



# Standard Practice for Aquatic Toxicity Testing of Lubricants: Sample Preparation and Results Interpretation<sup>1</sup>

This standard is issued under the fixed designation D 6081; 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 practice covers procedures to be used in the preparation of lubricants or their components for toxicity testing in aquatic systems and in the interpretation of the results of such tests.

1.2 This practice is suitable for use on fully-formulated lubricants or their components which are not completely soluble at the intended test treat rates. It is also suitable for use with additives, if the additive is tested after being blended into a carrier fluid at the approximate concentration as in the intended fully-formulated lubricant. The carrier fluid shall meet the above solubility criterion, be known to be minimally toxic in the toxicity test in which the material will be tested, and be known to have a chemical composition similar to the rest of the intended fully-formulated lubricant.

1.3 Samples prepared in accordance with this practice may be used in acute or chronic aquatic toxicity tests conducted in fresh water or salt water with fish, large invertebrates, or algae. This practice does not address preparation of samples for plant toxicity testing other than algae.

1.4 Standard acute and chronic aquatic toxicity procedures are more appropriate for lubricants with compositions which are completely soluble at the intended test treat rates **(1,2,3,4,5)**.<sup>2</sup>

1.5 This practice is intended for use with lubricants or lubricant components of any volatility.

1.6 This practice does not address any questions regarding the effects of any lubricant or lubricant component on human health.

1.7 *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.*

<sup>1</sup> This practice is under the jurisdiction of ASTM Committee D-2 on Petroleum Products and Lubricants and is the direct responsibility of Subcommittee D02.12 on Environmental Standards for Lubricants.

Current edition approved May 10, 1998. Published September 1998.

<sup>2</sup> The boldface numbers in parentheses refer to the list of references at the end of this standard.

## 2. Referenced Documents

### 2.1 ASTM Standards:

D 1129 Terminology Relating to Water<sup>3</sup>

D 1193 Specification for Reagent Water<sup>3</sup>

E 729 Guide for Conducting Acute Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians<sup>4</sup>

E 943 Terminology Relating to Biological Effects and Environmental Fate<sup>4</sup>

## 3. Terminology

### 3.1 Definitions:

3.1.1 *acute toxicity test, n*—a comparative toxicity test in which a representative subpopulation of organisms is exposed to different treat rates of a test material and is observed for a short period usually not constituting a substantial portion of their life span.

3.1.2 *chronic toxicity test, n*—a comparative toxicity test in which a representative subpopulation of organisms are exposed to different treat rates of a test material and is observed for a period of time which constitutes a major portion of their life span.

3.1.3 *ecotoxicity, n*—propensity of a test material to produce adverse behavioral, biochemical, or physiological effects in non-human organisms or populations.

3.1.4 *effect load XX (ELXX), n*—a statistically or graphically estimated loading rate of test material that is expected to cause one or more specified effects in XX % of a representative subpopulation of organisms under specified conditions.

3.1.4.1 *Discussion*—This terminology should be used instead of the standard ECXX when the test material is not completely soluble at the test treat rates.

3.1.5 *inhibition load XX (ILXX), n*—a statistically or graphically estimated loading rate of test material that is expected to cause a XX % inhibition of a biological process (such as growth or reproduction) which has an analog as opposed to a digital measure.

3.1.5.1 *Discussion*—An example of a digital measure would be alive/dead. This terminology (ILXX) should be used instead

<sup>3</sup> *Annual Book of ASTM Standards*, Vol 11.01.

<sup>4</sup> *Annual Book of ASTM Standards*, Vol 11.05.

of the standard ICXX when the test material is not completely soluble at the test treat rates.

3.1.6 *lethal load XX (LLXX), n*—a statistically or graphically estimated loading rate of test material that is expected to be lethal to XX % of a representative subpopulation of organisms under specified conditions.

3.1.6.1 *Discussion*—This terminology should be used instead of the standard LCXX when the material is not completely soluble at the test treat rates.

3.1.7 *loading rate, n*—the ratio of test material to aqueous medium used in the preparation of WAF, WSF, or mechanical dispersion and in the interpretation of the results of a toxicity study with a poorly water-soluble lubricant or lubricant component.

3.1.8 *mechanical dispersion, n*—a low energy aqueous medium produced by continuous stirring of the test solution and containing both dissolved and undissolved components of the test material.

