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Standard Guide for Assessing the Hazard of a Material to Aquatic Organisms and Their Uses[1]

This standard is issued under the fixed designation E1023; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

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.

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 and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

D1129 Terminology Relating to Water

E724 Guide for Conducting Static Acute 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

E1022 Guide for Conducting Bioconcentration Tests with Fishes and Saltwater Bivalve Mollusks

IEEE/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*—the quotient of an appropriate measure of the acute toxicity (usually 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*—the net uptake of a material from water and from food.

3.1.3 *environmental concentration (EnC)*—the concentration, duration, form, and location of a material in environmental waters, sediments, or the food of aquatic organisms.

3.1.4 *hazard assessment*—the identification of the adverse effects likely to result from specified releases(s) of a material.

3.1.5 *maximum acceptable toxicant concentration (MATC)*—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.

3.1.6 *no-observed-effect concentration (NOEC)*—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.

3.1.7 *safety factor*—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 and E729, and Practice E1022. For an explanation of units and symbols, refer to IEEE/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 likely to result. Unavailable necessary information concerning environmental concentrations and adverse effects is obtained through a stepwise program that starts with inexpensive information and progresses to expensive information if necessary. 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 reassessed. 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 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.

[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.

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

6.2.2 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

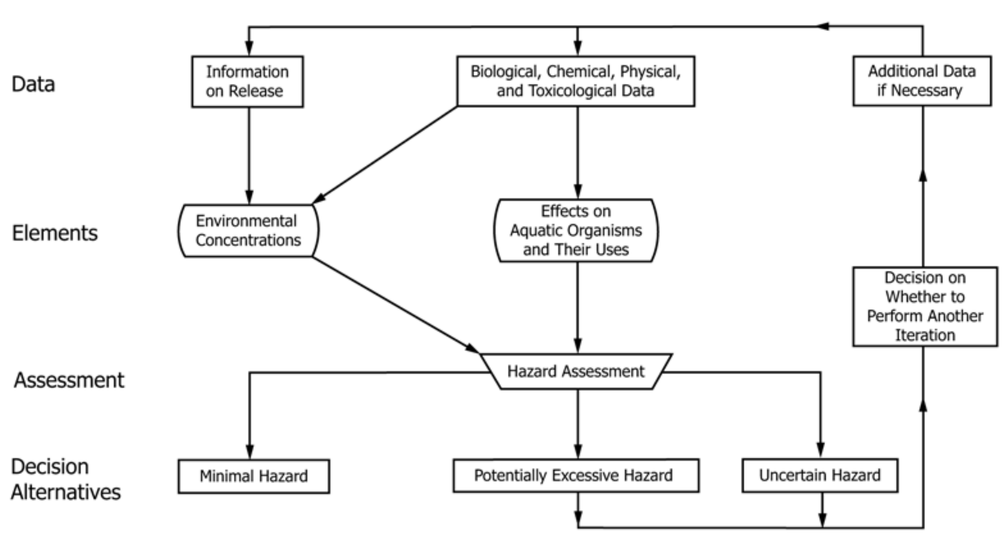

FIG. 1 Flow-Chart of an Iteration

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 be too high 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 acquatic 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).

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,

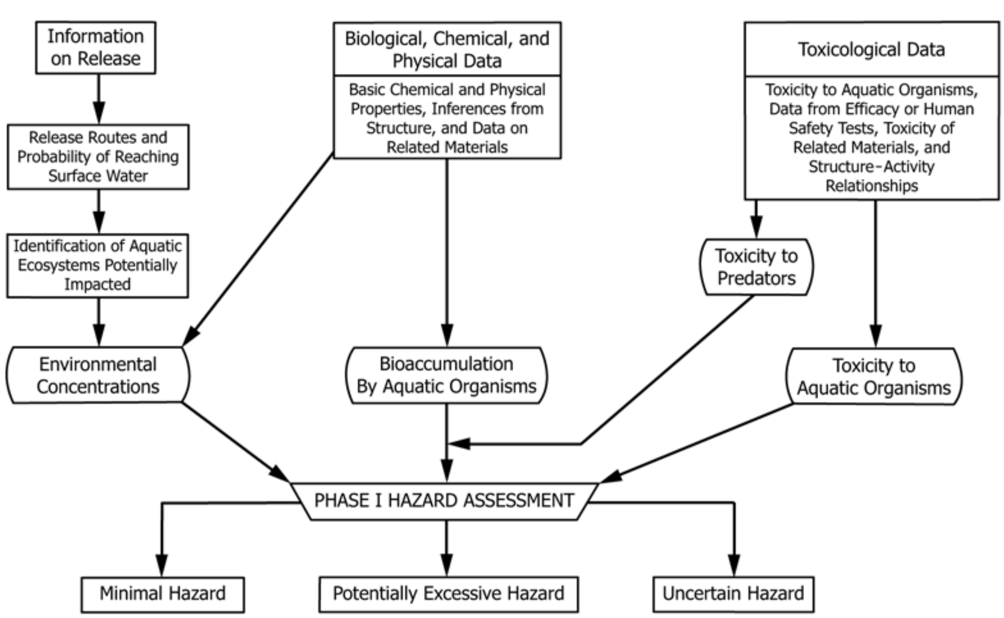

FIG. 2 Phase I—Use of Low-Cost (Existing) Information

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. 3)

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 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

