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Standard Guide for Assessing the Hazard of a Material to Aquatic Organisms and Their Uses¹

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1. Scope

- 1.1 This guide describes a stepwise process for using information concerning the biological, chemical, physical, and toxicological properties of a material to identify adverse effects likely to occur to aquatic organisms and their uses as a result of release of the material to the environment. The material will usually be a specific chemical, although it might be a group of chemicals that have very similar biological, chemical, physical, and toxicological properties and are usually produced, used, and discarded together.
- 1.2 The hazard assessment process is complex and requires decisions at a number of points; thus, the validity of a hazard assessment depends on the soundness of those decisions, as well as the accuracy of the information used. All decisions should be based on reasonable worst-case analyses so that an appropriate assessment can be completed for the least cost that is consistent with scientific validity.
- 1.3 This guide assumes that the reader is knowledgeable in aquatic toxicology and related pertinent areas. A list of general references is provided (1).²
- 1.4 This guide does not describe or reference detailed procedures for estimating or measuring environmental concentrations, or procedures for determining the maximum concentration of test material that is acceptable in the food of predators of aquatic life. However, this guide does describe how such information should be used when assessing the hazard of a material to aquatic organisms and their uses.
- 1.5 Because assessment of hazard to aquatic organisms and their uses is a relatively new activity within aquatic toxicology, most of the guidance provided herein is qualitative rather than

quantitative. When possible, confidence limits should be calculated and taken into account.

- 1.6 This guide provides guidance for assessing hazard but does not provide guidance on how to take into account social considerations in order to judge the acceptability of the hazard. Judgments concerning acceptability are social as well as scientific, and are outside the scope of this guide.
 - 1.7 This guide is arranged as follows:

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¹ This guide is under the jurisdiction of ASTM Committee E50 on Environmental Assessment, Risk Management and Corrective Action and is the direct responsibility of Subcommittee E50.47 on Biological Effects and Environmental Fate.

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² Boldface numbers in parentheses refer to the list of references at the end of this standard.

Appendix X1 Production, Use, Disposal, and Other Release

- 1.8 This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.
- 1.9 This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.

2. Referenced Documents

- 2.1 ASTM Standards:³
- D1129 Terminology Relating to Water
- E724 Guide for Conducting Static Short-Term Chronic Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivalve Molluscs
- E729 Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians
- E943 Terminology Relating to Biological Effects and Environmental Fate (Withdrawn 2023)⁴
- E1022 Guide for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Mollusks
- E1191 Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids
- E1193 Guide for Conducting *Daphnia magna* Life-Cycle Toxicity Tests
- E1218 Guide for Conducting Static Toxicity Tests with Microalgae
- E1241 Guide for Conducting Early Life-Stage Toxicity Tests with Fishes
- E1415 Guide for Conducting Static Toxicity Tests With Lemna gibba G3 ch. a/catalog/standards/sist/1425e3d
- E1706 Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates
- IEEE/ASTM SI 10 American National Standard for Use of the International System of Units (SI): The Modern Metric System

3. Terminology

- 3.1 Definitions of Terms Specific to This Standard:
- 3.1.1 *acute-chronic ratio*, *n*—the quotient of an appropriate measure of the acute toxicity (for example, the 96 h LC50) of a material to a species divided by the result of a life-cycle, partial life-cycle, or early life-stage test in the same water on the same material with the same species.
- 3.1.2 *bioaccumulation*, *n*—the net uptake of a material from water and from food.

