Designation: E 1850 – 97

Standard Guide for Selection of Resident Species as Test Organisms for Aquatic and Sediment Toxicity Tests[1]

This standard is issued under the fixed designation E 1850; 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 along with Guide E 1192 and guidance from the U.S. Environmental Protection Agency **(1,2)**[2] covers the use of resident species in toxicity testing, particularly if site-specific information is desired. For example, in those systems where particular species are considered to be economically or aesthetically important, it might be more appropriate to utilize resident species for testing **(3)**. For this reason, the USEPA allows development of site-specific chemical standards, using resident species, in order to reflect local conditions **(1)**. This guide is designed to guide the selection of resident species for use as test organisms in aquatic and sediment toxicity tests. It presupposes that the user is familiar with the taxonomy of aquatic and benthic species and has some field experience.

1.2 Because toxicological information is often limited for many aquatic species, it is assumed that the majority of testing applications will be acute tests. Therefore, much of the guidance presented in this guide pertaining to the species selection process is applicable when acute toxicity testing is the desired goal. However, the principles discussed in this guide pertain to chronic toxicity test applications as well, although it should be clearly understood that such testing requires substantially greater effort, time, and resources than acute testing.

1.3 The procedures for selecting resident species in toxicity testing are necessarily general at this time because information is often lacking for specific taxa or groups of taxa. This guide attempts to give specific information when appropriate.

1.4 This guide is not intended to be inclusive. References listed provide a starting point from which to approach the literature. This guide deals solely with aquatic toxicity test situations. Terrestrial, arboreal, or atmospheric species are not considered in this guide.

1.5 This guide is arranged as follows:

1.6 *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.* All safety precautions and health-related practices are the responsibility of the user. Specific safety practices are suggested in Section 8.

2. Referenced Documents

2.1 *ASTM Standards:*

D 4229 Test Method for Conducting Static Acute Toxicity Tests on Waste-Waters with Daphnia[3]

D 4401 Practice for Collecting Benthic Macroinvertebrates with Petersen Grab Sampler[4]

D 4407 Practice for Collecting Benthic Macroinvertebrates with Orange Peel Grab Sampler[4]

D 4556 Guide for Selecting Stream-Net Sampling Devices for Collecting Benthic Macroinvertebrates[4]

D 4557 Practice for Collecting Benthic Macroinvertebrates with Surber and Related Type Samplers[4]

[1] This guide is under the jurisdiction of ASTM Committee E47 on Biological Effects and Environmental Fateand is the direct responsibility of Subcommittee E47.01on Aquatic Assessment and Toxicology.

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[2] The boldface numbers given in parentheses refer to a list of references at the end of the text.

[3] Discontinued; see *1989 Annual Book of ASTM Standards,* Vol 11.04.

[4] *Annual Book of ASTM Standards*, Vol 11.05.

D 4558 Practice for Collecting Benthic Macroinvertebrates with Drift Nets[4]
E 724 Guide for Conducting Static Acute Toxicity Tests Starting with Embryos of Four Species of Saltwater Bivalve Molluscs[4]
E 729 Guide for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians[4]
E 1191 Guide for Conducting Life-Cycle Toxicity Tests with Saltwater Mysids[4]
E 1192 Guide for Conducting Acute Toxicity Tests on Aqueous Effluents with Fishes, Macroinvertebrates, and Amphibians[4]
E 1193 Guide for Conducting Renewal Life-Cycle Toxicity Tests with *Daphnia magna*[4]
E 1210 Test Method for Fluorescent Liquid Penetrant Examination Using the Hydrophilic Post-Emulsification Process[5]
E 1218 Guide for Conducting Static 96-h Toxicity Tests with Microalgae[4]
E 1241 Guide for Conducting Early Life-Stage Toxicity Tests with Fishes[4]
E 1367 Guide for Conducting Solid Phase 10-Day Static Sediment Toxicity Tests with Marine and Estuarine Amphipods[4]
E 1383 Guide for Conducting Sediment Toxicity Tests with Freshwater Invertebrates and Supporting Annexes[4]
E 1415 Guide for Conducting Static Toxicity Tests with *Lemna Gibba* G-3[4]
E 1440 Guide for an Acute Toxicity Test with the Rotifer *Brachionus*[4]
E 1463 Guide for Conducting Static and Flow-Through Acute Toxicity Tests with Mysids from the West Coast of the United States[4]
E 1498 Guide for Conducting Sexual Reproduction Tests with Seaweeds[4]
E 1525 Guide for Designing Biological Tests with Sediments[4]
E 1562 Guide for Conducting Acute, Chronic, and Life-Cycle Aquatic Toxicity Tests With Polychateous Annelids[4]
E 1563 Guide for Conducting Static Acute Toxicity Tests with Echinoid Embryos[4]
E 1611 Guide for Conducting Sediment Toxicity Tests with Marine and Estuarine Polychaetous Annelids[4]
E 1706 Test Methods Measuring the Toxicity of Sediment-Associated Contaminants with Fresh Water Invertebrates[4]

3. Terminology

3.1 *Definitions:* The words "must," "should," "may," "can," and "might" have very specific meanings in this guide. "Must" is used to express an absolute requirement. "Should" is used to state that the specified condition is recommended and ought to be met if possible. Although a violation of one "should" is rarely a serious matter, violation of several will often render the results questionable. Terms such as "desirable," or "might be desirable" are used in conjunction with less important factors. "May" is used to mean "is (are allowed to)," "can" is used to mean "is (are) able to," and "might" is used to mean "could possibly." Thus, the classic distinction between "may" and "can" is preserved, and "might" is never used as a synonym for either "may" or "can."

3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *impaired water body or site*—a body of water or site which exhibits decreased structural or functional biological integrity, or both, given the geomorphic habitat available. This is typically measured as a decrease in the number of species present or decreased biological productivity compared to sites similar in size and habitat and having few anthropogenic influences.