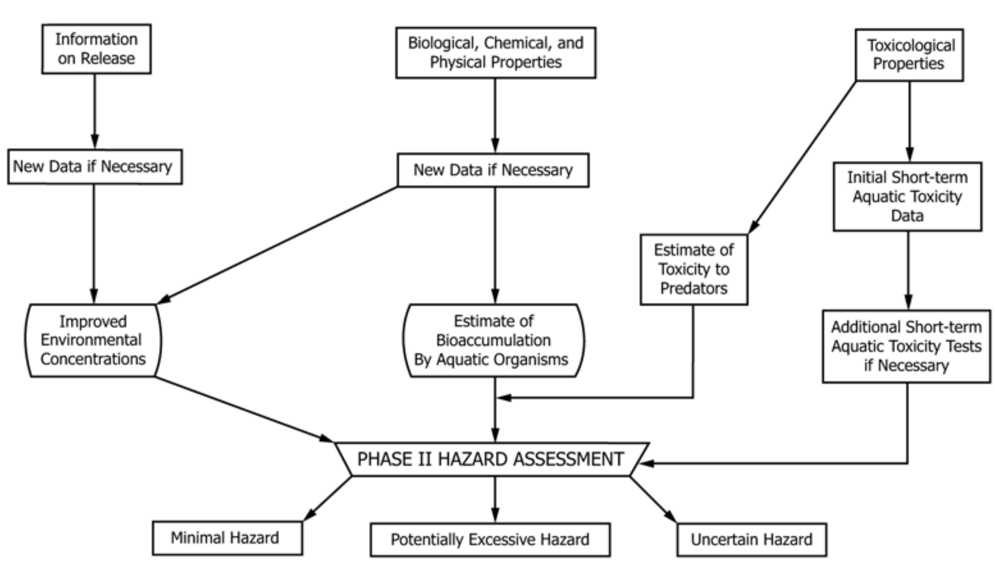

FIG. 3 Phase II—Use of Medium-Cost Information

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 (**3**).

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

TABLE 1 Factors Affecting Design of Expanded Short-Term Toxicity Testing Program

Factor	Implication for Testing
A) *Depletion of Concentrations in Static Tests:*	
Volatility, sorption, or solubility losses may be significant; material may exert significant oxygen depletion; degradation may reduce test concentrations.	Flow-through test needed with the same species used in static tests.
B) *Static and Flow-Through Results Differ Significantly:*	
1) Flow-through test gives lower acute value.	1) Use flow-through for other species. Chemically monitor test concentrations. Determine if factor decreasing toxicity in static tests has environmental significance (that is, degradation, sorption).
2) Flow-through test gives higher acute value.	2) Determine if factor increasing toxicity is material related (that is, more toxic degradation product) or test related (that is, low D.O.).
C) *Relationship of LC50 to Environmental Concentration (EnC):*	
1) All available LC50s are more than 100 000 times the EnC.	1) Additional acute tests probably unnecessary.
2) At least one LC50 is less than 100 000 times the EnC.	2) Additional acute tests may be necessary depending on the nature of the test material, the taxonomic range of the species tested, the range of the acute values, and differences between the acute values and the EnC (see 8.7.1.2).
D) *Differences in Response Between Species:*	
1) No unreasonable differences between taxa.	1) Additional acute tests unnecessary with particular genera.
2) Unreasonable or unexpected differences between taxa.	2) Conduct tests with other species in sensitive families.
E) *Chemical and Physical Properties of Test Material:*	
1) Material non-ionic and water soluble.	1) No special test conditions necessary.
2) Hardness may reduce solubility.	2) Test in harder water.
3) Material has limited solubility under "standard" test conditions.	3) Test at higher temperature; check effect of solubilizing.
4) Material causes excessive pH change at test concentrations.	4) Test in buffered water.
5) Degradation appears to alter toxicity substantially.	5) Test effect of delaying introduction of test organisms and monitor, control, or renew test solutions.
6) Solubility or sorption indicates association with solids or sediments.	6) Conduct test(s) with benthic species.
F) *Location Considerations:*	
1) Unusual species or important ones of unknown sensitivity may be exposed to significant concentrations.	1) Conduct test(s) with this special species if important and available.
2) Valuable fishery may be exposed to significant concentrations.	2) Conduct test(s) with important species or best representatives.
G) *Special Toxicological Information:*	
1) Material is effective pesticide.	1) Conduct test(s) with a non-target species phylogenetically related to target species.

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:*

8.7.1 Hazard may be judged minimal if most of the following are supported, and none are contradicted, by available data:

8.7.1.1 Similar materials are generally accepted as biologically innocuous at estimated or measured EnCs.

8.7.1.2 LC50s and EC50s are sufficiently above the water-column EnCs. For some materials, some species are more than 1000 times more sensitive than others (**4**), and some acute-chronic ratios are above 100 (**5**). Both the acute-chronic ratios and ranges of sensitivities seem to be less for nonpesticide organic chemicals (**6**). Therefore, unless the material is a nonpesticide organic chemical, if an acute test has been conducted with only one species and the relative sensitivity of that species to the test material is unknown, hazard should be judged minimal only if the LC50 or EC50 is more than 100 000 times the EnC. The greater the variety of species with which acute tests have been conducted, the smaller the factor can be (**7, 8**). Except possibly for nonpesticide organic chemicals, an acute–chronic ratio less than 100 should not be used unless it has been experimentally determined, especially if the material takes more than a few days to reach steady-state in a bioconcentration test or has a low depuration rate.

8.7.1.3 Aquatic species do not show any unusual symptoms, patterns of sensitivity, concentration-effect curves, or time-effect curves.

8.7.1.4 Water-column EnCs are below concentrations that are known to cause chronic toxicity.

8.7.1.5 EnCs are unlikely to affect aquatic plants unacceptably.

8.7.1.6 Available data strongly indicate that bioaccumulation will not be a problem, either because the EnC is low, the BCF is low, for example, below 100, or because the material has low toxicity to consumers of aquatic life.

8.7.1.7 Toxicological data obtained from human safety testing are reassuring.

8.7.1.8 A review of the items in 6.3.2 is reassuring.

8.7.2 The hazard should be judged potentially excessive if any of the following are true:

8.7.2.1 Acute toxicity occurs to important or other appropriate species at concentrations near or below the water-column EnCs.

8.7.2.2 Acute-chronic ratios, indications of cumulative toxicity during acute tests, or sublethal effects make unacceptable chronic effects likely at EnCs.

8.7.2.3 EnCs are likely to cause unacceptable effects on aquatic plants.

8.7.2.4 Partitioning data indicate that bioconcentration will probably occur to a degree likely to be detrimental to uses or consumers of aquatic organisms.