- 3.1.3 *bioconcentration factor (BCF)*, *n*—a ration of the net accumulation of a substance by an aquatic organism to the concentration in solution.
- 3.1.4 environmental concentration (EnC), n—the concentration, duration, form, and location of a material in environmental waters, sediments, or the food of aquatic organisms.
- 3.1.5 *hazard assessment, n*—the identification of the adverse effects likely to result from specified releases(s) of a material.
- 3.1.6 maximum acceptable toxicant concentration (MATC), n—the highest concentration of a material that would have no statistically significant observed adverse effect on the survival, growth, or reproduction of the test species during continuous exposure throughout a life-cycle or partial life-cycle toxicity test. Such tests usually indicate that the MATC is between two tested concentrations.
- 3.1.7 no-observed-effect concentration (NOEC), n—the highest tested concentration of a material at which the measured parameters of a specific population of test organisms under test conditions show no statistically significant adverse difference from the control treatment. When derived from a life-cycle or partial life-cycle test, it is the same as the lower limit on the MATC.
- 3.1.8 *safety factor*; *n*—the quotient of a toxicologically significant concentration divided by an appropriate EnC.
- 3.2 For definitions of other terms used in this guide, refer to Terminology E943 and D1129, Guides E724, E729, and E1022. For an explanation of units and symbols, refer to IEEE/ASTM SI 10.

4. Summary of Guide

4.1 This guide describes an iterative process for assessing the hazard of a material to aquatic organisms and their uses by considering the relationship between the material's measured or estimated environmental concentration(s) and the adverse effects that may to result, with an understanding that laboratory testing results may differ from effects that occur in the environment where conditions affecting toxicity may differ. Necessary information concerning environmental concentrations and adverse effects is obtained through a stepwise program that starts with more general and economical information and progresses to expensive more intensive (and potentially more costly) information, as needed to meet specific project or investigative goals. At the end of each iteration the estimated or measured environmental concentration(s) are compared with information on possible adverse effects to determine the adequacy of the available data for assessing hazard. If it is not possible to conclude that hazard is either minimal or potentially excessive, the available data are judged inadequate to characterize the hazard. If desired, appropriate additional information is identified and obtained, so that hazard can be further assessed. The process is repeated until the hazard is adequately characterized.

5. Significance and Use

5.1 Adverse effects on natural populations of aquatic organisms and their uses have demonstrated the need to assess the

³ For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

⁴ The last approved version of this historical standard is referenced on www.astm.org.



hazards of many new, and some presently used, materials. The process described herein will help producers, users, regulatory agencies, and others to efficiently and adequately compare alternative materials, completely assess a final candidate material, or reassess the hazard of a material already in use.

- 5.2 Sequential assessment and feedback allow appropriate judgments concerning efficient use of resources, thereby minimizing unnecessary testing and focusing effort on the information most pertinent to each material. For different materials and situations, assessment of hazard will appropriately be based on substantially different amounts and kinds of biological, chemical, physical, and toxicological data.
- 5.3 Assessment of the hazard of a material to aquatic organisms and their uses should never be considered complete for all time. Reassessment should be considered if the amount of production, use, or disposal increases, new uses are discovered, or new information on biological, chemical, physical, or toxicological properties becomes available. Periodic review will help assure that new circumstances and information receive prompt appropriate attention.
- 5.4 If there is substantial transformation to another material, the hazard of both materials may need to be assessed.
- 5.5 In many cases, consideration of adverse effects should not end with completion of the hazard assessment. Additional steps should often include risk assessment, decisions concerning acceptability of identified hazards and risks, and mitigative actions.
- 5.6 Because this practice deals mostly with adverse effects on aquatic organisms and their uses, it is important that mitigative actions, such as improved treatment of aqueous effluents, not result in unacceptable effects on non-aquatic organisms. Thus, this standard should be used with other information in order to assess hazard to both aquatic and non-aquatic organisms.

6. Four Basic Concepts

6.1 The Iteration (see Fig. 1)—The basic principle used in this hazard assessment process is the repetitive or iterative comparison of measured or estimated EnCs of a material with concentrations that cause adverse effects. When available data are judged inadequate, needed data are identified. Unless the hazard assessment is terminated, necessary additional information is obtained and used with all other pertinent information to reassess hazard. The process is repeated until hazard is adequately characterized.