3.2.2 *indigenous species*—a species that is likely to occur at a specified site for some portion of its life span as a native species.

3.2.3 *key species*—a species that is of special concern for ecological or economic reasons.

3.2.4 *resident species*—a species that is present at a specified site for some portion of its life span.

3.2.5 *surrogate species*—a species that can be studied to produce results to estimate toxicity responses of other species that are not tested directly **(4)**. Frequently, published standard testing procedures, established through nationally recognized agencies or societies such as ASTM, OECD, Environment Canada, and USEPA, have been developed for these species.

4. Summary of Guide

4.1 A list of resident species is compiled from published literature on the natural history of the area, bioassessments of the receiving body of water, species lists compiled by individuals or agencies, maps, and taxonomic keys.

4.2 The list of species is reduced by first defining the objectives of the study and the decisions to be made, followed by a stepwise procedure to determine which species to test. This procedure includes consideration of factors such as ease of handling and testing, availability, sensitivity, and a variety of other concerns (see Section 6).

5. Significance and Use

5.1 The USEPA's policy for whole-effluent monitoring stresses, an integrated approach to toxicity testing **(1, 5)** tests and other measures of toxicity, should be systematically employed and should be related to certain aquatic-system factors, such as the type of habitats available (benthic and water column), flow regime, and physicochemical quality of the site water and sediment. The determination of toxicity is generally accomplished with a few surrogate species for four major reasons: a regulatory agency can compare test results between sites and over time in order to help prioritize enforcement efforts, tests using these species are relatively inexpensive since the organisms can be cultured year-round under laboratory conditions, the reliability of test methods utilizing surrogate species is better established than for other species, and surrogate species are better integrated into toxicity identification evaluations than other species. However, in systems where surrogate species are not found, erroneous predictions might be obtained of environmental impact or water and sediment quality impairment based on toxicity tests using surrogate species **(6)**.

[5] *Annual Book of ASTM Standards*, Vol 03.03.

5.2 This guide is intended to assist researchers and managers in selecting appropriate resident species for site-specific toxicity assessments. This guide could be used to select a resident species for use in predicting the potential toxic effects of a substance in certain types of aquatic environments. Another use might be for selecting a number of indigenous species from the aquatic community, that when tested, might indicate potential toxic effects of the test substance or material on the ecological integrity of that community. Selection of a suitable test species is very important because species might respond quite differently to toxic compounds **(7)**. Species suggested as test organisms by regulatory agencies might not occur in the receiving waters of interest and their sensitivity to a toxic substance might not be representative of the sensitivity exhibited by resident species. Since aquatic ecosystem structure and function is often determined by a few key species **(8, 9, 10, 11)**, toxicological tests with these resident species might be very important.

5.3 This guide can be used in the selection of representative test species for certain site-specific assessments, such as the Resident-Species Criteria Modification Procedure **(1)**, the Recalculation Procedure **(12)**, and ecological risk assessment studies.

5.4 This guide can be used as a general framework for researchers who desire to develop or modify existing toxicity test methods for previously untested species.

5.5 Researchers in countries other than the United States and Canada might obtain useful information from this guide regarding potential test species or test methods for sites of local interest.

6. Species Selection Process

6.1 *Collection of Information*—To select a resident species for toxicity tests, one must first determine what species are likely to occur at the location of interest. This can be determined by examining historical species data for the site that predates contamination, or by examining recent or historical data for nearby reference sites of similar size and habitat type. From these lists, select species that can be handled in the laboratory and for which test data are known, or species with close relatives for which data are available to demonstrate sensitivity to the contaminant of interest. Methods suggested include the following:

6.1.1 *Bioassessments*—Quantitative sampling of macroinvertebrates, fish, algae, and macrophytes, see Guides D 4229 and D 4407 **(11, 12, 13)** located outside point and non-point sources of pollutants can yield information on the types of common species available as potential test organisms. If a site containing potential pollutants is the object of study, a bioassessment performed both within and outside of the suspected impaired area might reveal species-specific population trends which might be correlated to toxicity. Species that exhibit decreases in abundance or biomass, or both, within or downstream of the suspect area might represent sensitive resident species that could be utilized in toxicity testing. Factors such as time of sampling, similarity of habitat regimes, and the number of samples taken might influence the accuracy of this approach (see Guide D 4556, Practice D 4557, and Practice D 4558). Studies of community structure **(13)** can be conducted to determine abundance and dominance of species. Such studies can provide lists of potential test species, as well as suggest suitable organism and laboratory maintenance procedures.

6.1.1.1 Bioassessments can also have significant application to the USEPA Recalculation Procedure **(1, 12)** that allows deletion of nonresident species from the National Water Quality criteria database. Bioassessments can be used to determine the types of species and taxonomic families capable of naturally existing in the water body of interest **(13, 14)**. Following the procedures outlined later in this guide, suitable test species can be identified, using bioassessments to replace missing data in the recalculated database for a given pollutant. Resident species data could then fulfill the minimum USEPA data requirements for developing water quality criteria **(1)**.

6.1.2 *Historical Survey of Study Site*—Records of past biological surveys or published fish harvesting documents can be compared with recent surveys or bioassessments, or both. Decreases in certain species over time might result from environmental degradation due to the presence of toxic materials or enhancement due to decreasing contaminant concentrations or nutrient enrichment. Such species may be candidate resident species for site-specific toxicity testing. It would be desirable not to use species that are believed to have been affected primarily by habitat changes (due to dams, extreme storms, fires, or other natural disturbances) or biological disturbances (introduction of exotic species or parasites). In general, it is desirable to utilize a species for which there exists information concerning its ecology, sensitivity, and life history. Many species have been used successfully in a variety of experimental settings to assess water or sediment toxicity (see Guides E 729, E 1192, and E 1525, and see Appendix X1). Methodological information gathered from such studies might be useful in the selection of a suitable species for testing.