8.7.2.5 If any of the above are true, the items listed in 6.3.3 should be reviewed. If there is continuing interest in the material, Phase III is necessary.

8.7.3 Hazard should be judged uncertain if some of the following are true:

8.7.3.1 Concentrations that are acutely toxic to aquatic animals are less than 100 000 times the water-column EnCs (but see 8.7.1.2).

8.7.3.2 Experience with similar materials is limited or mixed, so that definitive input from this source is lacking.

8.7.3.3 Efficacy studies or human safety evaluations show developmental or unusual biological activity.

8.7.3.4 Release pattern and stability of the material indicates probable long-term exposure.

8.7.3.5 Partitioning data indicate that bioaccumulation might result in concentrations in aquatic organisms that are toxic to predators.

8.7.3.6 If hazard is judged uncertain and there is continuing interest in the material, Phase III is necessary.

9. Phase III—Use of High-Cost Information (see Fig. 4)

9.1 Because of the substantial increase in time, effort, and money required for tests considered in Phase III, it is particularly important in this phase that the hazard assessment program be tailored to the individual material in order to obtain the most useful information in the least expensive, scientifically sound manner. If tests are conducted, a representative and well-characterized sample of test material is essential (see Appendix X3). Careful consideration of biological, chemical, and physical properties is required so that:

9.1.1 Stock solutions, flow rates, dilution water, etc., allow maintenance of desired test concentrations,

9.1.2 Analytical monitoring will adequately describe exposure, and

9.1.3 Appropriate interpretation and extrapolation of test results to environmental conditions is possible.

9.2 *Refined Estimates of Environmental Concentrations*—Unless it has already been done, a thorough modelling effort of the fate of the material should be performed using stability and rate constants and partition coefficients (see Appendix X6). It is especially important to predict peak concentrations, concentrating mechanisms, and sinks. If the material of concern or a similar material is already in use, field monitoring should be used to validate the model.

9.3 *Chronic Toxicity to Aquatic Animals*—The more frequently recommended or considered types of long-term tests are listed in Appendix X8. Selection of the most appropriate test(s) should take into account several factors:

9.3.1 *Stability of Material*—If biological or chemical stability of the test material is marginal, but a chronic test with an animal species is necessary, practical considerations usually dictate conducting the shorter early life-stage test. Even with high flow rates, maintenance of concentrations of unstable

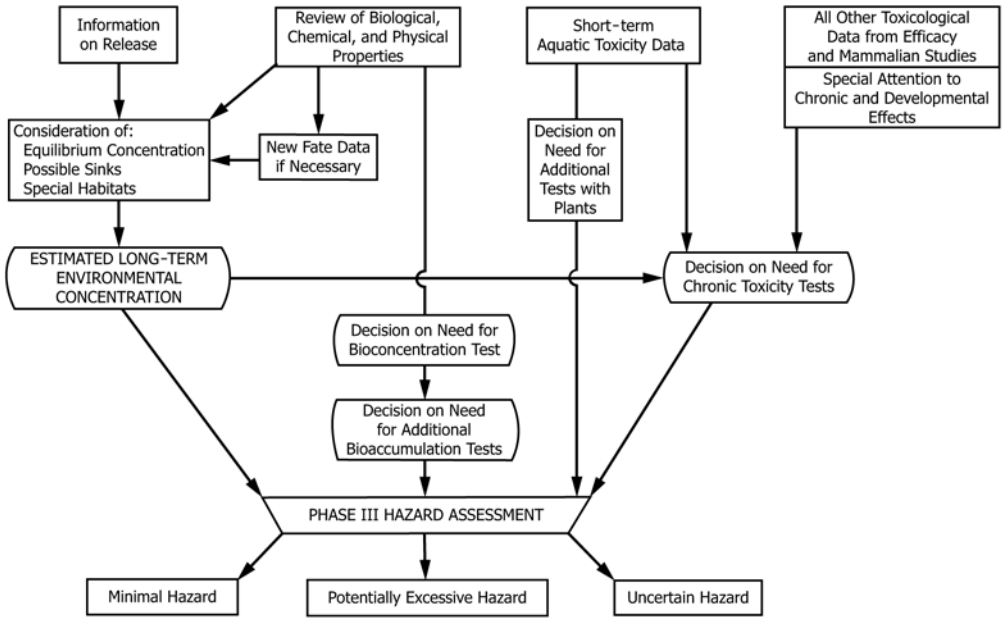

FIG. 4 Phase III—Use of High-Cost Information

materials in test chambers is often impractical over extended periods. Reassuringly, metabolic and other degradation processes generally limit the concentration, extent, and duration of such unstable materials in the environment.

9.3.2 *Species Sensitivity*—If acute toxicity data indicate unusual sensitivity of a particular trophic level, family, or species to the test material, a test should be conducted with the phylogenetically closest species for which a chronic test method exists.

9.3.3 *Target Species Toxicity*—If the material is a pesticide, a test should be conducted with the species most closely related to the target species for which a chronic test method exists.

9.3.4 *Environmental Exposure Areas*—If saltwater areas are of concern, species representative of such waters should be used in chronic tests. Similarly, if EnCs in cold, clean waters pose a major concern about salmonid populations, salmonids deserve serious consideration because they are sensitive to many materials, and they can be used in early life-stage tests.

9.3.5 *Acute Toxicity Divergence by Species*—If results of acute toxicity tests present an unusual pattern or show large differences in sensitivity between species, chronic testing should probably include more than one species. The species used will depend on the hypothesis used to explain the unusual or unexpected differences.

9.3.6 *Environmental Concentrations*—When chronically toxic concentrations closely approach the EnC, more extensive chronic testing should be considered.

9.3.7 *Agency Guidelines*—Assessment of materials subject to regulatory review by the U.S. Environmental Protection Agency or other agencies will need to take into account species or test preferences indicated in agency guidelines.

