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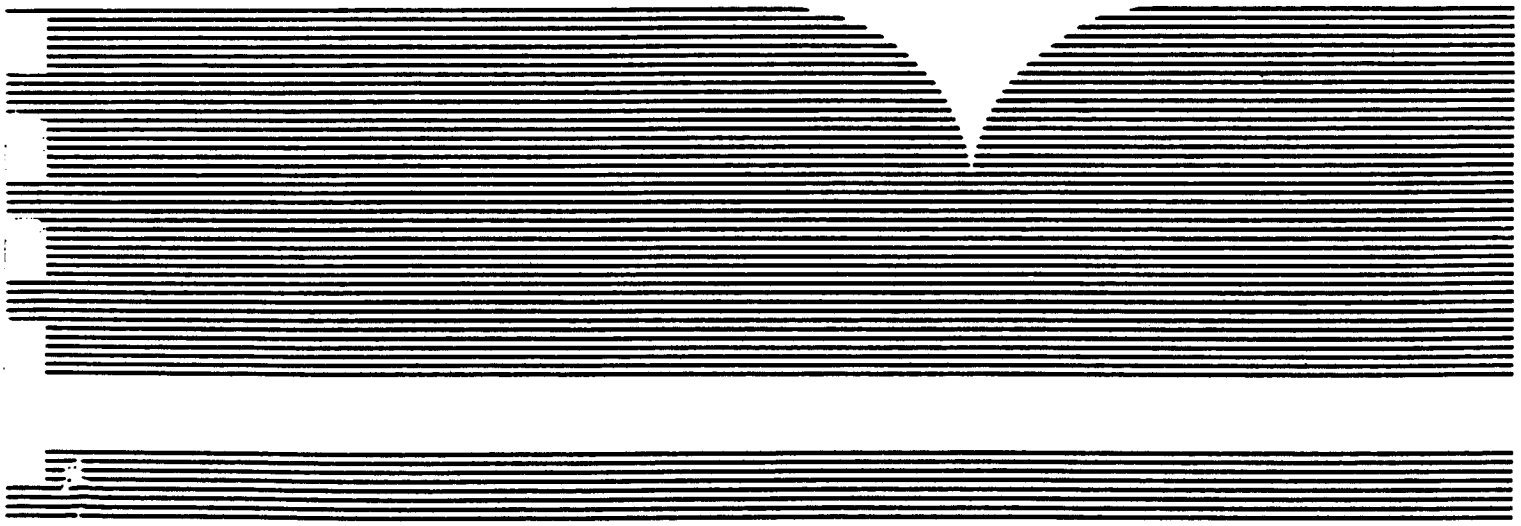

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Guidelines for Deriving Numerical Aquatic Site-Specific Water Quality Criteria by Modifying National Criteria

by

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16. ABSTRACT

A major goal of the U.S. Environmental Protection Agency is to directly link regulatory decision-making regarding priority water bodies to the capacity of those water bodies to receive wastewater discharges and still maintain acceptable water quality. To assist states in achieving this goal in a consistent, cost-effective manner, the Office of Research and Development (ORD) has developed a new approach to water quality criteria derivation with the report "Guidelines for Deriving Numerical Aquatic Site-Specific Water Quality Criteria by Modifying National Criteria."

These guidelines provide a series of protocols for modifying national water quality criteria to reflect local environmental conditions. The national criteria, because they are to protect the biological integrity of all water bodies, serve as benchmarks and may require adjustments for site-specific applications. The new protocols take into account site-specific variations in species composition, physical factors, and chemical water quality variables. Consideration of local conditions assures that criteria for a given water body are tailored specifically to its aquatic life and uses.

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INTRODUCTION

Relationship to the National Guidelines

These Guidelines for Deriving Numerical Aquatic Site-Specific Water Quality Criteria by Modifying National Criteria (hereinafter referred to as the Site-Specific Guidelines) are the next steps evolving from the Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Life and Its Uses (U.S. Environmental Protection Agency, 1983) (hereinafter referred to as the National Guidelines).

In that the Site-Specific Guidelines follow from the National Guidelines, an understanding of the National Guidelines and the national criteria document for the material of interest is a prerequisite for understanding and use of the Site-Specific Guidelines. The derivation of a site-specific criterion for freshwater or saltwater aquatic life will generally evolve from national criteria that are available for a limited number of chemicals (Appendix 1). Site-specific criteria derived by these guidelines may be the same as, or higher or lower than national criteria.

In the absence of a national criterion, additional data may be generated so that the minimum data set requirements of the National Guidelines are met and a national or site-specific criterion may be calculated.

The national water quality criteria have been developed using guideline procedures that have undergone extensive scientific review regarding their general applicability. States may choose to apply these criteria directly or to modify them according to site-specific criteria guidelines. Whenever decisions are sought regarding modification of these criteria, the assistance of those biologists, chemists, hydrologists, and toxicologists most knowledgeable of the local species and conditions is essential to the proper evaluation of exposure assessment and population at risk.

Rationale for the Site-Specific Guidelines

National criteria may be underprotective or overprotective because: (1) The species at the site are more or less sensitive than those included in the national criteria data set; (2) the physical and/or chemical characteristics of the water at the site alters the biological availability and/or toxicity of the material. Therefore, it is appropriate that the individual Site-Specific Guidelines procedures address each of these conditions separately, as well as the combination of the two.

Site-specific criterion derivation may be justified because species at the site may be more or less sensitive than those in the national criterion document. For example, the national criteria data set contains data for trout, salmon, or penaeid shrimp, aquatic species that have been shown to be especially sensitive to some materials. Because these or other sensitive species may not occur at a particular site, they may not be representative of those species that do occur there. Conversely, there may exist at a site untested sensitive species that are ecologically or economically important and would need to be protected. Secondly, differences in physical and chemical characteristics of water have been demonstrated to ameliorate or enhance the biological availability and/or toxicity of chemicals in freshwater and saltwater environments. Alkalinity, hardness, pH, suspended solids and/or salinity influence the concentration(s) of the toxic form(s) of some heavy metals, ammonia and other chemicals. For some materials, hardness or pH-dependent national criteria are available for fresh water. No salinity-dependent criteria have been derived because most of the saltwater data for heavy metals has been developed in high salinity waters. However, in some estuarine sites where salinity may vary significantly, the

development of salinity-dependent site-specific criteria for metals of local interest may be appropriate.

The effect of seasonality on the physical and chemical characteristics of water and subsequent effects on biological availability and/or toxicity of a material, may also justify seasonally dependent site-specific criteria. The major implication of seasonally dependent criteria is whether or not the "most sensitive" time of the year coincides with that time for which the flow is the basis for waste treatment facilities design or NPDES permits. That is, if the physical and chemical characteristics of the water during low flow seasons increases the biological availability and/or toxicity of the chemical of concern, the permit limitations may be more restrictive than if the converse relationship were to apply.