6.2 Two Elements:

6.2.1 The first element in assessing the hazard of a material to aquatic organisms and their uses is the EnCs of the material. For some existing materials the EnCs may be measured, but in most hazard assessments the concentrations, durations, forms, and locations of the material are predicted by starting with information on its anticipated or actual release and then taking into account its biological, chemical, and physical properties. The release may be from a single event, such as an application of a pesticide, or a series of events, such as the production, use, and disposal of a deicer. A material may have three kinds of EnCs in a body of water, because it might occur in the water column, in sediment, and in food of aquatic organisms. In addition, EnCs may be different for different kinds of surface waters, different geographic areas, and different seasons of the year. Also, determination of EnCs may have to consider total versus available and short-term peak concentrations versus long-term average concentrations. Each iteration considers the potential of a particular EnC to cause adverse effects, but the assessment of a material is not complete until the hazard of each and every EnC of that material has been adequately assessed. EnCs may aid in selecting appropriate aquatic species to be used in tests, identifying and designing tests to be conducted, choosing test concentrations, and interpreting results. Determination of EnCs should take into account not only

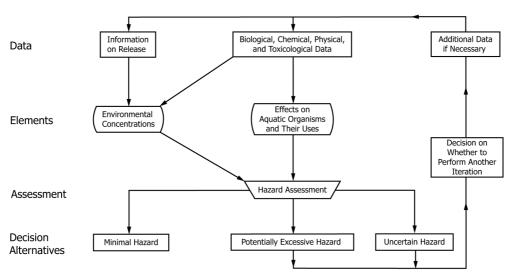


FIG. 1 Flow-Chart of an Iteration

all pertinent probable means of release, but also dilution, transport and transformations, sinks and concentrating mechanisms, and degradation and degradation products.

- 6.2.2 If there is a potential for biological organisms of concern to be exposed EnCs, then the second element essential to assessing hazard is the possible adverse effects on aquatic organisms and their uses. For convenience, such effects can be placed in four categories:
 - 6.2.2.1 Acute and chronic toxicity to aquatic animals,
- 6.2.2.2 Effects on uses of aquatic organisms, including such effects as flavor impairment and accumulation of unacceptable residues,
- 6.2.2.3 Effects on aquatic plants, including toxicity and stimulation, and
 - 6.2.2.4 Other effects on aquatic animals, such as avoidance.

6.3 Possible Decisions:

- 6.3.1 In each iteration, information concerning possible adverse effects is used to decide whether the hazard due to a particular EnC is minimal, potentially excessive, or uncertain. If the safety factor is large, that is, if the unacceptable concentration is much greater than the EnC, hazard should be judged minimal. If the safety factor is low, for example, if the unacceptable concentration is below the EnC and therefore the safety factor is less than 1, the hazard should be judged potentially excessive because it is likely that the EnC will cause an unacceptable effect on aquatic organisms or their users. If hazard cannot be judged either minimal or potentially excessive, it is uncertain. The necessary minimum size of the safety factor for judging the hazard of an EnC to be minimal will vary from iteration to iteration because it will depend on (a) the amount, quality, and kind of data available concerning the EnC and possible adverse effects and (b) the degree of confidence in the validity of any extrapolations and assumptions that were used. The necessary minimum safety factor will especially depend on the appropriateness, range, and number of aquatic species for which data are available. For this hazard assessment process to produce valid results, it is particularly important that EnCs and adverse effects not be underestimated (see 6.4.5).
- 6.3.2 A decision of minimal hazard should account for the following considerations:
- 6.3.2.1 The specified releases of the material will not result in concentrations that are acutely toxic to appropriate and sensitive aquatic animals that will be exposed.
- 6.3.2.2 Any expected long-term concentrations of the material in surface waters will not be chronically toxic to appropriate and sensitive aquatic animals.
- 6.3.2.3 Unacceptable effects on aquatic plants will probably not occur.
- 6.3.2.4 There is no indication that bioaccumulation will result in concentrations in aquatic organisms that would adversely affect users of the organism.
- 6.3.2.5 The material, its impurities, and any environmental transformation products are well enough understood that "ecological surprises" are unlikely.