9.4 *Use of Acute-Chronic Ratios*—Measured or estimated acute–chronic ratios are used to predict the results of chronic tests with species of fishes and invertebrates with which appropriate acute tests have been conducted but chronic tests have not. Ratios for some materials and species are between 1 to 3 and most are less than 100. For a particular material, species that are taxonomically similar and species with similar acute sensitivities are more likely to have similar acute-chronic ratios. The more chronic data available for species sensitive to the material and similar materials, the greater the ratio of measured and estimated chronic values to EnCs, and the greater the agreement between available chronic data, the more acceptable it is to use an acute-chronic ratio instead of conducting a chronic test.

9.5 *Toxicity to Aquatic Plants*—When short-term algal tests (see 8.4) indicate that an EnC may affect algae, a long-term algal test (**8**) is usually desirable. If tests with algae are not completely reassuring, tests with vascular plants, such as the freshwater *Lemna* sp. (**9**), *Elodea* sp., and *Potomogeton* sp. or the saltwater *Thalassia* sp. or *Sargassum* sp., are desirable.

9.6 *Bioconcentration*—If the information available from Phases I and II indicates that bioconcentration might result in unacceptable effects on uses or consumers of aquatic organisms, it may be necessary to experimentally determine the BCF (see Practice E1022). If the predicted or measured BCF is low or the material is known to be extremely unstable, easily metabolized, or not very toxic to consumers, the bioaccumulation hazard is minimal and experimental determination of the BCF should not be necessary. If the predicted BCF is high and the material is known to be stable and relatively toxic to consumers of aquatic organisms, hazard is probably excessive and an experimentally determined BCF may not be necessary. If the predicted BCF is medium or high, the material is reasonably toxic to consumers, and factors of uptake or metabolism are uncertain or unknown, experimental determination of the BCF is probably necessary. If a species shows a marked increase in sensitivity during a chronic test, this might indicate that the organisms are accumulating the material and are unable to metabolize, excrete, or harmlessly store it. Then a bioconcentration test is probably desirable.

9.7 *Bioaccumulation from Food*—Bioconcentration only accounts for uptake by aquatic organisms directly from water, but uptake from food is another route for bioaccumulation. A review (**10**) indicated that for aquatic species directly exposed to a test material in water, the added body burden from dietary exposure was statistically indistinguishable or qualitatively insignificant when compared to that obtained directly from water, with only one exception (DDT). However, indications of the importance of uptake from sources other than water have been reported for kepone (**11**), endrin (**12**), PCBs (**13**), and mercury (**14**), and general models of food chain transfer have been developed (**15**).

9.7.1 Some laboratory test procedures to evaluate uptake by aquatic organisms from food and other complex interactions have been developed (**16**), but these methods require substantial biological and chemical effort. Studies of the importance of uptake from food by aquatic organisms are probably only necessary for materials with very low depuration rates. Studies of uptake from food may not even be necessary for a material with a very low depuration rate if the material has been shown to have low toxicity to predators.

9.8 *Phase III Hazard Assessment:*

9.8.1 A judgment of minimal hazard to aquatic organisms and their uses is probably appropriate if all of the following are true:

9.8.1.1 The measured or estimated MATCs for sensitive species are enough greater than the EnCs for appropriate habitats that the estimated confidence intervals do not overlap.

9.8.1.2 The BCF is less than 100 or the toxicity of the material to consumers of aquatic organisms is so low that concentrations of the material and its metabolites in aquatic organisms should not cause unacceptable effects on predators, including humans.

9.8.1.3 Exposures of aquatic organisms are likely to be incidental or temporary and depuration so rapid that there is little likelihood of adverse effects due to chronic toxicity or bioaccumulation.

9.8.1.4 No other information indicates a cause for concern.

9.8.1.5 A review of the items in 6.3.2 is reassuring.

9.8.2 Hazard should be judged potentially excessive if any of the following are true: (*a*) An appropriate MATC is below an EnC in surface water; or (*b*) Concentrations of the material or its metabolites in aquatic organisms are likely to cause unacceptable effects on predators. Before hazard is judged potentially excessive, 6.3.3 should be reviewed.

9.8.3 In some cases, hazard may still be uncertain, or it may be known to be borderline. In such situations, small-scale field trials with biological and chemical monitoring may be desirable to provide additional information on fate, acute and chronic toxicity, bioaccumulation, and other possible effects such as avoidance, flavor impairment, or subtle effects on aquatic communities or predators.

APPENDIXES

(Nonmandatory Information)

X1.

The portions of a complete testing program that are less likely to be under direct control of an aquatic toxicologist are covered in Appendix X1 – Appendix X6. Their placement in appendixes should not be considered an indication of low importance. Various statements in the description of the hazard assessment process have emphasized the importance of using such information when designing aquatic tests and interpreting results. A hazard assessment program cannot be acceptable if it neglects information on release and properties of the test material, because reliable EnCs are required at all points in the process. Also, mammalian and other toxicological data often developed by other groups should be reviewed to help in planning aquatic toxicity tests and in deciding whether bioaccumulation by aquatic organisms should be a major concern. Additionally, some information related to aquatic tests is supplied in Appendix X7 and Appendix X8.

PRODUCTION, USE, DISPOSAL, AND OTHER RELEASE

X1.1 Hazard can be assessed for a specific release of a material, such as a specific use of a pesticide, but hazard assessment should usually take into account production, disposal, and other uses because these may add to the EnCs or increase the temporal and geographical regions of concern. For materials already in production, information on existing production, use, and disposal should be obtained. For new materials and new uses of existing materials, estimates must suffice.

X1.2 *Production*—Amount of total production should be known or estimated so that a mass balance of all releases can be performed. Location of production will be necessary to consider transportation to use and disposal areas.

X1.3 *Major Use*—Amount for major use can be estimated directly from production plans or indirectly from (*a*) the percentage in a product and an estimate of the amount of the product, (*b*) per capita use of the material, or (*c*) amount of another material that might be replaced by the material of concern.

X1.4 *Other Uses*—Consideration of additional uses should not be neglected in arriving at total use, especially if use or disposal patterns of major and minor uses overlap.

X1.5 *Similar Materials*—These may be considered, either as a method of estimating use of a new material, or in considering possible impact on total use, where it is appropriate to combine existing and new uses.

X1.6 *Form of Material*—The physical form of the material or the product containing it is important in assessment of human safety and should not be overlooked in consideration of environmental hazard. Water-soluble liquids are more readily available to aquatic organisms, but leaching of solids, emulsification of water-insoluble liquids or solids, and precipitation of gases or aerosols also represent routes that can cause exposure of aquatic species to materials and can even make exposures possible over wider areas.

X1.7 *Type of Container*—The type of container and its size may limit potential concentrations in the environment. Such consideration should be reviewed, including accidental spillage in transit, in use, or from container disposal. Spillage of relatively toxic materials could cause a localized but severe hazard to aquatic organisms, especially during transport by water.

X1.8 *Geographical Pattern*—Most materials are produced and disposed in one or more discrete locations, but the geographical pattern of use will vary widely. Some pesticides have small areas of use, whereas most household products are used nationwide. Geographical areas should be considered because of their interrelationships with soil, temperature, rainfall, etc.

X1.9 *Time Pattern*—A full assessment of time factors involved in production, use, disposal, and other release should be considered when estimating EnCs of a material. Whereas some materials will be produced, used, and disposed uniformly throughout the year, the total production of others may be used, applied, and disposed in a season or much shorter time and therefore might cause higher concentrations at these times. Regularity of use should be assessed in determining potential for release to the environment. Seasonal drainage and precipitation patterns will affect runoff and dilution ratios and may allow high concentrations to become available at intervals.

X1.10 *Method of Use or Application*—For pesticide-type products, application rates and mode of application are usually well specified, thereby making total use reasonably easy to calculate. Hand sprayers or powder spreaders will usually restrict the amounts immediately reaching surface waters, whereas power sprays and aerial spraying will result in proportionately higher direct fallout without immediate waters and decreasing amounts in downwind waters.

X2. BIOLOGICAL CONSIDERATIONS

X2.1 Organisms can affect the form and location of a material through degradation and uptake. Uptake by aquatic organisms is covered explicitly in 7.4, 8.6, 9.6, and 9.7.

X2.2 *Microbial Degradation*—The ability of microbial systems to degrade organic chemicals is often an important factor in reducing the concentration entering or persisting in surface waters. Although there are exceptions, biological degradation of organic materials usually not only decreases toxicity, but also increases water solubility and decreases bioaccumulation. Procedures for measuring microbial degradation range from basic screening tests to procedures that model environmental systems such as waste treatment plants and surface waters (**17**). As indicated in X5.1, special care is necessary in designing appropriate tests for materials with known or suspected antimicrobial efficacy. Testing should avoid artifacts resulting from unreasonably high concentrations.

X2.3 Tests of microbial degradation should parallel as closely as possible the kinds of situations that are anticipated from projected patterns of production, use, disposal, and other release. For agricultural chemicals, degradation tests in soils and possibly in surface waters may be most appropriate. For materials that are disposed of in household waste treatment systems, models of biological waste treatment systems are appropriate, supplemented in some situations by further degradation in surface waters.

X2.4 For many materials, primary biodegradation, which modifies the properties of the material, may be adequate. For others, the degree and rate of more complete mineralization need to be determined. In all cases, the nature and toxicity of the residue must be considered. The more complete the information about degradation by biological processes, and the more complete the adequacy of data supporting lack of interference with such processes, the more certain can be the hazard assessment. A substantial effort may be needed to determine the routes of degradation and the products. Such effort should be undertaken only if justified by the structure, properties, and amount of concern about environmental exposure. The complexity of chemical determinations may, in some cases, make a toxicity test of the partially degraded material a preferred and appropriate alternative.

X2.5 The potential for chemical or physical interactions with biological processes should be considered. Materials that have low solubility in water or cause precipitation when added to water can show a rate of degradation in improperly designed tests that is not indicative of the environmental situation. On the other hand, chemical or photochemical reactions of biologically resistant materials can produce intermediates that are utilized by microorganisms. Such interactions should be considered in fate studies. Bacterial metabolism can be an important factor in reducing concentrations of organic materials. For those subject to biological waste treatment processes, 80 to 95 % of the material entering raw sewage may be converted to simpler chemicals or even mineralized, thus substantially reducing the EnCs. Further degradation in the receiving water can be expected and its rate may be important to determine.

X2.6 *Metabolism by Other Organisms*—Metabolites are often identified in studies of toxicity to mammals and in efficacy tests. Information from such studies and on the metabolism of related materials should provide indications of metabolites that may be formed in aquatic organisms.

X3. CHEMICAL CONSIDERATIONS

X3.1 *Positive Identification*—Complete chemical characterization of the test material is important, but is often difficult to obtain. Although short-term tests may have to be conducted with incompletely characterized materials, use of such samples in longer and more expensive tests is questionable. In all cases the sample history, preparation procedure, and all pertinent results of chemical analyses should be a matter of record, and pertinent details should accompany any report of test results.

X3.2 *Pure versus Technical Grade*—Although reagent-grade (or better) individual chemicals have considerable appeal in basic research, their use in hazard assessment may be unrealistic and could provide misleading results. Many industrial chemicals contain a mixture of isomers, homologues, or varied length polymer chains, as well as impurities or by-products. It is highly desirable in some cases to compare the toxicity of the technical-grade material with that of the major component. Use of reagent-grade materials can simplify the development of structure-activity correlations, which may then allow estimates of the toxicity of more complex mixtures. Comparison of reagent-grade and technical-grade materials may also provide useful information on whether some component or impurity is causing most of the toxicity. In many cases, separate measurement of the toxicity of each component would require so massive a research effort that a test on the technical-grade material, plus knowledge that the majority of components are similar in structure and can be assumed to have similar effects, must suffice. The test material should be made from controlled raw materials, by a controlled process, and subject to regular quality control. These safeguards should provide adequate assurance of usefulness of results, unless hazard is borderline.