Definition of Site

Since the rationales for the Site-Specific Guidelines are usually based on potential differences in species sensitivity, physical and chemical characteristics of the water, or a combination of the two, the concept of site must be consistent with this rationale.

A site may be a single point source discharge or quite large. If water quality effects on toxicity are not a consideration, the site will be as large as a generally consistent biogeographic zone permits. In this case, for example, large portions of the Chesapeake Bay, Lake Michigan, or the Ohio River may each be considered as one site because their respective aquatic communities do not vary substantially. Unique populations or less sensitive use within sites may justify a designation as a distinct site (site within a site). When sites are large, the necessary data generation can be more economically supportable.

If the selected species of a site are toxicologically comparable to those in the national criteria data set for a material of interest, and physical and/or chemical water characteristics are the only factors supporting modification of the national criteria, then the site would be defined on the basis of expected changes in the material's biological availability and/or toxicity due to physical and chemical variability of the site water.

Two additional considerations in defining a site are: 1) viable communities must occur, or be historically documented, in order to select resident species for use in deriving site-specific criteria, and 2) the site must contain acceptable quality dilution water if site water will be required for testing (to be discussed later in these Guidelines).

For the purpose of the Site-Specific Guidelines, the term "selected resident species" is defined as those species that commonly occur in a site including those that occur only seasonally (migration) or intermittently (periodically returns or extends its range into the site). It is not intended to include species that were once present in that site and cannot return due to physical habitat alterations.

Selection of a resident species should be designed to account for differences between the sensitivities of the selected resident species and those in the national data set. There are several possible reasons for this potential difference. The principal reason is that the resident communities in a site may represent a more or less narrow mix of species due to a limited range of natural environmental conditions (e.g., temperature, salinity, habitat, or other factors affecting the spatial distribution of aquatic species). The number of resident species will generally decrease as the size of the site decreases.

A second potential reason for a real difference in sensitivity could be the absence of most of the species or groups of species (e.g., families) that are traditionally considered to be sensitive to certain, but not all, materials (e.g., trout, salmon, saltwater penaeid shrimp, and Daphnia magna). Predictive relative species sensitivity does not apply to all materials, and the assumption that sensitive species are unique rather than representative of equally sensitive untested species is tenuous. A final reason could be that the resident species may have evolved a genetically based greater resistance to high concentrations of a material, but no data have been presented to demonstrate such a genetic difference. A few instances of increased resistance have been suggested but they may be due to an acclimation of individual organisms to a stress. However, such an acclimation, should it occur, would be transitory.

Assumptions

There are numerous assumptions associated with the Site-Specific Guidelines, most of which also apply to and have been discussed in the National Guidelines. A few need to be emphasized. The principal assumption is that the species sensitivity ranking and toxicological effect endpoints (e.g., death, growth, or reproduction), derived from appropriate laboratory tests will be similar to those in site situations. Another assumption is that protection of all of the site species all of the time is not necessary because aquatic life can tolerate some stress and occasional adverse effects.

It is assumed that the Site-Specific Guidelines are an attempt to protect more correctly the various uses of aquatic life by accounting for toxicological differences in species sensitivity or the biological

availability, and/or toxicity of a material at specific sites. Modification of the data set must always be scientifically justifiable and consistent with the assumptions, rationale, and spirit of the National Guidelines.

Site-specific criteria are designed to be used by the States to develop water quality standards, mixing zone standards, or toxicity based effluent standards. The development of such standards should take into account additional factors such as the use of the site, and social, legal, and economic considerations as they impact the site, the environmental and analytical chemistry of the material, the extrapolation from laboratory data to site situations, and the relationship between the species for which data [illegible] and the species in the body of water which is to be protected.

Heavy Metal Speciation

The national criteria for metals are established primarily using laboratory data in which reported effect concentrations have been analyzed primarily as total, total recoverable, or acid extractable metal concentrations. Consequently, the national criteria are expressed as total recoverable metal. Metals exist in a variety of chemical forms in water. Available toxicological data have demonstrated that some forms are much more toxic than others. Most of the toxicity appears to reside in the soluble fraction and, potentially, in the easily labile, nonsoluble fraction. The national criteria values may be unnecessarily stringent if applied to total metal measurements in waters where total metal concentrations include a preponderance of metal forms which are highly insoluble or strongly complexed. Derivation of criteria based on metal forms is not possible at this time because adequate laboratory or field data bases do not exist in which metal toxicity is partitioned among the various metal forms. Analysis

of total and soluble metal concentrations when soluble metal is added to site water may indicate that the metal is rapidly converted to insoluble forms or to other forms with presumed low biological availability. Under these circumstances, derivation of a site-specific criterion based on site-water effect in either the indicator or resident species procedures will probably result in less stringent criteria values.

Use of the indicator species or resident species procedures is encouraged for derivation of site-specific criteria for those metals whose biological availability and/or toxicity is significantly affected by variation in physical and/or chemical characteristics of water. Measurement of both total recoverable and soluble metal concentrations during toxicity testing is recommended.

Plant and Other Data

In the published criteria documents, no national criterion is based on plant data or "Other Data" (e.g. flavor impairment, behavioral, etc.). For some materials, observed effects on plants occurred at concentrations near the criterion. The following procedures do not contain techniques for handling such data, but if a less stringent site-specific criterion is derived, those data may need to be considered.

PROCEDURES

There are three procedures in these Site-Specific Guidelines for modifying the national criterion which is composed of both a maximum concentration and a 30-day average concentration. These procedures are:

A. The recalculation procedure for the derivation of a site-specific criterion to account for differences in resident species sensitivity to a material.

The indicator species procedure for the derivation of a site-specific criterion for a material to account for differences in biological availability and/or toxicity of a material caused by physical and/or chemical characteristics of a site water.

C. The resident species procedure for the derivation of a site-specific criterion to account for differences in resident species sensitivity and differences in the biological availability and/or toxicity of a material due to physical and/or chemical characteristics of a site water.

The following is the sequence of decisions to be made before any of the above procedures are initiated:

- Define the site boundaries.
- Determine from the national criterion document and other sources if physical and/or chemical characteristics are known to affect the biological availability and/or toxicity of a material of interest.
- If data in the national criterion document and/or from other sources indicate that the range of sensitivity of the selected resident species to the material of interest is different from that range for the species in the national criterion document and variation in physical and/or chemical characteristics of the site water is not expected to be a factor, use the recalculation procedure (A).
- If data in the national criterion document and/or from other sources indicate that physical and/or chemical characteristics of the site water may affect the biological availability and/or toxicity of the material of interest, and the selected resident species range of sensitivity is similar to that for the species in the national criterion document, use the indicator species procedure (B).

- If data in the national criterion document and/or from other sources indicated that physical and/or chemical characteristics of the site water may affect the biological availability and/or toxicity of the material of interest, and the selected resident species range of sensitivity is different from that for the species in the national criterion document, use the resident species procedure.

The following Figures 1 and 2 are generalized flow charts for these Guidelines.