- 6.3.2.6 Any episodic non-planned exposure of aquatic organisms to toxic concentrations resulting from spills or other accidents would probably be temporary and limited in geographical scope.
- 6.3.2.7 No long-term environmental sinks are expected where the material might be concentrated and cause a delayed and perhaps difficult-to-reverse problem.
- 6.3.2.8 The possibility of exacerbating factors is small. For example, could transformation products or synergism cause problems? Could an estimated EnC, acute-chronic ratio, or bioconcentration factor (BCF) be too low?
- 6.3.3 The hazard of an EnC is considered potentially excessive if the safety factor is so low, for example, below 1, that the EnC is expected to cause one or more unacceptable effects. Before hazard is judged potentially excessive, available data should be critically reviewed and thorough consideration should be given to possible mitigating factors such as the following:
- 6.3.3.1 Could the EnC have been estimated to be higher than it occurs in the environment because degradation or partitioning were not adequately considered?
- 6.3.3.2 Could toxicity have been caused by an impurity in the material that could be removed or would not persist in the environment?
- 6.3.3.3 Could the availability of the material in the environment be lower than in the test?
- 6.3.3.4 Could restriction on the amount, type, time, or location of release realistically reduce an EnC that is too high? Could spatial or temporal limitations on use preclude long-term toxicity or bioaccumulation (2)?
- 6.3.3.5 Are the tested species appropriate for the respective EnCs?
- 6.3.3.6 Could a BCF estimated from chemical or physical properties be higher than the actual value?
- 6.3.3.7 Could an estimated MATC be too low because the acute-chronic ratio used was too high?
- 6.3.3.8 Would the limiting adverse effects observed in toxicity tests be meaningful in the environment?
- 6.3.4 If hazard is judged either potentially excessive or uncertain and there is continuing interest in the material, additional information should be selectively obtained to answer the most critical question for the least cost that is consistent with good science. An appropriate balance should be maintained between consideration of EnCs and adverse effects.
- 6.4 The Phased Approach—This hazard assessment process is divided into three phases, which differ mainly with respect to the cost of obtaining necessary information. As many iterations as necessary are used within each phase to help make the best decision concerning whether to stop the hazard assessment or to proceed to the next phase. If all of the information needed concerning EnCs and effects is already available, the cost of that phase is negligible. The purpose of a cost-effective hazard assessment process is to ensure that all hazards receive adequate consideration for the least cost.
- 6.4.1 The purpose of Phase I is to make an initial assessment of hazard using available information concerning release and biological, chemical, physical, and toxicological properties. It

may be possible to determine that hazard is minimal. If not and there is continuing interest in the material, Phase II is necessary.

- 6.4.2 Depending upon data available in Phase I, Phase II may require additional time and effort to obtain specific information to provide better information concerning EnCs or effects, or both. The necessary additional information will differ widely depending on the available data and the properties of the material. Depending upon the EnCs for water and sediment, it may be necessary to conduct short-term toxicity tests with species representative of different trophic levels and habitats. The relationships of the EnCs to toxic concentrations are the important factors in deciding whether short-term testing is adequate to determine that hazard is minimal. If not and there is continuing interest in the material, the assessment should proceed to Phase III.
- 6.4.3 Phase III may require extensive time and effort to obtain needed additional information on release, long-term toxicity, or bioaccumulation. Because of the high cost of additional information needed in this phase, it is particularly important that each new piece of information initiate the iterative review and assessment process.
- 6.4.4 A decision on hazard to aquatic organisms can usually be based on information developed by using this three-phase laboratory testing process. For some materials, however, field testing or monitoring may be needed to confirm the assessment.
- 6.4.5 Because of the nature of this phased hazard assessment process, it is extremely important that neither EnCs nor effects be underestimated in any phase. The estimates may be high by factors of 10 or 100, but they must not be too low. A material can only be judged to have minimal hazard in Phases I or II without the high-cost consideration of EnCs and effects in Phase III, if care was taken to assure that neither EnCs nor effects were underestimated in Phases I and II. The intent of this phased approach is to allow a scientifically valid judgment