X3.3 *Formulated Mixtures*—When specific materials are to be mixed to improve efficacy, some testing to compare the toxicities of the mixture and the major active ingredient should be considered to ensure against the possibility of unacceptable synergistic behavior. Minor adjustments in mixtures usually should not require further testing, especially if lack of interaction has previously been established.

X3.4 *Complex Mixtures*—When the hazard of very complex mixtures is assessed, complete chemical characterization may be impractical. Without structure information and the kind of extrapolation it allows, a greater breadth and depth of data may be necessary to supply the same degree of information.

X3.5 *Chemical Reactivity*—Information on chemical structure is important as the initial step in the determination of the reactivity of the material, and early involvement of a chemist knowledgeable in dilute solution chemistry might be helpful.

X3.5.1 *Acid-Base Properties*—Materials ionizing to form strongly acidic or basic solutions can adversely affect sensitive membranes; tests using concentrated solutions may produce artifacts that are not representative of more typical buffered environmental situations. Such acidic and basic solutions can sometimes affect test apparatus if it is not properly designed.

X3.5.2 *Reaction with Metals*—Strongly acidic or basic materials and others capable of reacting with metals can affect the form of metals present in test solutions and suspended particles. Appropriate safeguards should be taken and appropriate experiments planned to determine the importance of this factor under simulated but realistic conditions.

X3.5.3 *Photodegradation*—Photodegradation has been particularly significant for those agricultural chemicals for which terrestrial exposure to sunlight is appreciable and chemical and biological breakdown processes are extremely slow. For such chemicals, photodegradation studies may be appropriate, but the results may not be easy to apply **(17).**

X3.5.4 *Chlorination and Ozonation*—Materials that are not mineralized in waste treatment plants may be subjected to chlorination or ozonation. In addition, materials that reach public water supplies will probably be subjected to chlorination.

X3.5.5 *Other Reactions*—Hydrolysis, oxidation, reduction, esterification, precipitation, complexation, and a variety of other reactions should be considered when handling samples, making stock solutions, and conducting tests to ensure that the material actually tested is what the investigator believed to be present.

X3.5.6 If reactions affect a substantial fraction of the material, it may be important to determine whether the reactions are pH- and temperature-dependent and to identify the reaction products and their biological, chemical, physical, and toxicological properties.

X3.6 *Chemical Similarity*—Availability of pertinent information on related materials may provide information useful in early selection and planning of the most critical experiments. Such information may provide a pattern of expected results and a logical framework for extrapolation of results to untested conditions or to untested species. Caution must be taken when extrapolating and some minimal toxicity checks should be considered.

X3.7 *Chemical Analysis Methods*—The usefulness of results of many tests (not only those dealing with toxicity but also those involving partitioning and environmental fate) is often dependent upon suitable analytical determinations of the concentration and identity of the test material. The need for analysis begins, of course, with the initial characterization of the material, its components, and its impurities. Difficult as this may be, it becomes successively more complicated as one moves from a simple laboratory system to the more complex and dilute situations of waste effluents, receiving waters, soil systems, and plant and animal tissues, where concentrations of interest can range downward from parts per million to fractions of a part per billion. Further, in complex and unknown systems, partial degradation products, similar chemicals, natural materials, and chemical interactions may affect analytical results or complicate interpretation because of interferences or uncertainties of recovery.

X3.8 The time and effort required to develop sensitive, specific, and reliable analytical procedures will, in many situations, dictate that testing should go forward with methods substantially less than perfect. In such cases, several points should be kept in mind:

X3.8.1 Development of specific and sensitive analytical methods should be justified by the probable need. When hazard can be adequately assessed without such methods and complicated testing is unlikely, a simple analytical method may suffice.

X3.8.2 When analytical limitations are real, they should be recognized and appropriate care taken both in design and interpretation of experimental results. In some cases, biological assays can be substituted for other types of measurement, and may obviate, or at least postpone, the need for chemical measurements in difficult systems. Simplification of systems and experiments may be needed.

X3.8.3 The usefulness of radiolabeled materials should be carefully considered, because they provide sensitivity and the ability to track and monitor recovery of labeled material from complex systems. However, the non-specific nature of radioactive measurements can limit usefulness when interference by impurities or degradation products is possible. The position of the label should be carefully considered to provide maximum and accurate information. The utility of labeled materials should not lead to their routine use, because their contribution to a specific assessment program may not justify their cost.

X3.8.4 Analytical procedures should always be completely documented and appropriately referenced. Documentation should include known limitations of the methods and guidance on appropriate interpretation of results based on them.

X4. PHYSICAL CONSIDERATIONS

X4.1 *Solubility in Water*—Ideally, aquatic toxicity and bioconcentration tests are conducted without additives in the dilution water. The form of the test material in both stock solutions and dilution water under test conditions should be determined. In such determinations, special consideration should be given to very hard, very alkaline, and salt waters.

X4.2 *Solubility in Organic Solvents*—If water solubility is limiting in stock solutions, water-miscible organic solvents may be useful. Selection of the most appropriate solvent for minimum use and effect may require development of solubility data in a variety of solvents.

X4.3 *Partition Coefficient*—Information about solubility in water or in polar and non-polar organic solvents may allow estimation of the octanol-water partition coefficient (**10**). The correlation covering a wide range of solubilities allows a prediction within one order of magnitude, which is usually adequate for early estimates. From estimated or measured octanol-water partition coefficients, it is then possible to estimate the BCF (**3**). An estimated BCF will allow a preliminary assessment of whether bioaccumulation might be an important factor in the assessment program.

X4.4 *Sorption on Solids*—Materials that are polymeric, surface active, or of low solubility in water are among those that may be strongly sorbed to solids (**17**). Such sorption definitely represents a complicating factor in many aquatic tests, but recognition of the problem and use of proper precautions and analyses should minimize the confusion that might otherwise result. Information about sorption onto specific surfaces may be important in selection of construction materials for testing and sampling apparatus. Sorption is also a process which might prevent materials from developing appreciable concentrations in surface waters by being removed in waste treatment systems or held by soils or other natural solids. Sorption may result in substantial concentrations in sediment or soil, thus making tests with benthic or terrestrial species necessary. The potential for desorption form solids during and after transport to other locations and types of waters should also be considered.