A. Recalculation procedure for the derivation of a site-specific criterion to account for differences between selected resident species and other species.

1. Summary: This recalculation procedure allows modifications in the national data set on the basis of eliminating data for species that are not resident at that site. When the recalculation procedure for the site-specific Final Acute Value results in a reduction in the national data base below the minimum data set requirements, additional resident species testing in laboratory water is necessary.

2. Rationale: This procedure is designed to account for any real difference between the sensitivity range of species represented in the national data set and species found at a site.

3. Conditions:

   - If acute toxicity data for resident species are insufficient to meet the minimum data set requirements of the National Guidelines, additional acute toxicity data in laboratory water for untested resident species would be needed before a calculation of the site-specific criterion could be made.

F I G U R E 1
Generalized Flow Chart for Deriving Numerical Site-Specific Criteria for the Protection of Aquatic Life and Its Uses.

This is an illustration of procedures that can be used to derive the *Maximum Concentration* of a two-part criterion. Derivation of the 30-day Average Concentration is illustrated in Figure 2.

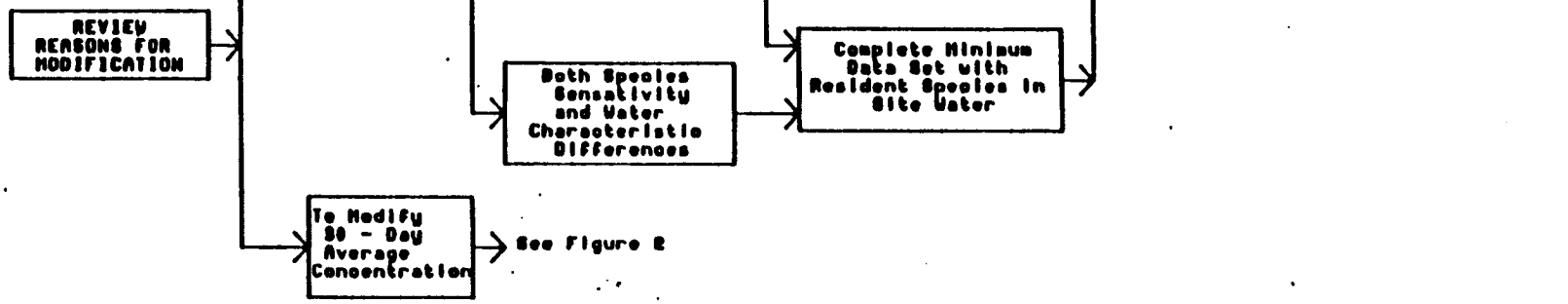
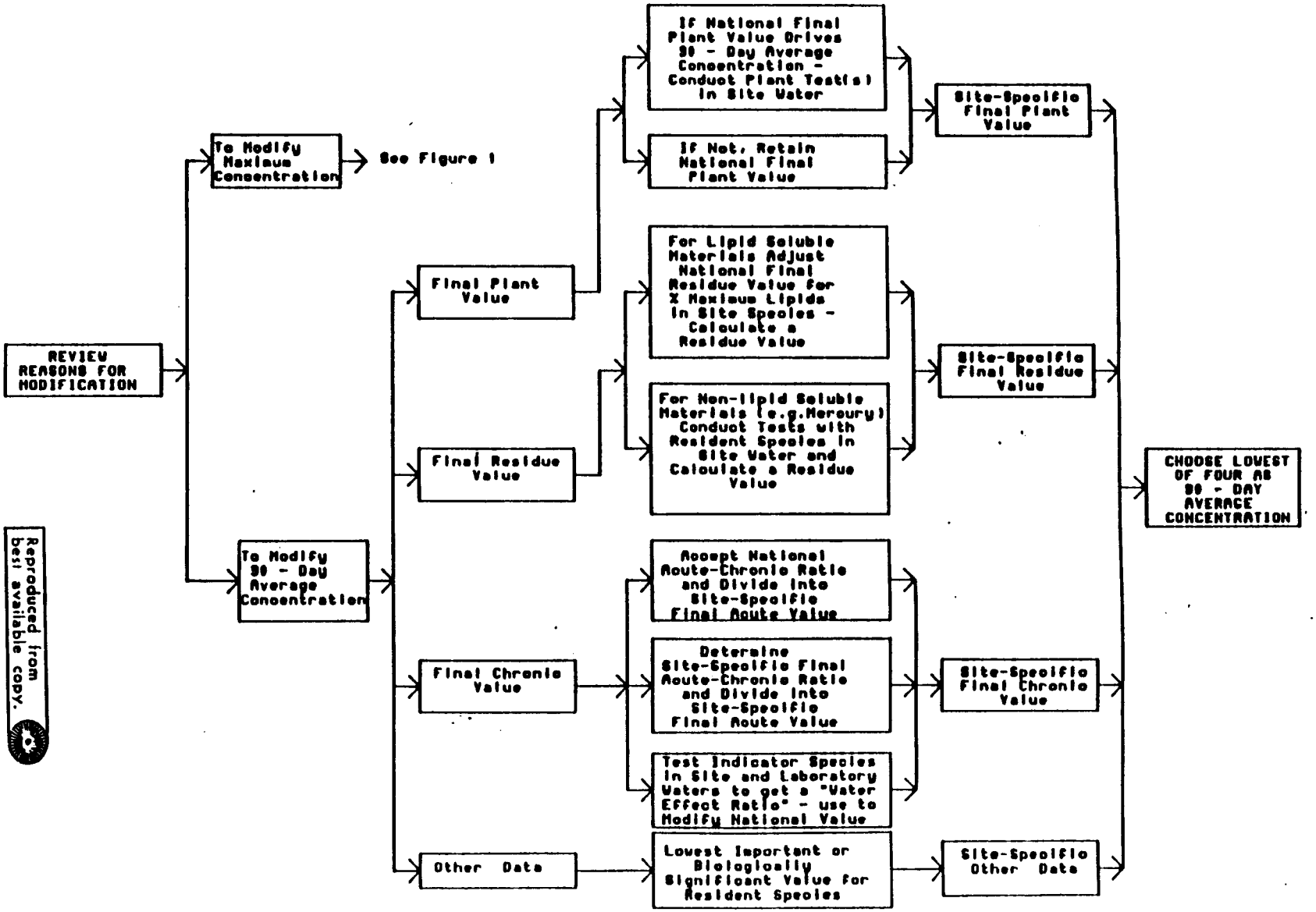


FIGURE 2
Generalized Flow Chart for Deriving Numerical Site-Specific Criteria for the Protection of Aquatic Life and Its Uses.

This is an illustration of procedures that can be used to derive the *30-day Average Concentration* of a two-part criterion. Derivation of the Maximum Concentration is illustrated in Figure 1.