that hazard is minimal as early (and inexpensively) as possible for as many materials as possible, but the more refined (and costly) consideration of EnCs and effects can be avoided only if the less costly approaches definitely do not underestimate hazard. The sequential use of iterations and phases is also designed to ensure that hazard is not judged potentially excessive because estimates of EnCs and effects are unnecessarily high.

6.4.6 Appropriate estimates of EnCs, toxicity, and bioaccumulation usually have to be based on incomplete data. Two techniques for attempting to ensure that such estimates are not too low are to perform a worst-case analysis or to make a best estimate and apply an uncertainty factor. Estimates used herein are based on reasonable worst-case analyses.

7. Phase I—Use of Low-Cost (Existing) Information (see Fig. 2)

- 7.1 Collection of Available Data—The initial step in assessment of the hazard of a material to aquatic organisms and their uses is to assemble all available pertinent information concerning the following:
- 7.1.1 Temporal and geographical patterns and amounts of planned release, from such things as production, use and disposal, and the potential for accidental release (see Appendix X1).
- 7.1.2 Biological properties concerning effects of organisms on the material, especially concerning degradation, uptake, transfer, and storage (see Appendix X2).
- 7.1.3 Structure, characterization, and chemical reactions of the test material, with emphasis on those chemical properties likely to affect testing procedures, EnCs, and effects (see Appendix X3).
- 7.1.4 Physical properties, with particular emphasis on solubility, sorption, and volatility (see Appendix X4).

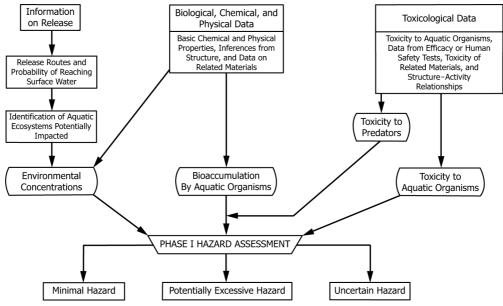


FIG. 2 Phase I—Use of Low-Cost (Existing) Information

- 7.1.5 Toxicity of the material or similar materials to aquatic organisms, target organisms, and consumers of aquatic organisms (see Appendix X5).
- 7.2 Initial Estimates of Environmental Concentrations—Based on available information on actual or planned release and biological, chemical, and physical properties, an initial estimate should be made of the concentrations likely to be found in surface water(s), sediment(s), and food(s) of aquatic organisms (see Appendix X6). In Phase I, it is usually appropriate to assume that degradation and deactivation are negligible.
- 7.3 Initial Estimate of Toxicity to Aquatic Organisms—Based on chemical structure, information on similar materials, and available data on toxicity to aquatic plants and animals, an initial assessment should be made as to whether the material is biologically inactive or presents special concerns. In some cases enough data on the acute toxicity of the material or very similar materials may be available to allow a good estimate of concentrations likely to adversely affect aquatic organisms.
- 7.4 Initial Estimate of Bioaccumulation by Aquatic Organisms—For an organic material its structure, or its solubility in water and organic solvents, will allow a first estimate of bioaccumulation (see Appendix X4).
- 7.5 Phase I Hazard Assessment—By using the information on EnCs and effects, hazard should be assessed as either minimal, potentially excessive, or uncertain.
- 7.5.1 *Minimal Hazard*—Hazard to aquatic organisms can usually be judged minimal if any one of the following conditions exists:
- 7.5.1.1 Only research quantities of the material are anticipated.
- 7.5.1.2 Release patterns are such that substantial aquatic exposure is very unlikely.
- 7.5.1.3 Existing evidence indicates that the material and its degradation products are toxicologically inactive to plants and animals.