6.1.3 *Ecoregion Species Lists*—Lists of species, by geographical (in the case of saltwater) or watershed location **(14, 15)** and books on taxonomy, detailing distribution locations of species, are numerous and generally available (see references in Appendix X2). Review of a list for the area of interest obtained through local and state fisheries and other natural resource agencies can provide additional potential test species. However, species lists may contain “ephemeral” or extremely rare species that might be inappropriate to test. These are often species at the fringe of their distribution and are only present when unusually favorable habitat conditions occur in a particular year. There are also many instances where the taxonomy of species may have been questionable. Therefore, it might be more useful to evaluate resident species that are relatively frequent when selecting a test species. Archives containing aerial photographs and infrared photographs are useful for determining wetland plant identifications.

6.1.4 *Taxonomic Studies*—References are available that discuss relative species sensitivity to pollutants (see Appendix X2). Some of the initial research on the ecology and response to stress/pollution of certain resident species has already been conducted **(16, 17, 18)**.

6.1.5 If any of the preceding information sources indicate that surrogate species or closely related species occur in the site of interest, then surrogate species tests should probably be

used. Further species selection processes discussed in this guide might be unnecessary. This is because the surrogate species tests already satisfy all of the selection criteria discussed in this guide.

6.2 *Obtaining Resident Species for Toxicity Testing*:

6.2.1 The ability to perform toxicity tests with resident species will depend on the availability of a sufficient number of organisms, similar in age or size, or both, and history, in order to maximize test precision (see Guide E 729). Some freshwater and saltwater species can be cultured or purchased from a supplier (see Ref **(33)** in Guide E 729), although these might be different genetic strains and therefore potentially different in sensitivity than species collected locally. Appendix X1 lists some examples of non-surrogate species that have been successfully cultured or maintained in a laboratory, or both. In some locations, certain species are sufficiently abundant to allow collection of organisms with similar ages for toxicity testing purposes **(19, 20, 21)**. The organisms must be collected from reference site conditions; that is, outside of potential or actual impact.

6.2.2 Methods for collection of resident organisms will depend on the habitat of the species and possibly on the species itself. Practices D 4401, and D 4557, and Test Method E 1201 are examples of references that describe suitable methods for collecting freshwater and saltwater organisms. Many references in this guide and in Appendix X2 have information on the habitat and appropriate collection methods for various freshwater or saltwater species. In all cases, care should be taken to minimize handling stress on organisms collected from the field. For this reason, non-destructive sampling methods might be preferred over other methods; that is, nets, seines, hand-picking, cores, and bottle samplers might involve less handling of organisms than pumps, kick sampling, dredging, or electrofishing. Regardless of the method of collection, field-collected organisms must be quarantined and acclimated to laboratory conditions prior to testing in order to ensure that healthy organisms are used in testing (see Guide E 729) **(2)**.

6.2.3 Rare or endangered species, as well as most game fishes, must not be collected or used in toxicity tests without prior approval of appropriate federal or state agencies.

6.2.4 The necessary federal or state collection permits, or both, must be acquired prior to collecting resident species.

6.2.5 Field-collected organisms, or organisms obtained from an outside supplier, need to be handled with care once they arrive at the laboratory. It is desirable at first to match laboratory conditions to those under which the organisms had been living previously (for example, similar temperature, pH, alkalinity, salinity, and so forth). Guide E 729 and other ASTM references previously cited in this guide should be consulted for further guidance on organism acclimation and holding procedures.

6.2.6 Field-collected organisms should be representative of the organisms that could occur at the study site based on habitat features available and historic species records for the region and should not have been previously exposed to hazardous materials, contaminants, or pathogens. Therefore, field-collected organisms should be obtained from "clean" areas, well outside of the influence of point- and nonpoint sources. As one check on the appropriateness of a certain species population for toxicity testing, priority pollutant analyses of the site water, sediment, or organism tissues should be used to determine whether organisms have had prior exposure to source-related pollutants. Since many aquatic species can disperse over relatively long distances during different life stages, it might be difficult in certain situations to ensure that field-collected test organisms have not had prior exposure to some toxicant. Furthermore, prior exposure to toxicants might be related to a particular life stage of the organism which might or might not be known. Therefore, in addition to obtaining organisms from relatively "clean" locations, field-collected organisms should be maintained, or preferably cultured, under known "clean" conditions prior to use in testing.

6.2.7 In addition to the surrogate species commonly used, several non-surrogate species have been successfully cultured in the laboratory (for example, the freshwater parthenogenic mayfly *Cloeon triangulifer* **(22)**, the rotifer *Brachionus acuticornis* (see Guide E 1440 and Ref **(23)**), the frogs *Hyla crucifer* **(21)** and Bufo spp. **(24)**, and the marine polychaetes *Neanthes arenaceodentata* (see Guides E 1562 and E 1611) and *Capitella capitata* **(25)** (see Guide E 1562), and in commercial aquaculture facilities (for example, *Mya arenaria, Crassostrea gigas, Crassostrea virginica*, certain freshwater molluscs and crustacea, and several saltwater and freshwater fish species) thereby minimizing the possibility of pre-exposure to toxicant. However, it should be recognized that species cultured under constant laboratory conditions, whether originally resident to a site or not, might not yield predictive test results if seasonally influenced effects are important. Also, a species that has been subjected to continuous laboratory culturing for multiple generations may not exhibit the same sensitivity to a toxicant as a wild population.