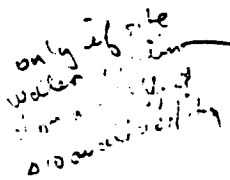
- Certain families or organisms have been specified to be represented in the National Guidelines acute toxicity minimum data set (e.g., Salmonidae in fresh water and Penaeidae or Mysidae in salt water). If this or any other requirement cannot be met because the family or other group (e.g., insect or benthic crustacean in fresh water) is not represented by resident species, select a substitute(s) from a sensitive family represented by one or more resident species and meet the 8 family minimum data set requirement. If all the families at the site have been tested and the minimum data set requirements have not been met use the most sensitive resident family mean acute value as the site-specific Final Acute Value.
- Due to the emphasis this procedure places on resident species testing when the minimum data set has not been met, there may be difficulty in selecting resident species compatible to laboratory testing. Some culture and/or handling techniques may need to be developed.
- No chronic testing is required by this procedure since the national acute-chronic ratio will be used with the site-specific Final Acute Value to obtain the site-specific Final Chronic Value.
- For the lipid soluble chemicals whose national Final Residue Values are based on Food and Drug Administration (FDA) action levels, adjustments in those values based on the percent lipid content of resident aquatic species is appropriate for the derivation of site-specific Final Residue Values.

- For lipid-soluble materials, the national Final Residue Value is based on an average 11 percent lipid content for edible portions for the freshwater chinook salmon and lake trout and an average of 10 percent lipids for the edible portion for saltwater Atlantic herring. Resident species of concern may have higher (e.g., Lake Superior siscowet, a race of lake trout) or lower (e.g., many sport fish) percent lipid content than used for the national Final Residue Value.
- For some lipid-soluble materials such as polychlorinated biphenyls (PCB) and DDT, the national Final Residue Value is based on wildlife consumers of fish and aquatic invertebrate species rather than an FDA action level because the former provides a more stringent residue level (see National Guidelines for details). Since the data base on the effects of ingested aquatic organisms on wildlife species is extremely limited, it would be inappropriate to base a site-specific Final Residue Value on resident wildlife species. Consequently, site-specific modification for those materials is based on percent lipid content of resident species consumed by humans.
- For the lipid-soluble materials whose national Final Residue Values are based on wildlife effects, the limiting wildlife species (mink for PCB and brown pelican for DDT) are considered acceptable surrogates for resident avian and mammalian species (e.g., herons, gulls, terns, otter, etc.). Conservatism is appropriate for those two chemicals, and no less restrictive modification of the national Final Residue Value is appropriate.

The site-specific Final Residue Value would be the same as the national value.

4. Details of Procedure:

- If the minimum data set requirements are met as defined in the National Guidelines or through substitution of one or more sensitive resident family(ies) for non-resident family(ies) or group(s) required in the National Guidelines, calculate a site-specific Final Acute Value using all available resident species data in the national document and/or from other sources. If all the families at the site have been tested and the minimum data set requirements have not been met use the most sensitive resident family mean acute value as the site-specific Final Acute value.
- If the minimum data set requirements are not met, satisfy those requirements with additional testing of resident species in laboratory water.



- a) If all species in a family at the site have been tested, then their Species Mean Acute Values should be used to calculate the site-specific Family Mean Acute Value and data for non-resident species in that family should be deleted from that calculation.

b) If all resident species in that family have not been tested, the site-specific Family Mean Acute Value would be the same as the national Family Mean Acute Value.

- To derive the site-specific maximum concentration divide the site-specific Final Acute Value by 2.
- Divide the site-specific Final Acute Value by the national Final Acute-Chronic Ratio to obtain the site-specific Final Chronic Value.

- When a site-specific Final Residue Value can be derived for lipid soluble materials controlled by FDA action levels, the following recalculation equation would be used:

  site-specific Final Residue Value =

$$\frac{\text{FDA action level}}{(\text{mean normalized BCF from criterion document})\ (\text{appropriate \% lipids})}$$

  where the appropriate percent lipid content is based on consumed resident species. A recommended method to determine the lipid content of tissues is given in Appendix 2.

- For PCB and DDT whose national Final Residue Values are based on wildlife consumers of aquatic organisms, no site-specific modification procedure is appropriate.

- In the case of mercury (a non-lipid-soluble material), a site-specific Final Residue Value can be derived by conducting acceptable bioconcentration tests with edible aquatic resident species using accepted test methods (Appendix 2) or the national value can be accepted as the site-specific value. For a saltwater residue value, use a bivalve species (the oyster is preferred), and for a freshwater value, use a fish species. These taxa yield the highest known bioconcentration factors for metals. The following recalculation equation would be used:

  site-specific Final Residue Value = $\frac{\text{FDA action level}}{\text{site-specific BCF}}$

- The lower of the site-specific Final Chronic Value and the site-specific Final Residue Value becomes the site-specific 30-day average concentration unless plant or other data indicate a lower value is appropriate. If a problem is identified,

judgment should be used in establishing the site-specific criterion.

5. Limitations:

- Whatever the results of this recalculation procedure may be, a decision should be made as to whether the numerical differences, if any, are sufficient to warrant changes in the criterion.
- The number of families used to calculate any Final Acute Value significantly affects that value. Even though the four lowest Family Mean Acute Values (most sensitive families) are most important in that calculation, the smaller N is, the lower the Final Acute Value. Consequently, if none of the four most sensitive families are changed or deleted, any reduction in N will result in a lower Final Acute Value. Changes in or deletions of any of the four lowest values, regardless of whether N is changed, may result in a higher or lower Final Acute Value.
- Site-specific or national Final Residue Values based on FDA action levels may not precisely protect that use since the FDA action levels are adverse (i.e., loss of marketability).
- Bioaccumulation, except in field studies, does not add to the laboratory-derived bioconcentration factors because the laboratory procedures preclude food chain uptake. Consequently, some residue levels obtained by laboratory studies of bioconcentration (direct uptake of the material from water) may underestimate potential effects encountered at a site. The magnitude of site-specific bioconcentration factors obtained in the laboratory, therefore, may be insufficient to protect the public from the effects of the ingested material of concern.

Indicator Species Procedure

B. Indicator species procedure for the derivation of a site-specific criterion for a material to account for differences in the biological availability and/or toxicity of a material due to physical and/or chemical characteristics of a site water.

1. Summary: This procedure is based on the assumption that physical and/or chemical characteristics of water at an individual site may influence biological availability and/or toxicity of a material. Acute toxicity in site water and laboratory water is determined using species resident in the site, or acceptable nonresident species, as indicators or surrogates for species found at the site. The difference in toxicity values, expressed as a water effect ratio, is used to convert the national maximum concentration for a material to a site-specific maximum concentration from which a site-specific Final Acute Value is derived.

This procedure also provides three ways to obtain a site-specific Final Chronic Value. It may be (1) calculated (no testing required) if an applicable Final Acute-Chronic Ratio for a given material is available in the national criteria document. This ratio is simply divided into the site-specific Final Acute Value to obtain the site-specific Final Chronic Value; (2) obtained by performing two acute and chronic toxicity tests which include both a fish and invertebrate species (resident or non-resident) in site water. Acute-chronic ratios are calculated for each species, and the geometric mean of these ratios is then divided into the site-specific Final Acute Value to obtain the site-specific Final Chronic Value; and (3) obtained by performing chronic toxicity tests with at least one fish and one invertebrate (resident or non-resident) in both

laboratory water and site water and calculating a geometric mean chronic water effect ratio which is used to modify the national Final Chronic Value.