X4.5 *Effects of Surfactants*—When surfactants are used in formulations, determinations of their effect in combination with the active ingredient are usually appropriate. In certain environments, low concentrations of natural and synthetic surfactants may be present and may need to be considered.

X4.6 *Volatility*—Highly volatile materials create certain testing problems, including the potential for laboratory accidents. For such materials, vapor pressure and flash point data should be obtained early in the program. Flow-through tests with aquatic organisms and determination of the actual concentration present in test solutions should be considered.

X4.7 *Temperature*—Most biological, chemical, physical, and toxicological properties are affected by temperature to at least some extent. Thus, it should be taken into account when considering EnCs and effects.

X4.8 *Other Physical Properties*—Specific gravity and viscosity can be important and should be considered if they may substantially affect rate and completeness of dispersal or the temporary or permanent location of the material within the aqueous environment.

X5. TOXICOLOGICAL CONSIDERATIONS

X5.1 *Microbial Effects*—Materials designed for, or possessing, antimicrobial efficacy should be carefully assessed in situations where antimicrobial activity might be undesirable, such as in biological waste treatment operations and surface water and soil systems. Usually, dilution of antimicrobial materials in these systems will reduce antibacterial action, but in systems where biodegradability of the material is being tested, excessive concentrations could result in inhibition.

X5.2 *Toxicity to Target Species*—Some of the materials of highest concern are those developed as agents for control of nuisance terrestrial or aquatic species. The most obvious lesson gained from their efficacy for such purposes is that their effects on related or analogous desirable non-target aquatic species should be studied. For example, materials effective as insecticides should be suspect for their effects on aquatic arthropods; herbicides should be tested for effects on algae and other aquatic plants. The mode or rate by which toxic effects are manifested or the target organs implicated in efficacy studies may provide useful input for tests with non-target species. Also, conditions maximizing efficacy need to be explored in order to identify special environmental concerns. Effects on any species by materials of similar chemical structure should also be utilized in planning programs and interpreting data on new materials.

X5.3 *Human Safety Data*—Information developed with mammalian species primarily for human safety assessments should be reviewed, not only for its direct input to tests with aquatic species, but also to determine if bioaccumulation by aquatic species might result in substantial doses to humans or other predators. Information on toxicity to non-aquatic species is also required when concentrations in drinking water or in food crops may be of concern. Data on toxicity to mammalian species often attain a level of sophistication that at present is rarely reached in aquatic toxicology. Accumulated mammalian studies on selected test animal models has lead to a more complete understanding of normal function and pathology, so that target organs or systems can be more reliably identified and the mode of toxicity deduced. Developmental studies and those at the cellular level can also supply useful information. A review of these accumulated toxicological data would appear appropriate, but has often been overlooked or disregarded by aquatic toxicologists.

X5.4 *Ancillary Toxicological Data*—Care must be exercised in using data developed for efficacy or mammalian safety in the assessment of hazard to aquatic organisms. Some data from these sources may be very useful when making a preliminary assessment, making decisions about the need for certain kinds of tests, and selecting aquatic species that are most appropriate. Although all these ancillary data should be surveyed, particular attention should be focused on the following questions:

X5.4.1 Is there any indication of a cumulative toxicological effect?

X5.4.2 Does relative sensitivity between species suggest any group of aquatic species that might be particularly sensitive to the test material?

X5.4.3 Do reproductive tests show effects at concentrations unusually low in relation to those that are acutely toxic to the same species?

X5.4.4 Does the mode of action or the life stage for which the material is designed suggest aquatic species or life stages that are likely to be most sensitive?

X5.4.5 Do sorption and metabolic studies with other species provide useful information about body burdens, metabolic pathways, etc., that should be considered in aquatic testing?

X5.4.6 Do cellular or fetal abnormalities or mutagenic effects occur at concentrations that suggest a need for reproductive studies with aquatic species?

X5.4.7 Do effects on growth or behavior suggest that similar effects are likely with aquatic species?

X5.4.8 Does the effect of mode of exposure on toxicitysuggest that availability of the material may be substantially affected by means of exposure or interaction with environmental factors?

X5.4.9 Does the toxicity to potential consumer species suggest that any significant residues accumulated in aquatic species might pose a hazard to higher species?

X6. ESTIMATING ENVIRONMENTAL CONCENTRATIONS

X6.1 *Potential Routes to Water*—Direct planned application to water or incidental application from aerial spraying of adjacent land areas represent the simpler situations and allow direct computation of anticipated initial EnCs. More uncertainty exists when concentrations in water depend on the degree and rate of leaching of material from soil. Experimental determinations in simulated soil leaching systems are generally required and calculations depend on local topography, soil, weather conditions, and the chemical and physical properties of the test material. As an initial approach, data on similar materials may be used to estimate EnCs for new materials.

X6.1.1 For materials used within the home, a determination should be made of the fraction that would be disposed of directly to waste treatment plants. Subsequent estimates should be made of the amounts that could reach receiving waters after waste treatment, not only in effluents but also by leaching from landfills used for sludge disposal. Alternately, in non-sewered areas, the material may reach receiving waters after discharge via home treatment systems or by direct discharge to surface waters without treatment.

X6.1.2 In an industrial situation two factors should be considered. Discharge in aqueous effluents is usually regulated, but may require the development of supporting toxicity data for new materials to determine appropriate discharge limits. Such data are also important in determining precautions necessary to minimize hazards of an accidental spill during manufacture or shipment and to develop acceptable practices for container washout or disposal.

X6.1.3 Many materials, although manufactured in substantial quantity, may be used, converted, controlled, or disposed of in a manner that limits their potential to reach water, thereby making exposure of aquatic organisms of little concern.