- 7.5.1.4 The material decomposes rapidly, for example, in 1 h or less, in water to materials of known low toxicity and bioaccumulation.
- 7.5.1.5 Toxicity is known for materials of similar structure, and together with structure-toxicity correlations, a reasonable estimate of the toxicity of the material can be made. Also, concentrations expected to cause long-term toxicity are substantially above EnCs, and concern about bioaccumulation is low because of the material's properties or because the EnC is low or both. Hazard due to bioaccumulation can usually be considered minimal if chemical or physical properties indicate that the BCF is low, for example, less than 100.
- 7.5.1.6 Generally, if any one of these conditions is satisfied, and review of the items in 6.3.2 is reassuring, hazard may be judged minimal because the safety factor will be high.
- 7.5.2 Potentially Excessive Hazard—A decision of potentially excessive hazard is usually appropriate if (a) EnCs exceed concentrations that cause acute toxicity or (b) Bioaccumulation will probably result in adverse effects on important consumers of aquatic organisms. Before hazard is judged to be potentially excessive, the items listed in 6.3.3 should be reviewed. If there is continuing interest in the material, Phase II must be considered.
- 7.5.3 *Uncertain Hazard*—For most new materials, available information will not be adequate to allow a conclusion of minimal or potentially excessive hazard, and so hazard will have to be judged uncertain. If there is continuing interest in the material, Phase II must be considered.

8. Phase II—Use of Medium-Cost Information (see Fig.

8.1 Whereas Phase I involves collection and analysis of data already available Phase II will probably require at least some medium-cost efforts to obtain better information on EnCs and effects. It is usually prudent to review all available toxicological information (see Appendix X5) and to obtain some estimate of toxicity to humans before undertaking tests with aquatic

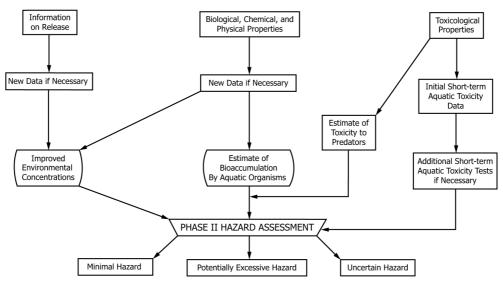


FIG. 3 Phase II—Use of Medium-Cost Information

organisms. An initial review of Phase II should indicate the most cost-effective place to start. This initial review might also indicate that the hazard assessment should be terminated because the necessary testing program will probably be more costly than can be justified by the possible utility of the material.

8.2 Improved Estimates of Environmental Concentrations— The EnCs used in Phase I may have been obtained with only minimal information on release, and little or no information on biological, chemical, and physical properties that determine environmental fate (see Appendix X6). In Phase II, inexpensive appropriate tests should be undertaken to obtain important data on biological, chemical, and physical properties that are not already available. Tests of biodegradation, hydrolysis, oxidation, reduction, photodegradation, volatility, and sorption may be appropriate and allow improved estimates of EnCs. If degradation is substantial, degradation products and their properties should be considered. Although sorption may reduce the concentration in the water column, it will probably increase the concentration in sediment, and thus tests with benthic species may be desirable. Assumptions and data used to derive EnCs should be carefully examined to determine the confidence that should be placed in them. If the material is already in use, some environmental monitoring may be appropriate.

8.3 Acute Toxicity to Aquatic Animals—Unless appropriate data are already available, some acute aquatic toxicity tests will normally be necessary for materials likely to reach water in a substantial quantity. Initial toxicity results are often necessary to estimate the scope of the assessment process. Unless data are already available, it is prudent to determine chemical and physical properties of the test material in water (see Appendix X3 and Appendix X4) in order to select appropriate test methods and conditions. Selection of the initial acute aquatic toxicity test will depend upon the nature of the material, expected exposure locations, and any available indications of the relative sensitivities of species.