2. Rationale: This procedure is designed to compensate for site water which may markedly affect the biological availability and/or toxicity of a material. Major factors affecting aquatic toxicity values of many materials, especially the heavy metals, have been identified. For example, the carbonate system of natural waters (pH, hardness, alkalinity, and carbon dioxide relationships) has been the most studied and quantified with respect to effects on heavy metal biological availability and/or toxicity in freshwater; however, the literature indicates that in natural systems organic solutes, inorganic and organic colloids, salinity and suspended particles also play an important but less quantifiable role in the biological availability and/or toxicity of heavy metals to aquatic life. This procedure provides a means of obtaining a site-specific Final Chronic Value for a material when the acute-chronic ratios in the national criteria document are thought to be inapplicable to site-specific situations.

3. Conditions:

   - There is no reason to suspect that the resident species sensitivity is different from those species in the national data set.
   - The toxic response seen in the tests used in the development of the national water quality criterion would be essentially the same if laboratory test water required in this procedure had been used instead.

- Differences in the toxicity values of a specific material determined in laboratory water and site water may be attributed to chemical (e.g., complexing ligands) and/or physical (e.g., adsorption) factors that alter the biological availability and/or toxicity of the material.
- Selected indicator species directly integrate differences in the biological availability and/or toxicity of a material. They provide a direct measure of the capacity of a site water to increase or decrease toxicity values relative to values obtained in laboratory water.
- National Final Acute-Chronic Ratios for certain materials can be used to establish site-specific Final Chronic Values.
- A site-specific acute-chronic ratio, obtained in site water testing, reflects the integrated effects of the physical and/or chemical characteristics of water on toxicity values.
- The water effect ratio concept used in this procedure for modifying national Final Acute Values to site-specific situations is also applicable to modifying national Final Chronic Values to site-specific situations.

4. Details of Procedure:

- Test at least two indicator species, a fish and an invertebrate, using laboratory dilution water and site dilution water according to acute toxicity test procedures recommended in Appendix 2. For each species, use organisms from the same population and conduct the tests at the same time and, most importantly (except for the water source) under similar conditions (e.g., temperature, lighting, etc.). Measure the concentration of the material in

the acute toxicity tests; the concentration must be within the solubility limits of the material. To avoid solubility problems, species selected for testing should be among the most sensitive to the material of interest (screening tests may be necessary).

- Compare the laboratory and site water LC50 values for each indicator species to determine if they are different ($P \leq 0.05$) (see statistical procedure in Appendix 3). If the LC50 values are not different, then the national maximum concentration is the site-specific maximum concentration. If the LC50 values are different, calculate the water effect ratio for each species according to the following equation:

$$\text{Water Effect Ratio} = \frac{\text{Site Water LC50 Value}}{\text{Laboratory Water LC50 Value}}$$

Determine if the two ratios are statistically different ($P \leq 0.05$) (see Appendix 3).

- If the two ratios are not different calculate the geometric mean of the water effect ratios. The site-specific maximum concentration can be calculated by using this geometric mean water effect ratio in the following equation: site-specific maximum concentration = water effect ratio x the national maximum concentration (or x the national maximum concentration adjusted to a water characteristic of the laboratory water when appropriate).

If the two ratios are different, additional tests may have to be conducted to confirm or refute the data. In such cases professional judgment is appropriate in determining if some or none of the ratio data can be used to modify the national maximum concentration.

The site-specific maximum concentration is multiplied by 2 to obtain the site-specific Final Acute Value which is used to calculate the site-specific Final Chronic Value.

- If the national Final Acute-Chronic Ratio for the material of interest was used to establish a national Final Chronic Value, the site-specific Final Chronic Value may be calculated using the acute-chronic ratio in the following equation:

$$\text{Site-Specific Chronic Value} = \frac{\text{Site-Specific Acute Value}}{\text{Final Acute-Chronic Ratio}}$$

- If the national Final Acute-Chronic Ratio was not used to establish a national Final Chronic Value, the national Final Chronic Value may be used as the site-specific Final Chronic Value, or it may be measured by performing 2 acute and 2 chronic tests, (Appendix 2) using site water. Test at least one fish and one invertebrate species, and conduct an acute test using site water of similar quality. These data are used to calculate an acute-chronic ratio for each species. If these ratios are within a factor of 10, the geometric mean of the 2 acute-chronic ratios (the site-specific Final Acute-Chronic Ratio) is used to calculate the site-specific Final Chronic Value using the following equation:

$$\text{Site-Specific Final Chronic Value} = \frac{\text{Site-Specific Final Acute Value}}{\text{Site-Specific Final Acute-Chronic Ratio}}$$

After an acute/chronic ratio is determined for one species and if that ratio is within the range of the values used to establish the national acute-chronic ratio, it is recommended that the

site-specific ratio be used in recalculating the national ratio. This recalculated ratio would then be used as the site-specific Final Acute-Chronic Ratio in the above equation.

- A site-specific Final Chronic Value can be obtained by testing indicator species for chronic toxicity. Test at least two indicator species, a fish and an invertebrate, using laboratory dilution water and site dilution water according to chronic toxicity test procedures recommended in Appendix 2. For each species, use organisms from the same population, and conduct tests at the same time and most importantly (except for the water source) under similar conditions (e.g., temperature, lighting). The concentration of the material in the toxicity tests must be within the solubility limits of the material. To avoid solubility problems, species selected for testing should be among the most sensitive to the material of interest (screening tests may be necessary).

  Compare the laboratory and site water chronic values for each of the indicator species to determine if they are reasonably different (limits of chronic values do not overlap).

  If for a species the chronic values are not different, the water effect ratio = 1.0.

  If the chronic values are different, calculate the water effect ratio for each species according to the following equation:

  Chronic Water Effect Ratio =

$$\frac{\text{Chronic Value in Site Water}}{\text{Chronic Value in Laboratory Water}}$$

Calculate the geometric mean of the water effect ratios for the species tested.

If the mean water effect ratio is not different from 1.0, the national Final Chronic Value is the site-specific Final Chronic Value.

If the mean water effect ratio is different from 1.0, the site-specific Final Chronic Value can be calculated by using the following equation: site-specific Final Chronic Value = Chronic Water Effect Ratio x the national Final Chronic Value (or the national Final Chronic Value adjusted to a water quality characteristic of the laboratory water when appropriate).

The site-specific Final Chronic Value is used in the determination of the site-specific 30-day average concentration.

- The lower of the site-specific Final Chronic Value and the recalculated site-specific Final Residue Value (as described in the recalculation procedure) becomes the site-specific 30-day average concentration unless plant or other data (including data obtained from the site-specific tests) indicates a lower value is appropriate. If a problem is identified, judgment should be used in establishing the site-specific criterion.

5. Limitations:

- If filter feeding organisms are determined to be among the most sensitive to the material of interest from the national criteria document and/or other sources, and members of the same group are important components of the site food web, a member of that group, preferably a resident species, should be tested in order to discern differences in the biological availability and/or

toxicity of the material of interest due to ingested particulates.

- Site water for testing purposes should be obtained under typical conditions and can be obtained at any time of the day or season. Storm or flood impacted water is unacceptable as test water in the acute tests used to calculate water effect ratios and acute/chronic ratios but is acceptable test water for short periods of time in long-term chronic tests used to calculate these ratios. There are some special cases when storm impacted water is acceptable in acute toxicity testing for use in criteria development. For example, an effluent discharge may be allowed only during high water periods, or a non-point source of a chemical pesticide may be of most concern during storm-related runoff events.
- Site water must not be influenced by effluents containing the material of interest or effuents that may impact the material's bioavailability and/or toxicity. The site water should be used as soon as possible after collection in order to avoid significant changes in its physical and chemical characteristics. If diurnal cycles in water characteristics (e.g., carbonate systems, salinity, dissolved oxygen) are known to affect a material's biological availability and/or toxicity markedly, use of on-site flow-through testing is suggested; otherwise transport of water to off-site locations is acceptable. During transport and storage, care should be taken to maintain the quality of the water; however, certain conditions of the water such as pH and dissolved oxygen concentration may change and the degree of these changes should be measured and reported.

markedly affect the biological availability and/or toxicity of the material of interest.

3. Conditions:

   - Develop the complete acute toxicity minimum data set using site water and resident species.

4. Details of Procedure:

   - Complete the acute toxicity minimum data set test requirements using site water and derive a site-specific Final Acute Value.
   - The guidance for site water testing has been discussed in the indicator species procedure (B).
   - Certain families of organisms have been specified in the National Guidelines acute toxicity minimum data set (e.g., Salmonidae in fresh water and Penaeidae or Mysidae in salt water); if this or any other requirement cannot be met because the family or other group (e.g. insect or benthic crustacean) in fresh water is not represented by resident species, select a substitute(s) from a sensitive family represented by one or more resident species and meet the 8 family minimum data set requirement. If all the families at the site have been tested and the minimum data set requirements have not been met use the most sensitive resident family mean acute value as the site-specific Final Acute Value.
   - To derive the site-specific maximum concentration divide the site-specific Final Acute Value by two.
   - The site-specific Final Chronic Value can be obtained as described in the indicator species procedure (B). An exception is that a chronic water effect ratio should not be used to calculate a Final Chronic Value.

markedly affect the biological availability and/or toxicity of the material of interest.

3. Conditions:

   - Develop the complete acute toxicity minimum data set using site water and resident species.

4. Details of Procedure:

   - Complete the acute toxicity minimum data set test requirements using site water and derive a site-specific Final Acute Value.
   - The guidance for site water testing has been discussed in the indicator species procedure (B).
   - Certain families of organisms have been specified in the National Guidelines acute toxicity minimum data set (e.g., Salmonidae in fresh water and Penaeidae or Mysidae in salt water); if this or any other requirement cannot be met because the family or other group (e.g. insect or benthic crustacean) in fresh water is not represented by resident species, select a substitute(s) from a sensitive family represented by one or more resident species and meet the 8 family minimum data set requirement. If all the families at the site have been tested and the minimum data set requirements have not been met use the most sensitive resident family mean acute value as the site-specific Final Acute Value.
   - To derive the site-specific maximum concentration divide the site-specific Final Acute Value by two.
   - The site-specific Final Chronic Value can be obtained as described in the indicator species procedure (B). An exception is that a chronic water effect ratio should not be used to calculate a Final Chronic Value.

- Seasonal site-specific criteria can be derived if monitoring data are available to delineate seasonal periods corresponding to significant differences in water characteristics (e.g., carbonate systems, salinity, turbidity).
- The frequency of testing (e.g., the need for seasonal testing) will be related to the variability of the physical and chemical characteristics of site water as it is expected to affect the biological availability and/or toxicity of the material of interest. As the variability increases, the frequency of testing will increase.
- With the exception that storm or flood impacted water may be used in chronic toxicity tests, the limitations on the use of indicator species to derive a site-specific Final Chronic Value are the same as those for site-specific modification of a national Final Acute Value.

C. Resident species procedure for the derivation of a site-specific criterion to account for differences in resident species sensitivity and differences in biological availability and/or toxicity of a material due to variability in physical and chemical characteristics of a site water.

1. Summary: Derivation of the site-specific maximum concentration and site-specific 30-day average concentration would be accomplished after the complete acute toxicity minimum data set requirements have been met by conducting tests with resident species in site water. Chronic tests may also be necessary.
2. Rationale: This procedure is designed to compensate concurrently for any real differences between the sensitivity range of species represented in the national data set and for site water which may

- The lower of the site-specific Final Chronic Value and the recalculated site-specific Final Residue Value (as described in the recalculation procedure) becomes the site-specific 30-day average concentration unless plant or other data (including data obtained from the site-specific tests) indicates a lower value is appropriate. If a problem is identified, judgment should be used in establishing the site-specific criterion.

5. Limitations:

- The frequency of testing (e.g., the need for seasonal testing) will be related to the variability of the physical and chemical characteristics of site water as it is expected to affect the biological availability and/or toxicity of the material of interest. As the variability increases, the frequency of testing will increase.
- Many of the limitations discussed for the previous two procedures would also apply to this procedure.

This draft of the Site-Specific Guidelines was written by Anthony R. Carlson, William A. Brungs, Gary A. Chapman, and David J. Hansen under the direction of the Site-Specific Criteria Committee of George S. Baughman, William A. Brungs, Anthony R. Carlson, Ronald G. Garton, David J. Hansen, Douglas A. Lipka, Alan B. Rubin, and Rosemarie C. Russo. John H. Gentile, Robert L. Spehar, and Charles E. Stephan provided review and comments. These efforts were supported by the U.S. Environmental Protectin Agency's Environmental Research Laboratories in Athens, Georgia; Corvallis, Oregon; Duluth, Minnesota; Gulf Breeze, Florida; and Narragansett, Rhode Island. The Office of Water Regulations and Standards' Criteria and Standards Division and the Office of Research and Development's Office of Environmental Processes and Effects Research also supported these efforts.

REFERENCES

U.S. Environmental Protection Agency. 1983. Guidelines for deriving numerical national water quality criteria for the protection of aquatic life and its uses. Draft July 5, 1983. U.S. EPA, Environmental Research Laboratories at Duluth, MN; Gulf Breeze, FL; Narragansett, RI; and Corvallis, OR.

APPENDIX 1

FRESHWATER AND SALTWATER NATIONAL CRITERIA LIST

(x = criteria are available)

Chemical	Freshwater	Saltwater
Aldrin	x	x
Ammonia	x	-
Dieldrin	x	x
Chlordane	x	x
DDT & Metabolites	x	x
Endosulfan	x	x
Endrin	x	x
Heptachlor	x	x
Lindane	x	x
Toxaphene	x	x
Arsenic(III)	x	-
Cadmium	x	x
Chlorine	x	x
Chromium(VI)	x	x
Chromium(III)	x	-
Copper	x	x
Cyanide	x	-
Lead	x	-
Mercury	x	x
Nickel	x	x
Selenium(IV)	x	x
Silver	x	x
Zinc	x	x

APPENDIX 2

TEST METHODS

The following procedures are recommended for conducting tests with aquatic organisms, including fishes, invertebrates, and plants. These procedures are the state-of-the-art based on currently available information.

Because all details are not covered in the following procedures, experience in aquatic toxicology, as well as familiarity with the pertinent references listed, is needed for conducting these tests satisfactorily.

Requirements concerning tests to determine the toxicity and bioconcentration of a material in aquatic organisms are given in the National Guidelines.

A. ACUTE TESTS:

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1980. Standard methods for the examination of water and wastewater. 15th ed. American Public Heath Association, Washington, D.C. 1134 p.

American Society for Testing and Materials. 1980. Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians. Standard E 729-80, American Society for Testing and Materials, Philadelphia, Penn. 25 p.

American Society for Testing and Materials. 1980. Standard practice for conducting static acute toxicity tests with larvae of four species of bivalve molluscs. Standard E 724-80, American Society for Testing and Materials, Philadelphia, Penn. 17 p.

B. CHRONIC TESTS:

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1980. Standard methods for the examination of water and wastewater. 15th ed. American Public Health Association, Washington, D.C. 1134 p.

American Society for Testing and Materials. Proposed standard practice for conducting toxicity tests with early life stages of fishes. S. C. Schimmel (Task Group Chairman). American Society for Testing and Materials, Philadelphia, Penn. (latest draft).

American Society for Testing and Materials. Proposed standard practice for conducting Daphnia magna renewal chronic toxicity tests. R. M. Comotto (Task Group Chairman). American Society for Testing and Materials, Philadelphia, Penn. (latest draft).

American Society for Testing and Materials. Proposed standard practice for conducting Daphnia magna chronic toxicity tests in a flow-through system. W. J. Adams (Task Group Co-chairman). American Society for Testing and Materials, Philadelphia, Penn. (latest draft.)

American Society for Testing and Materials. Proposed standard practice for conducting life cycle toxicity tests with saltwater mysid shrimp. Susan Gentile and Charles McKenny (Task Group Co-chairman). American Society for Testing and Materials, Philadelphia, Penn. (latest draft.)

Benoit, D. A. 1982. User's guide for conducting life-cycle chronic toxicity tests with fathead minnows (Pimephales promelas). EPA-600/8-81-011, U.S. EPA, Environmental Research Laboratory, Duluth, Minn.

C. FISH LIPID ANALYSIS PROCEDURE:

Approximately 10 g tissue is homogenized with 40 g anhydrous sodium sulfate in a Waring blender. The mixture is transferred to a Soxhlet extraction thimble and extracted with a 1:1 mixture of hexane and methylene chloride for 3-4 hours. The extract volume is reduced to approximately 50 ml and washed into a tared beaker, being careful not to transfer any particles of sodium sulfate which may be present in the extract. The solvent is removed in an air stream and the sample is heated to 100° C for 15 minutes before weighing the sample.

The lipid content is calculated as follows:

$$\% \text{ lipid} = \frac{\text{total residue} - \text{tare weight}}{\text{tissue weight}} \times 100$$

U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, Duluth, MN 55804.

D. BIOCONCENTRATION FACTOR (BCF) TEST:

American Society for Testing and Materials. Proposed standard practice for conducting bioconcentration tests with fishes and saltwater bivalve molluscs. J. L. Hamelink and J. G. Eaton (Task Group Co-chairmen). American Society for Testing and Materials, Philadelphia, Penn. (latest draft.)

Veith, G. D., D. L. DeFoe, and B. V. Bergstedt. 1979. Measuring and estimating the bioconcentration factor of chemicals in fish. J. Fish. Res. Board Can. 36: 1040-1048.

E. PLANT TESTS:

American Public Health Association, American Water Works Association, and Water Pollution Control Federation. 1980. Standard methods for the examination of water and wastewater. 15th ed. American Public Health Association, Washington, D.C. 1134 p.

Lockhart, W. L. and A. P. Blouw. 1979. Phytotoxicity tests using the duckweed Lemna minor. pp. 112-118, IN: Toxicity tests for freshwater organisms. E. Scherer (ed.), Can. Spec. Publ. Fish. Aquat. Sci. 44. (Canadian Government Publishing Centre, Supply and Services Canada, Hull, Quebec, Canada K1A 059.)

Joubert, G. 1980. A bioassay application for quantitative toxicity measurements, using the green algae Selenastrum capricornutum. Water Res. 14: 1759-1763.

Miller, W. E., J. C. Greene, and T. Shiroyama. 1978. The Selenastrum capricornutum Printz algal assay bottle test - Experimental design, application, and data interpretation protocol. EPA-600/9-78-018, Environmental Research Laboratory-Corvallis, Corvallis, Oreg. 125 p.

Steele, R. L., and G. B. Thursby. A toxicity test using life stages of Champia parvula [Rhodophyta]. Presented at the Sixth Symposium on Aquatic Toxicology. Sponsored by the American Society for Testing and Materials Committee E-47 on Biological Effects and Environmetal Fate. 13-14 October 1981. American Society for Testing and Materials, Philadelphia, Penn.

U.S. Environmental Protection Agency. 1974. Marine algal assay procedure; bottle test. Eutrophication and Lake Restoration Branch, National Environmental Research Center, Corvallis, OR. 43 p.

APPENDIX 3

The following problems are addressed and examples are given:

(1) how to determine if two LC50 values are statistically significantly different, and

(2) how to determine if the difference between two pairs of LC50 values is statistically significant.

To determine if two LC50 values are statistically different (at $p \leq .05$):

(a) Obtain the 95% confidence limits for both LC50 values.

(b) If the confidence intervals do not overlap the two values are different.

(c) If one confidence interval encompasses the other the values are not different.

(d) If the confidence intervals partly overlap the values may be different. To ascertain if they are different further statistical analysis must be done.

If the above procedure does not indicate whether or not the LC50 values are statistically significantly different, examine the confidence interval of either the ratio or the difference of the two values. If the confidence interval of the ratio brackets one, the two LC50 values are not statistically significantly different; if the confidence interval does not bracket one, then there is a statistical difference. The difference between two LC50 values is not statistically significant if the confidence interval of the difference includes zero; if the confidence interval does not cover zero, then the difference is statistically non-zero.

The following example demonstrates how the ratio of the LC50 values can be compared when the estimated LC50 values are obtained by the Trimmed

Spearman-Karber Method. (See Hamilton et al. 1977 for a discussion of the Trimmed Spearman-Karber Method, including calculation of the variance.) The example presents a difference between laboratory and site LC50 values that is statistically significant.

Table 1a gives the estimated LC50 values with 95% confidence intervals for both the lab and site measurements. The LC50 values are obtained by using the Trimmed Spearman-Karber Method on the natural logarithm of the concentrations.

To determine if there is a statistically significant difference, it is essential to work with the metric in which the analysis was performed. In the example the metric is the natural logarithm of the concentration. The LC50 values in Table 1a were obtained from the results in Table 1b, which gives $\log_e$ LC50 values and variances.

The calculations for the ratio and its 95% confidence interval are given in Table 1c. Since the confidence interval does not cover one, the laboratory and site LC50 values are statistically significantly different.

To compare two pairs of LC50 values several different procedures are possible. The procedure that follows shows one way to compare the ratios of the LC50 values. Specifically, the variable that is examined is the difference of the ratio of LC50 values:

$$\frac{\log_e \text{LC50}_{\text{site 1}}}{\log_e \text{LC50}_{\text{lab 1}}} - \frac{\log_e \text{LC50}_{\text{site 2}}}{\log_e \text{LC50}_{\text{lab 2}}}.$$

(As stated before, it is necessary to work in the metric in which the analysis was performed. Since the Trimmed Spearman-Karber estimate is usually obtained from an analysis of the logarithm of the dose, the ratio above should be of the logarithms of the LC50 values.)

The following four steps may indicate whether or not the difference is significant (at $p \leq .05$) without calculating the confidence interval of the difference:

(1) Obtain the 95% confidence limits for both LC50 values.

(2) If the confidence intervals do not overlap the two values are different.

(3) If one confidence interval encompasses the other the values are not different.

(4) If the confidence intervals partly overlap the values may be different. To ascertain if they are different further statistical analysis must be done.

If the above four steps do not indicate whether or not the difference of the ratios is statistically significant, the confidence interval of the difference should be examined. If the confidence interval of the difference brackets zero, the difference is not statistically significant; if the confidence interval does not cover zero, the difference is statistically significant.

An example is given in Tables 2a-2c. Table 2a gives the estimated LC50 values with 95% confidence intervals for two sets of site and lab measurements. These results were obtained from Table 2b which gives the results in natural log units based on the Trimmed Spearman-Karber Method of estimation.

Table 2c demonstrates how to determine if the difference is statistically significant. In this example, the difference is not significant. Note that this result means that there is no evidence that there is a difference; it does not mean that two ratios are necessarily identical.

References:

Hamilton, M. A., R. C. Russo, and R. V. Thurston. 1977. "Trimmed Spearman-Karber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays". Environ. Sci. Technol. 11(7): 714-719. Correction 12(4): 417 (1978).

Ku, H. H. 1966. "Notes on the Use of Propagation of Error Formulas". J. of Research of the National Bureau of Standards - C. Engineering and Instrument 70C: 331-263—341-273.

Tables 1a-c Analysis of Lab and Site LC50 Values

Table 1a LC50 Values

Source	Estimated LC50	95% Confidence Interval
Lab	75	(55,104)
Site	130	(100,169)

Table 1b $\log_e$ LC50 Value

Source	$\log_e$ LC50	Variance
Lab	4.32	.0256
Site	4.87	.0169

Table 1c Calculation of Ratio of Site to Laboratory LC50 Values* and 95% Confidence Intervals

(i) Ratio = $\log_e$ LC50 site/$\log_e$ LC50 lab = 4.87/4.32 = 1.13

(ii) Variance of ratio =

$$\left(\frac{\log_e LC50_{site}}{\log_e LC50_{lab}}\right)^2 \left(\frac{\text{variance } \log_e LC50_{site}}{(\log_e LC50_{site})^2} + \frac{\text{variance } \log_e LC50_{lab}}{(\log_e LC50_{lab})^2}\right)$$

$$= \left(\frac{4.87}{4.32}\right)^2 \left(\frac{.0169}{(4.87)^2} + \frac{.0256}{(4.32)^2}\right)$$

$$= .0026$$

(iii) Confidence limit = 2 x (variance of difference)$^{1/2}$

= 2 x (.0026)$^{1/2}$ = .10

(iv) Confidence interval = ratio $\pm$ confidence limit

= 1.13 $\pm$ .10 = (1.03, 1.23)

(v) Since the confidence interval does not bracket one, the ratio of site to laboratory LC50 values is statistically significant at $\alpha \leq .05$.

* Note that in this example the ratios are of $\log_e$ LC50 values since the Trimmed Spearman-Karber Method of estimating LC50 values was used. This method estimates the LC50 based on the logarithm of the concentration, so the logarithm of the LC50 should be used here.

Tables 2a-c Analysis of the Lab and Site LC50 Values for Two Species

Table 2a LC50 Values

	Source	Estimated LC50	95% Confidence Interval
Species 1	Lab	75	(55,104)
	Site	130	(100,169)
Species 2	Lab	60	(48, 75)
	Site	90	(67,122)

Table 2b Log_e LC50 Values

	Source	Log_e LC50	Variance
Species 1	Lab	4.32	.0256
	Site	4.87	.0169
Species 2	Lab	4.10	.0121
	Site	4.50	.0225

Table 2c Calculation of Difference of Ratios Between Field and Site LC50 Values* and 95% Confidence Intervals

(i) Difference =

$$\frac{\log_e LC50_{site\ 1}}{\log_e LC50_{lab\ 1}} - \frac{\log_e LC50_{site\ 2}}{\log_e LC50_{lab\ 2}}$$

$$= \frac{4.87}{4.32} - \frac{4.50}{4.10} = 1.13 - 1.10 = .03$$

(ii) Variance of difference =

$$\text{variance}\left(\frac{\log_e LC50_{site\ 1}}{\log_e LC50_{lab\ 1}}\right) + \text{variance}\left(\frac{\log_e LC50_{site\ 2}}{\log_e LC50_{lab\ 2}}\right)$$

(where variance $\left(\frac{\log_e LC50_{site}}{\log_e LC50_{lab}}\right)$ is found as in Table 1c (ii)).

$$= .0026 + .0022 = .0049$$

(iii) Confidence limit = 2 x (variance of difference)$^{1/2}$

$$= 2 \times (.0049)^{1/2} = .14$$

(iv) Confidence interval = difference $\pm$ confidence limit

$$= .03 \pm .14 \ (-.11, .17)$$

(v) Since the confidence interval does bracket zero, there is not enough evidence to reject the hypothesis that the ratios are different.

* Note that in this example the ratios are of $\log_e$ LC50 values since the Trimmed Spearman-Karber Method of estimating LC50 values was used. This method estimates the LC50 based on the logarithm of the concentration, so the logarithm of the LC50 should be used here.