6.2.8 Appropriate species may include protozoans, other microfauna, macrophytes, algae, macroinvertebrates, and vertebrates. Many candidate species are cited in USEPA manuals **(2, 26)**, USEPA criteria documents, and documents specific to certain taxonomic orders such as Amphipoda, Emphemeroptera, Isopoda, Odonata, Pelecypoda, and Plecoptera **(12, 27)**. Representatives of these orders have been successfully used in a variety of toxicity test situations **(20, 21)**. Additionally, there are written procedures for using both microphytes and macrophytes in toxicity tests (see Guides E 1218 and E 1415 and Ref **(28)**).

6.3 *Criteria for Selection*:

6.3.1 Selection of species or life stages, or both, depends first on the purpose and scope of the study, and should be appropriate to the scientific inquiry. For example, early life stages of a species might be sensitive to a certain toxicant and readily acclimate to the laboratory environment. These organisms may be used in acute toxicity test or sublethal test designed to assess toxicity using developmental end points, but may not provide information on reproductive behavior. Studies designed to examine biological effects due to certain chemicals should use species that are representative of the assumed target community (for example, algae for algicides, insects for insecticides, and so forth). It might be desirable to use test species that represent a particular trophic level (for example,

primary producers, primary consumers, detritivores, and so forth) or feeding guilds (filter feeders, deposit feeders, algal scrapers, or predators **(29)**). The taxonomic identity of test species used must be determined by appropriate keys (see Appendix X2) and verified by an appropriate expert.

6.3.1.1 In further selecting of appropriate resident test species, the following selection criteria should be considered in order of importance:

6.3.2 *Ease of Organism Procurement and Laboratory Culture and Handling*—Species should be screened for ease of handling, ease of collection, and resistance to shock and handling (see 6.2). Preference might be given to those species that can be successfully cultured in the laboratory and are amenable to laboratory testing. Organisms for use in testing should not have had prior exposure to contaminants or other sources of stress (see 6.2.6). Potential criteria to determine whether a given batch of field-collected organisms is suitable for laboratory testing should include the following:

6.3.2.1 Survival of organisms several days after placement in the laboratory environment should indicate that the organism has adapted to the new environment.

6.3.2.2 Organisms must have no obvious physical abnormalities such as missing body parts or lesions.

6.3.2.3 Organisms should exhibit normal behavior (for example feeding or locomotory, if appropriate).

6.3.2.4 Reference toxicant tests should be performed to compare organism sensitivity (and indirectly their health) over time either with previously reported results or laboratory data being developed for that species and life stage (see section 6.5.1).

6.3.3 *Ease of Test Method Development*—Acute or chronic toxicity test procedures might exist for the species of interest or an ecologically similar species (see ASTM guides referenced in this guide and Refs **(2** and **26)**). In some cases, benthic or sediment-dwelling species can be successfully used in water column testing with the aid of chemically inert structures in test chambers to simulate the natural habitat of the species. For example, glass tubes have been used in aquatic tests for the burrowing mayfly *Hexagenia* **(30)**, and PVC tubes have been used as habitat shelters for the benthic mayfly *Stenonem* **(20)**. For sediment testing, care should be taken to provide an adequate natural or synthetic culture sediment having the appropriate particle size and other physical and chemical characteristics for the species of interest (see Guides E 1383 and E 1367).

6.3.4 *Potential Sensitivity to Pollutants*—A variety of references are available that categorize species in terms of general sensitivity to organic enrichment and other pollutants **(12, 16, 17, 18)**, and there are similar references available for groups (orders, families) of species (for example, Ephemeroptera **(7)**). It is desirable to utilize species for which data are available indicating their relative sensitivity to a given toxicant or class or toxicant.

6.4 *Test Performance Characterization*—To document the quality of the data produced from a given resident species toxicity test (and surrogate tests as well), and to determine its comparability with other species data for the same test material, test method performance characteristics should be determined, preferably prior to definitive screening of the substance of interest. The degree to which a resident species test yields meaningful data will depend on how well the test performance characteristics meet the data quality objectives of the study. Test performance characterization should include the following steps:

6.4.1 Collect and test different batches of the same species over time in order to obtain a measure of the variability associated with testing the particular species. The relative health and quality of test organisms can then be documented through an assessment of their behavioral repertoire and toxicity tests with a known toxicant or, preferably, different classes of toxicants (for example, heavy metals, chlorinated organic compounds, or PAHs) in which the toxicity effect is theoretically constant across tests. Repeated tests using standard or reference materials could be used to: compare the resident species test end point with existing data for standard surrogate test species (that is, data for the same toxicant can be compared to define relative sensitivity of the resident species tested) and define resident species test precision through the development of a reference toxicant control chart for the species and the test material being used **(2)**.

6.4.2 The appropriate exposure time required for testing should be determined and documented. Different taxonomic groups (for example, rotifers versus molluscs) or different life stages of the same species (for example, glochidia versus juvenile stage of bivalves) might require different exposure durations in order to obtain meaningful test end points. As a general rule (consistent with Guides E 729 and E 1192), guidance, aquatic acute toxicity tests should be at least 48 h in length for zooplankton species and 96 h for other species. Longer exposure periods might be necessary in sediment exposures (see Test Method E 1706 and Guides E 1367 and E 1611) and for species that are capable of avoiding pollutant exposure for short periods of time (juvenile and adult bivalves, for example).

6.4.3 If a hypothesis test is used, the statistical power of a particular toxicity test method (that is, the probability of the null hypothesis being accepted when in fact it is false [β error]) and the sensitivity of the test (that is, the probability of the null hypothesis being rejected when in fact it is true [$\propto$ error]) should be determined **(32)** in relation to the decision criteria or data quality objectives of the study. This information will provide a measure of test reliability given the method and test species used. For regression, probit, or logit-based end points such as LC_{50} or IC_{25}, test reliability and data quality of objectives are best stated in terms of the range of the 95 % confidence limit around the end point. The tighter the confidence intervals around the end point, the more reliable the test.