8.3.1 Acute Toxicity Test in Fresh Water—For most materials production, use, and disposal results in higher concentrations in fresh than in salt water, and fishes are almost always more commercially and recreationally important than invertebrates in fresh water. Thus, the initial acute toxicity test on a material is usually with a freshwater fish. Use of a standardized test (see Practice E729) with a commonly used species allows comparison of results with a substantial amount of data on other materials.

8.3.1.1 When an acute test with an aquatic invertebrate is needed, a static test with a daphnid should be considered in most situations because of the ready availability of daphnids from laboratory cultures. Use of a daphnid instead of a fish in the initial acute test can be particularly appropriate for insecticides, metals, and other classes of materials to which daphnids are often sensitive.

8.3.2 Acute Toxicity Test in Salt Water—When the test material can be expected to reach estuarine or near-shore ocean areas in quantities that could reasonably be of concern, aquatic species representing these ecosystems should be either included or substituted in the acute toxicity testing program at an early stage. Use of a grass shrimp, penaeid shrimp, or mysid,

rather than a fish, as the initial saltwater species is usually appropriate because these invertebrates are often more sensitive and represent important species. Further, the release pattern may make higher exposure concentrations of test material more likely for saltwater invertebrates than saltwater fishes. Mysids are often preferred because life-cycle tests, which may be necessary in Phase III, are easier to conduct with them than with grass shrimp (see Appendix X8).

8.3.2.1 When EnCs in salt water may be significant, an acute test with bivalve mollusc embryos and larvae (see Practice E724) is probably desirable because these are sensitive life stages of commercially and recreationally important species

8.3.2.2 When exposure in salt water is critical or when interaction of the test material with salt water is suspected, an acute test with a saltwater fish may also be desirable.

8.3.3 For most materials, the initial acute test is a static test. For some materials, a flow-through toxicity test should be conducted in addition to, or as an alternative to, the static test, particularly when an exposure longer than 96 h is desired or when sorption, degradation, hydrolysis, oxidation, reduction, volatilization, or oxygen demand make the static test questionable. Obvious advantages of the flow-through test are replenishment of test material, continual supply of oxygenated water, and removal of wastes.

8.4 Toxicity to Algae—Herbicides and materials with suspected phytotoxicity that are expected in water at substantial concentrations should be tested initially with a representative freshwater or saltwater, or both, algal species (see Guide E1218).

8.5 Expansion of Short-Term Testing—Depending upon the relation between the results of the initial test(s), the EnCs, and the nature of the material, the need for additional short-term toxicity tests should be considered. If short-term toxicity occurs at or below a water-column EnC, hazard is potentially excessive. For some materials, acute toxicity may only occur at concentrations so far above the EnC that additional short-term tests are not necessary. For most materials, however, Table 1 and Appendix X3, Appendix X4 and Appendix X8 should be consulted for additional considerations. In addition, observed physiological or behavioral changes should be reviewed for their significance. The relation between time and toxicity should be noted because it may influence decisions to extend test duration or perform long-term tests. The need to include other species or phyla should be based on the toxicological data, the likelihood of special species sensitivity, and the probability of exposure. High-volume materials that will reach surface waters on an extensive and continuing basis should be tested with more than the minimum number of species.

8.6 *Bioaccumulation*—If the Phase I estimate of bioaccumulation was based solely on chemical structure or solubility in water, an improved estimate is probably necessary if the material is lipophilic, persistent, or highly toxic. For organic materials, calculation of a BCF from an estimated or measured octanol-water partition coefficient usually will be sufficient in this phase (see Appendix X4).

8.7 Phase II Hazard Assessment: