American eel	<i>Anguilla rostrata</i>	A15
		Clupeidae 161700	Atlantic menhaden	<i>Brevoortia tyrannus</i>
	Gulf menhaden		<i>Brevoortia patronus</i>	A17
	Atlantic herring		<i>Clupea harengus</i> <i>Clupea harengus harengus</i> [*]	A17
	Pacific herring		<i>Clupea pallasii</i> <i>Clupea harengus pallasii</i> [*]	A17
	Herring		<i>Clupea harengus</i>	A17
	Engraulidae 553173	Northern anchovy	<i>Engraulis mordax</i>	A18
		Nehu	<i>Encrasicholina purpurea</i> [†] <i>tolephorus purpureus</i> [*]	nonresident [Hawaii only]
	Salmonidae 161931	Pink salmon	<i>Oncorhynchus gorbuscha</i>	A18
		Chum salmon	<i>Oncorhynchus keta</i>	A18
		Coho salmon	<i>Oncorhynchus kisutch</i>	A18
		Sockeye salmon	<i>Oncorhynchus nerka</i>	A19
		Chinook salmon	<i>Oncorhynchus tshawytscha</i>	A19
		Rainbow trout (Steelhead trout)	<i>Oncorhynchus mykiss</i> (Formerly <i>Salmo gairdneri</i>)	A19
		Atlantic salmon	<i>Salmo salar</i>	A19
	Gadidae 164701	Atlantic cod	<i>Gadus morhua</i>	A30
		Haddock	<i>Melanogrammus aeglefinus</i>	A30
	Cyprinodontidae 165629	Sheepshead minnow	<i>Cyprinodon variegatus</i>	A33
		Mummichog	<i>Fundulus heteroclitus</i>	A33
		Striped killifish	<i>Fundulus majalis</i>	A33
		Longnose killifish	<i>Fundulus similis</i>	A33
	Poeciliidae 165876	Mosquitofish	<i>Gambusia affinis</i>	A33
		Sailfin molly	<i>Poecilia latipinna</i>	A34
Atherinidae 165984	Inland silverside	<i>Menidia beryllina</i>	A34	
	Atlantic silverside	<i>Menidia menidia</i>	A34	
	Tidewater silverside	<i>Menidia peninsulae</i>	A34	
Gasterosteidae 166363	Threespine stickleback	<i>Gasterosteus aculeatus</i>	A35	
	Fourspine stickleback	<i>Apeltes quadracus</i>	A35	
Syngnathidae 166443	Northern pipefish	<i>Syngnathus fuscus</i>	A36	
Percichthyidae 170315	Striped bass	<i>Morone saxatilis</i> (<i>Roccus saxatilis</i> , Obs.)	A36	
Kuhliidae 168083	Mountain bass	<i>Kuhlia sandvicensis</i> [†]	nonresident [Hawaii only]	
Carangidae 168584	Florida Pompano	<i>Trachinotus carolinus</i>	A43	

Class	Family	Species		Reference
		Common Name	Scientific Name	
	Sparidae 169180	Pinfish	<i>Lagodon rhomboides</i>	A45
	Sciaenidae 169237	Spot	<i>Leiostomus xanthurus</i>	A46
		Atlantic croaker	<i>Micropogonias undulatus</i>	A46
		Red drum	<i>Sciaenops ocellatus</i>	A46
	Embiotocidae 169735	Shiner perch	<i>Cymatogaster aggregata</i>	A47
		Dwarf perch	<i>Micrometrus minimus</i>	A48
	Pomacentridae 170044	Blacksmith	<i>Chromis punctipinnis</i>	A48
	Labridae 170477	Cunner	<i>Tautoglabrus adspersus</i>	A49
		Bluehead	<i>Thalassoma bifasciatum</i>	A49
	Mugilidae 170333	Mullet	<i>Aldrichetta forsteri</i> ^f	nonresident
		Striped mullet	<i>Mugil cephalus</i>	A49
		White mullet	<i>Mugil curema</i>	A49
	Ammodytidae 171670	Pacific sand lance	<i>Ammodytes hexapterus</i>	A53
	Gobiidae 171746	Longjaw mudsucker	<i>Gillichthys mirabilis</i>	A54
		Naked goby	<i>Gobiosoma boscii</i>	A54
	Cottidae 167196	Tidepool sculpin	<i>Oligocottus maculosus</i>	A61
	Bothidae 172714	Speckled sanddab	<i>Citharichthys stigmaeus</i>	A64
		Summer Flounder	<i>Paralichthys dentatus</i>	A64
	Pleuronectidae 172859	Dab	<i>Limanda limanda</i> ^f	nonresident
		Plaice	<i>Pleuronectes platessa</i> ^f	nonresident
		English sole	<i>Parophrys vetulus</i>	A65
		Winter flounder	<i>Pseudopleuronectes americanus</i>	A65
	Balistidae 173128	Planehead filefish	<i>Monacanthus hispidus</i>	A66
	Tetraodontidae 173283	Northern puffer	<i>Sphoeroides maculatus</i>	A66

Footnotes for Saltwater Species

- ¹ Organisms not identified to species are considered resident only if obtained from wild populations in North America.
- ² This species should not be used because it might be too atypical.
- ³ This species might be established in portions of the southern United States.

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Appendix 2. Example Calculation of Final Acute Value, Computer Program, and Printouts

A. Example Calculation

N = total number of MAVs in data set = 8

Rank	MAV	ln(MAV)	ln(MAV) ²	P = R / (N+1)	√P
4	6.4	1.8563	3.4458	0.44444	0.66667
3	6.2	1.8245	3.3290	0.33333	0.57735
2	4.8	1.5686	2.4606	0.22222	0.47140
1	0.4	-0.9163	0.8396	0.11111	0.33333
Sum		4.3331	10.0750	1.11110	2.04875

$$S^2 = \frac{10.0750 - (4.3331)^2 / 4}{1.11110 - (2.04875)^2 / 4} = 87.134$$

$$S = 9.3346$$

$$L = [4.3331 - (9.3346)(2.04875)] / 4 = -3.6978$$

$$A = (9.3346) (\sqrt{0.05}) - 3.6978 = -1.6105$$

$$FAV = e^{-1.6105} = 0.1998$$

B. Example Computer Program in BASIC Language for Calculating the FAV

```

10 REM This program calculates the FAV when there are less than
20 REM 59 MAVs in the data set
30 X = 0
40 X2 = 0
50 Y = 0
60 Y2 = 0
70 PRINT "How many MAVs are in the data set?"
80 INPUT N
90 PRINT "What are the four lowest MAVs?"
100 FOR R = 1 TO 4
110 INPUT V

```

```

120         X = X + LOG(V)
130         X2 = X2 + (LOG(V)) * (LOG(V))
140         P = R / (N + 1)
150         Y2 = Y2 + P
160         Y = Y + SQR((X2 - X * X / 4))
170     NEXT R
180     S = SQR((X2 - X * X / 4) / (Y2 - Y * Y / 4))
190     L = (X - S * Y) / 4
200     A = S * SQR(0.05) + L
210     F = EXP(A)
220     PRINT "FAV = " F
230     END

```

C. Example Printouts from Program

How many MAVs are in the data set?

? 8

What are the four lowest MAVs?

? 6.4

? 6.2

? 4.8

? .4

FAV = 0.1998

How many MAVs are in the data set?

? 16

What are the four lowest MAVs?

? 6.4

? 6.2

? 4.8

? .4

FAV = 0.4365