6.4.4 The test method precision (that is, degree to which independent tests, using the same concentration of test material, elicit a similar response or test end point) should be determined **(32)** and compared in relation to the decision criteria or data quality objectives to the study. For certain applications, it might be desirable or necessary to determine test precision and test reproducibility prior to definitive testing of a particular test material.

6.4.5 The flow chart in Appendix X3 summarizes the factors

previously discussed in choosing a resident test species.

7. Interferences

7.1 A number of factors can impede or prevent selection and use of resident species for toxicity testing. The following should be considered when selecting a resident species and measuring its sensitivity during toxicity tests.

7.1.1 Handling of field-collected organisms resulting from collection or transport to the laboratory might cause excessive mortality or sublethal effects.

7.1.2 The age, health, and physical condition of organisms (for example, the presence of parasites, bacteria, and disease) collected from a resident population might not be adequately known.

7.1.3 Determination of species identity of resident organisms might be difficult without damaging the organisms.

7.1.4 The physical characteristics of the testing environment (such as water quality, temperature, water flow, light, cover, or the grain size of the test sediment) and food requirements might affect the organisms' ability to acclimate, recover from handling, or accept the laboratory environment conditions.

7.1.5 Unknown reproductive states at the time of collection might produce aberrant results due to interactions between breeding condition and metabolism or toxicity of contaminants.

7.1.6 The degree of contamination and the history of contamination at the collection site might not be adequately known.

7.1.7 The degree to which the organisms have been exposed to contaminants in areas other than where the organisms were collected is unknown.

8. Safety Precautions

8.1 Field-collection techniques might pose dangers to personnel. Safety provisions, such as the buddy system, complete pre-survey of the collection area, obtaining dam discharge schedules, tidal conditions, and other pertinent actions, should be considered. Personal floatation devices and protective clothing are required. Contact with sediments and water should be minimized. It might be desirable to require immunization for common waterborne diseases. All personnel should be made aware of safety precautions and potential hazards before any collection trip.

9. Report

9.1 The user should report why a particular choice of test species was made (that is, rationale for using a resident species) and the species selection process procedures used for collection, handling, and holding or culturing the organisms in the laboratory should also be well documented and recorded. The record should include the following information, either directly, or by reference to available documents:

9.1.1 Report the source of the test organisms including location and description of the collection site, if appropriate; or the supplier's name and location, collection methods; shipping procedures and conditions, date, and time of acquisition.

9.1.2 The history (including holding time prior to testing) and age/size of test organism(s), scientific name (and strain when appropriate), name of the person who identified the species, and the taxonomic key used for identification should be given. If a brood stock was used, observed specific diseases, disease treatments, holding, and acclimation procedures should be reported. Reasons for, and method of, selection of the species should be given.

9.1.3 A full description of the procedure and apparatus used in breeding, culturing, holding, and handling the organism should be reported. Volume and quality of the water and sediment used in the culture chamber and stocking density in the breeding chambers should be reported along with source and composition of food, feeding methods, frequency, and ration size.

9.1.4 The source of the culture water and sediment (if utilized), its chemical characteristics (including salinity, if appropriate), a description of any pretreatment (including sediment manipulations such as seiving or homogenizing), and results of any demonstration of the ability of the test species to survive and thrive (grow and reproduce) in the water or sediment should be reported.

9.1.5 A report and discussion of data on survival, growth, and behavior of the test organisms in the dilution water or sediment, or both, should be given in sufficient detail to allow for independent statistical analysis.

9.1.6 Results of reference toxicity tests and control chart should be reported.

10. Keywords

10.1 aquatic toxicity testing; bioassessment; indigenous species; resident species; sediment toxicity testing; site-specific monitoring

APPENDIXES

(Nonmandatory Information)

X1. POTENTIAL TEST SPECIES

X1.1 *Algae:*

X1.1.1 Algae, both microalgae and macroalgae (seaweeds), often comprise the major primary producers in aquatic systems, especially lentic waters. Therefore, they are an important component of food webs and overall ecosystem structure and function. Several saltwater and freshwater monocultures are commercially available and amenable to laboratory culture. Consult Guide E 1218 for further information on culturing and toxicity testing of microalgae and Guide E 1498 for culturing and testing of seaweeds.

X1.2 *Aquatic Floating Macrophytes:*

X1.2.1 Although there have been numerous studies which have monitored aquatic macrophyte growth or production in field water quality assessments, little information is available regarding culture and testing of aquatic macrophytes for toxicological studies. Some species of *Lemna* (duckweed) have been successfully used as surrogate test species for this group. (see Guide E 1415 and Refs **(28, 33,** and **34)**.

X1.3 *Protozoa*—Protozoans and other microbial species often comprise the largest portion of the total biomass in some aquatic systems. They are easily collected without specialized equipment and readily transported. Aquatic toxicity tests with flagellates are reported by Honig et al **(35)**. Cairns **(36)** detailed procedures for exposing protozoan communities to various concentrations of zinc and copper, and protozoans have been used for in-situ assessments **(37, 38, 39, 40)**. Several species are commercially available and amenable to laboratory culture.

X1.4 *Rotifera*—Rotifers represent a major component of the food chain in lentic and some lotic systems. As a group, they occupy a variety of niches including those as detrivores, predators, and primary consumers. Their relatively rapid population growth in laboratory culture make them useful for population-level ecotoxicological studies. Commercial kits are available which utilize cysts that can be used over prolonged time periods **(23)**, and ASTM test guidance is available for the genus *Brachionus* (see Guide E 1440).

X1.5 *Attached and Benthic Fauna:*

X1.5.1 Benthic organisms have been successfully used in a number of toxicology studies, including single-species and multi-species tests, artificial stream and microcosm studies, and mesocosm (both stream and pond) studies. Benthic species are potentially useful candidates for site-specific testing since many species have limited mobility and are, therefore, expected to be affected by water quality impairment. There are numerous references citing the sensitivity of benthic organisms to pollutants and their use in toxicology or water quality studies **(11, 12, 17, 21, 29**, **42, 43, 44, 45**, and **46)**. A large number of species have been used in toxicity testing **(42, 43, 44)**.

X1.5.2 A large number of species have been used in toxicity testing **(42, 43, 44)**.

X1.6 *Fish*—Fish have, historically, been one of the major groups of test organisms used in toxicity test. They are relatively visible to the public and are of both economic and recreational importance. Some beneficial or designated aquatic uses are specifically based on certain fish species. Several freshwater and saltwater species can be obtained through commercial suppliers and hatcheries. Young of the year of a particular species can often be obtained from the field in relatively large numbers depending on time of year and water conditions.

X1.7 *Amphibia*—The egg and larval stages of several species of amphibia have proven useful in toxicological and water quality assessment studies. This group can be particularly important in wet-weather or temporary aquatic systems where many other aquatic species (particularly fish) might be absent **(20, 24)**. In these systems, amphibia might represent the only vertebrates and the highest aquatic trophic level. Furthermore, since many of these species spend the greater part of their life cycle in terrestrial systems, amphibia have the potential to act as vectors of bioaccumulative pollutants to terrestrial reptiles, mammals, and birds. Several species are available in various life stages (including eggs) from commercial suppliers. Some species can be cultured year-round in the laboratory.

TABLE X1.1 Examples of Resident Species

NOTE 1—Table X1.1 contains examples of resident species for which test methods and, in some cases, culturing procedures have been developed. This table is not meant to be all inclusive. See legend at the end of the table for explanations of symbols used.

Taxonomic Name	Water Type[A]	Organism Source[B]	Test Type[C]	Test Media[D]	Geographic Range[E]	References
Microalgae						
Anabaena flos-aquae	F	Cu	G	W	U	Guide E 1218
Ankistrodesmus sp.	F	Cu	G	W	U	Guide E 1218
Scenedesmus pannonicus	F	Cu	G	W	U	Guide E 1218
Selenastrum capricornutum	F	Cu	G	W	U	Guide E 1218

TABLE X1.1 *Continued*

Taxonomic Name	Water Type[A]	Organism Source[B]	Test Type[C]	Test Media[D]	Geographic Range[E]	References
Skeletonema costatum	M	Cu	G	W	U	Guide E 1218
Macroalagae and Macrophytes						
Champia sp.	M	Cu	R	W	U	**(4, 7)** Guide E 1498
Macrocystis pyrifera	M	Cu	R	W	U	**(48, 49)** Guide E 1498
Lemna gibba	F	Cu	G	W	U	**(28, 33, 34)** Guide E 1415
Rotifera						
Philodina acuticornis	F	F	A	W	U	**(41)**
Brachionus rubens	F	Cu	A	W/S	U	**(23)**
Branchionus plicatilis	M	Cu	A/C	W/S	U	Guide E 1440
Keratella sp.	F	Cu	A	W	U	**(44)**
Asplanchna sieboldi	F	Cu	A	W	U	**(50)**
Protozoa						
Chilomonas paramecium	F	Cu	A	W	U	**(35, 36, 37)**
Bufo arenarum	F	Cu/F	A/C/E/G	W/S	U	**(24)**
Heliophrya sp.	F	F	A	W	U	**(38)**
Coelenterata						
Hydra attenuata	F	Cu	A	W	U	**(51)**
Annelida						
Polychaeta						
Neanthes arenaceodentata	M	Cu	A/C	W/S	U	**(25, 52)** Guides E 1562, E 1611
Neanthes virens	M	Cu	A/C	W/S	U	**(25, 52)** Guides E 1562, E 1611
Capitella capitata	M	F	A/C	W/S	U	**(25)** Guide E 1562
Dinophilus gyrociliatus	M	F	A/C	W/S	U	**(25)** Guide E 1562
Oligochaeta						
Tubificoides frasia	F	F	A/C	W/S	U	**(53)**
Tubifex tubifex	F	Cu	A/C	W/S	U	Guide E 1383
Limnodrilus hoffmeisteri	F	Cu	A/C	W/S	U	Guide E 1383
Mollusca						
Gastropoda						
Physa spp.	F	F	A/G	W/S	U	**(44)**
Pelecypoda						
Mytilus edulis	M	Cu	A/C/E	S	U	Guide E 724
Corbicula fluminea	F	F	A/G	W/S	E	**(19, 54)**
Crassostrea gigas	M	Cu	A/C/E	S	W	Guide E 724
Crassostrea virginica	M	Cu	A/C/E	S	E	Guide E 724
Haliotis rufescens	M	F	A/C/E	S	U	Guide E 724
Crustacea						
Cladocera						
Daphnia sp.	F	Cu	A/C	W/S	U	**(2, 55)** Guides E 1193, E 1383
Ceriodaphnia dubia	F	Cu	A/C	W/S	U	**(2)** Guide E 1383
Copepoda						
Acartia tonsa	M	Cu	A	W	U	**(47, 56)**
Ostracoda						
Cypris subglobosa	F	F	A	W/S	U	**(55)**
Amphipoda						
Hyalella azteca	F/M*	Cu	A/C	W/S	U	**(57)** Test Methods E 1706
Gammarus lacustris	F	Cu	A/C	W/S	E	**(39, 58)**
Crangonyx spp.	F	F	A/C	W/S	E	**(21)**
Diporeia	F	F	A/C	W/S	GL	**(59)** Guide E 1383
Rhepoxynius abronius	M	F	A	S	W/(E)	Guide E 1367
Corophium insidiosum	M	F	A/C	W/S	W	**(60)**
Grandidierella japonica	M	F	A	W/S	W	Guide E 1367
Eohaustorius estuarius	M	F	A/C	W/S	E	Guide E 1367
Leptocheirus plumulosus	M	F/Cu	A	W/S	E	Guide E 1367
Ampelisca abdita	M	F	A/C	W/S	E	Guide E 1367
Mysidacea						
Neomysis spp.	M	Cu	A/C	W/S	W	Guides E 1191, E 1463
Holmesimysis costata	M	Cu	A/C	W/S	W	Guides E 1191, E 1463
Mysidopsis bahia	M	Cu	A/C	W/S	E	**(2, 47)** Guide E 1191
Decapoda						
Palaemonetes spp.	F	Cu	A/C	W/S	E	**(2)**
Procamberus clarkii	F	Cu	A/C	W/S	E	**(21)**
Echinodermata						
Strongylocentrotus purpuratus	M	F	E	W	W	**(61)** Guide E 1563
Arbacia punctulata	M	Cu	E	W	U	**(17, 62)** Guide E 1563
Insecta						
Hexagenia limbata	F	(Cu)	A/C	W/S	E	**(30, 63)** Guide E 1383
Stenonema sp.	F	F	A/C	W/S	U	**(20)**
Ephemerella sp.	F	F	A	W	U	**(64)**
Isonychia bicolor	F	F	A/C	W	E	**(19)**
Cleon triangulifer	F	Cu	A/C	W	E	**(22)**
Acroneuria sp.	F	F	A	W	U	**(65)**

TABLE X1.1 *Continued*

Taxonomic Name	Water Type[A]	Organism Source[B]	Test Type[C]	Test Media[D]	Geographic Range[E]	References
Leuctra sp.	F	F	A	W	U	**(19)**
Pteronarcys sp.	F	F	A	W	U	**(65)**
Odonata	F	F	A	W	U	**(66)**
Brachycentrus sp.	F	F	A	W	U	**(65)**
Chironomus riparius	F	Cu	A/C	W/S	U	**(57)** Guide E 1383
Chironomus tentans	F	Cu	A/C	W/S	U	**(67)** Guide E 1383, Test Methods E 1706
Amphibia						
Rana temporaria	F	Cu	A	W	E	**(68)** Guide E 729
Rana pipiens	F	Cu	A/C	W/S	E	**(21)**
Hyla crucifer	F	Cu	A/C	W/S	E	**(21)**
Bufo spp.	F	Cu	A/E	W	U	**(24, 68, 69)**
Fish						
Carassius auratus	F	F	A	W	U	**(71)**
Lepomis sp.	F	F	A/G	W	U	**(19, 21)** Guide E 729
Morone saxitilis	F	Cu	A/C	W	U	**(72)**
Fundulus sp.	M	Cu	A/C	W	U	**(2, 47)** Guides E 729, E 1241
Oncorhynchusmy Kiss	F	Cu	A/E	W	U	Guide E 729
Menidia spp.	M	Cu	A/C	W	E	**(2, 47, 73)** Guides E 729, E 1241
Cyprinodon variegatus	M	Cu	A/C	W	E	**(2, 47)** Guides E 729, E 1241
Atherinops affinis	M	F	A/C	W	W	**(73)**
Pimephales promelas	F	Cu	A/C	W/S	E	**(2, 26)** Guides E 729, E 1241

[A] F = freshwater, M = marine.

[B] Cu = species has been cultured in a laboratory or aquaculture facility; F = field-collected.

[C] A = acute; C = chronic (life cycle); E = embryo/larval; G = survival/growth; R = reproduction.

[D] W = water column; S = sediment.

[E] U = ubiquitous; E = eastern United States; W = western United States; GL = Great Lakes.

X2. TAXONOMIC KEYS—PARTIAL LISTING (1,2)

X2.1 *All Categories*—See Ref **(74)**. Dichotomus keys and descriptions.

X2.2 *Invertebrates (General)*—See Refs **(75-77)**.

X2.3 *Aquatic Insects*—See Refs **(78-82)**.

X2.4 *Macrophytes (Plants)*—See Refs **(83, 84)**.

X3. FLOW CHART OF FACTORS TO CONSIDER FOR SELECTING A RESIDENT TEST SPECIES

I. Define Study Objectives and Types of Species of Interest (i.e., salmonid, filter-feeder, benthic invertebrate, etc.).

II. Field Bioassessment and/or Information Search to identify resident species of interest or concern.

III. Collate List of Potential Test Species based on information above. For each potential species, determine the following factors:

IV. **A. Availability of species**

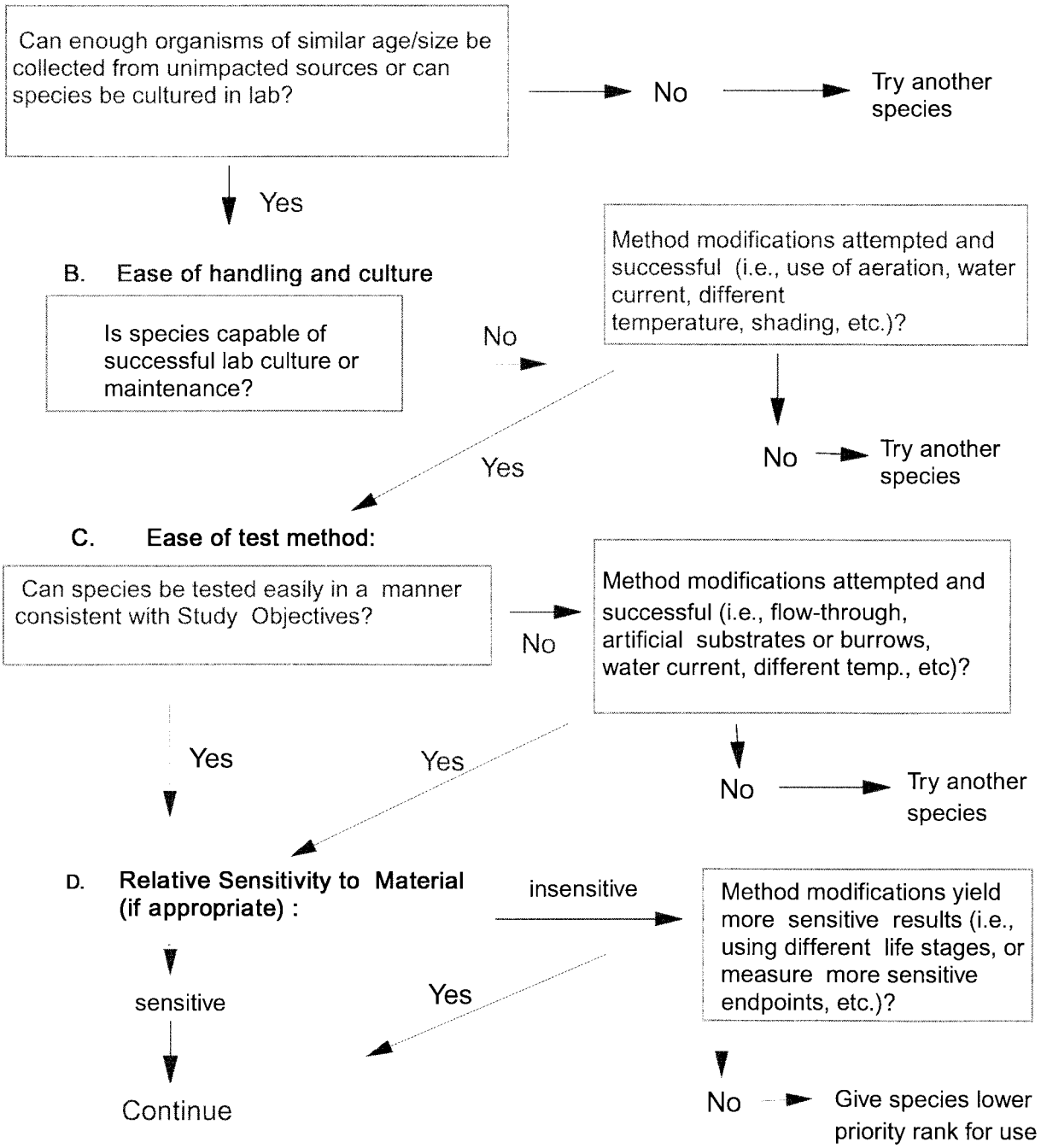

FIG. X3.1 Flow Chart of Factors to Consider for Selecting a Resident Test Species

Appendix X3. Flow Chart (continued)

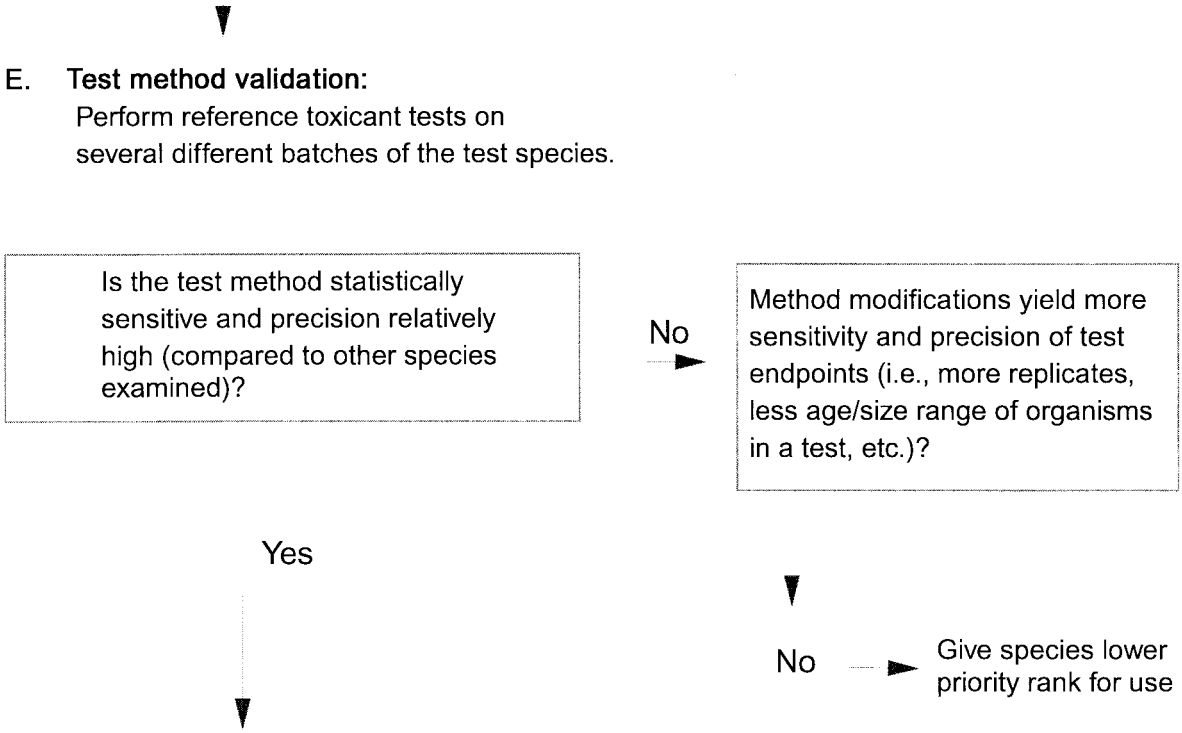

FIG. X3.1 Flow Chart of Factors to Consider for Selecting a Resident Test Species *(continued)*

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