X6.2 *Types of Water*—Consideration of the possible routes to water naturally leads to a delineation of the types of water that could be affected. If the amounts and patterns of production, use, disposal, and other release are such that a material could be expected to reach only local farm ponds or marginal quality local streams, then only a few toxicity tests with typical species may be necessary to assess hazard and provide needed directions for safe use and disposal. Broadly distributed materials reaching high quality or major freshwater streams and lakes require consideration of a greater variety of test species and the possibility that more subtle effects could be important. Therefore, a program that will allow a more careful assessment of the hazards in a variety of freshwater situations would be required. If substantial concentrations of the material will reach estuarine or ocean waters, the test program should include commercially and ecologically important species typical of these waters. Whereas direct or proximate discharge to an estuary obviously requires such consideration, the possibility of very stable materials traveling long distances into estuaries should not be overlooked. The possibility of differing chemical interactions in salt waters should also be considered.

X6.3 Prediction of EnCs often proceeds from use of simple models and assumptions in Phase I to complex compartmental models in Phase III (**18**).

X7. SELECTION OF TEST SPECIES

X7.1 The primary consideration should be relevancy to the hazard assessment:

X7.1.1 *Habitat*—Does the hazard appear more likely for species in the water column or for those of the benthic community?

X7.1.2 *Types of Water*—Is a specific type of water (fresh, alkaline, estuarine, ocean) of special concern?

X7.1.3 *Temperature*—Are warmwater or coldwater species of more concern?

X7.2 If relevancy considerations are not critical, other factors should be taken into account. Positive attributes of species are:

X7.2.1 Widespread distribution in the environment,

X7.2.2 Availability of data on sensitivity to a variety of materials,

X7.2.3 Commercial and recreational importance,

X7.2.4 Availability on a regular basis or ability to maintain or breed healthy specimens on a continuing basis,

X7.2.5 Appropriate sensitivity to similar materials, and

X7.2.6 Recommendations by technical and regulatory organizations.

X7.3 A selected strain of a species may be useful under circumstances where genetic uniformity is advantageous. Rare or endangered species should normally not be considered.

X8. LONG-TERM TOXICITY TESTS

X8.1 *Fish Life-Cycle Tests:*

X8.1.1 *Fathead Minnow (***19***)*—This approximately 9-month test with *Pimephales promelas* Rafinesque allows exposure beginning with embryos or newly hatched fry through reproduction and exposure of next generation embryos and fry. This provides not only exposure of sensitive stages, but also allows assessment of effects on growth and reproduction in the determination of an MATC.

X8.1.2 *Sheepshead Minnow (***20, 21***)*—This test with *Cyprinodon variegatus* Lacepede is a 5 to 6 month saltwater counterpart of the fathead minnow life-cycle test.

X8.2 *Brook Trout Partial Life-Cycle Test* **(22)**—This test begins with juvenile *Salvelinus fontinalis* (Mitchell). Because of longer maturation time, larger test equipment, and more exacting water requirements, it should be used only when assessment of a salmonid fishery resource is critical.

X8.3 *Early Life-Stage Tests* **(23)**—This test starts with exposure of fish embryos and continues with exposure of fry or juveniles through their early development. Although it does not provide a total life-cycle exposure and therefore does not provide a full assessment of reproductive factors, this test usually provides exposure during the most sensitive life stage. Results of these tests are generally useful estimates of comparable life-cycle tests with the same species **(24).** In addition, early life-stage tests can be conducted with a wider variety of fishes than can life-cycle or partial life-cycle tests.

X8.4 *Daphnid Life-Cycle Test* **(24, 25)**—Life-cycle tests with species in this family are shorter than those with fishes and offer, therefore, substantial savings in time, as well as advantages in smaller test equipment and easier availability of the organisms. When chronic data with a freshwater invertebrate are desired, a species in this family usually is the first choice. The NOEC obtained using *Daphnia magna* has been shown to correlate well for metals, pesticides, and other chemicals with that obtained from fathead minnow life-cycle tests **(26).**

X8.5 *Mysid Life-Cycle Test* **(27)**—A life-cycle test with a species in the family Mysidae is the estuarine counterpart of the daphnid life-cycle test.

X8.6 *Grass Shrimp Life-Cycle Test* **(21)**—A life-cycle test with *Palaemonetes pugio* may be useful when a reproduction study with a saltwater invertebrate in a different family is desired.

X8.7 *Midge Long-Term Test* **(28)**—Various species in the widely distributed family Chironomidae are often used for long-term studies when a benthic species is desired in an extensive program or when there is a need to test a material of limited solubility.

X8.8 *Alternative Species and Experimental Procedures*—Individual investigators have used a variety of other species and tests in an attempt to study sublethal or long-term adverse effects on survival, growth, reproduction, and species competitive position. Before using new species, the criteria for selection of test species discussed in Appendix X7 should be carefully evaluated and the practical requirement of maintaining the species through longer or more critical periods should be realistically considered. Deviations from the suggested test species and recommended procedures for the hazard assessment of new materials should be based on sound reasons because of the added complications, the unknown risks in execution and interpretation, and the likelihood of less general acceptance.

X8.9 *Functional Tests*—Most of the more traditional and widely used chronic toxicity tests study adverse effects on survival, growth, and reproduction of individual species of different families or trophic levels, and are intended to produce data to be used to protect these species and the structure of the aquatic ecosystem. Some of the new tests **(31)** are designed to measure functions of simple biological systems, individual species, or groups of species. These tests have the intent of measuring physiological functions indicative of the well-being of the species or community. There are a great variety of such tests and they measure such diverse activities as community microbial activity, photosynthetic activity, community metabolism, enzyme activity, mobility or swimming, respiration and breathing patterns, and avoidance. These tests attempt to detect adverse effects on sensitive functions of individual species or communities or subtle changes in species or communities that are predictive of important long-term adverse effects.

X8.9.1 Although a number of these tests have proven useful as diagnostic or predictive tests with specific materials in particular situations, general application in hazard assessment is questionable at this time because extrapolation of measured effects from such tests to the environment is too uncertain. Further research on such approaches and some use of functional tests in extensive assessment programs could allow comparison with more established tests, and should ultimately lead to a more definitive measure of their value.

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