3.1.9 *toxicity, n*—the propensity of a test material to produce adverse behavioral, biochemical, or physiological effects in a living organism.

3.1.10 *water accommodated fraction (WAF), n*—the predominantly aqueous portion of a mixture of water and a material poorly soluble in water which separates in a specified period of time after the mixture has undergone a specified degree of mixing and which includes water, dissolved components, and dispersed droplets of the poorly soluble material.

3.1.10.1 *Discussion*—The composition of the WAF depends on the ratio of poorly soluble material to water in the original mixture as well as on the details of the mixing procedure.

3.1.11 *water soluble fraction (WSF), n*—the filtrate or centrifugate of the water accommodated fraction which includes all parts of the WAF, except the dispersed droplets of the poorly soluble material.

### 3.2 *Definitions of Terms Specific to This Standard:*

3.2.1 *dilution water, n*—the water used in the aquatic test under consideration which may contain organic or inorganic components depending on the test requirements.

3.2.2 *exposure matrix, n*—the WAF, WSF, or mechanical dispersion to which test organisms are exposed for toxicity testing.

3.2.3 *test systems, n*—the test organisms plus the exposure matrix and the test chambers themselves.

3.2.4 *water, n*—a reagent which is defined by Type II of Specification D 1193.

3.3 Definitions of additional terms used in this practice can be found in Terminologies D 1129 and E 943.

## 4. Summary of Practice

4.1 This practice discusses various techniques for the preparation of test material for toxicity testing of poorly water-soluble lubricants or their components under acute or chronic exposure conditions with fish, large invertebrates, or algae.

## 5. Significance and Use

5.1 This practice gives techniques to use in the preparation of lubricants or lubricant components for acute or chronic aquatic toxicity tests. Most lubricants and lubricant components are difficult to evaluate in toxicity tests because they are

mixtures of chemical compounds with varying and usually poor solubility in water. Lubricants or lubricant component mixtures should not be added directly to aquatic systems for toxicity testing because the details of the addition procedure will have a large effect on the results of the toxicity test. Use of the techniques described in this practice will produce well characterized test systems which will lead to tests with meaningful and reproducible results.

5.2 The toxicity of mixtures of poorly soluble components cannot be expressed in the usual terms of lethal concentration (or the similar terms of effect concentration or inhibition concentration) because the mixtures may not be completely soluble at treat levels which lead to toxic effects. The test material preparation techniques given in this practice lead to test results expressed in terms of loading rate, which is a practical and meaningful concept for expressing the toxicity of this type of material.

5.3 One of the recommended methods of material preparation for lubricants or their components is the mechanical dispersion technique. This particular technique generates turbulence, and thus, it should not be used for poorly-swimming organisms.

## 6. Preparation of Exposure Matrix

6.1 Experimental design considerations for fish and invertebrates will determine the test species, number of loading rates tested, and number of organisms per treatment. Experimental design considerations for algae will determine the test species, number of loading rates tested, and appropriate initial biomass per treatment.

6.2 An acute or chronic test intended to allow calculation of an LLXX, ILXX, or ELXX will typically consist of a dilution water or test medium control and a series of at least five loading rates of test material.

6.3 Test material should be added directly to the dilution water to prepare the exposure matrix. Use of a solvent or chemical dispersant is not recommended because these may alter the nature of the exposure to the test material and lead to non-representative ecotoxicity relationships.

### 6.4 *Preparation of Water-Accommodated Fraction (WAF):*

6.4.1 Components dissolved in the water phase or entrained as stable droplets in the water phase are evaluated in a WAF study (6). Individual WAFs must be generated for each test exposure loading. Serial dilutions of a single WAF are not appropriate due to differential solubility of constituents at low exposure loads (6,7). A schematic of the WAF setup is provided in Fig. 1.

6.4.2 Vessels for WAF preparation should be of suitable shape and size for the experimental design. The vessels should be of sufficient size to maintain the vortex intensity and optimize surface area contact between the test material and the water. Height to diameter ratio for the WAF preparation at initiation should be between 1:1 and 2:1, and solutions should not exceed 20 L per individual preparation. If larger volumes of test solution are required for experimental design, separate WAF preparations of the same test load may be combined. Aspirator bottles or other vessels with outlet ports at the bottom are preferable for WAF collection, if the test material is less dense than water. If a glass siphon is used to remove the WAF,

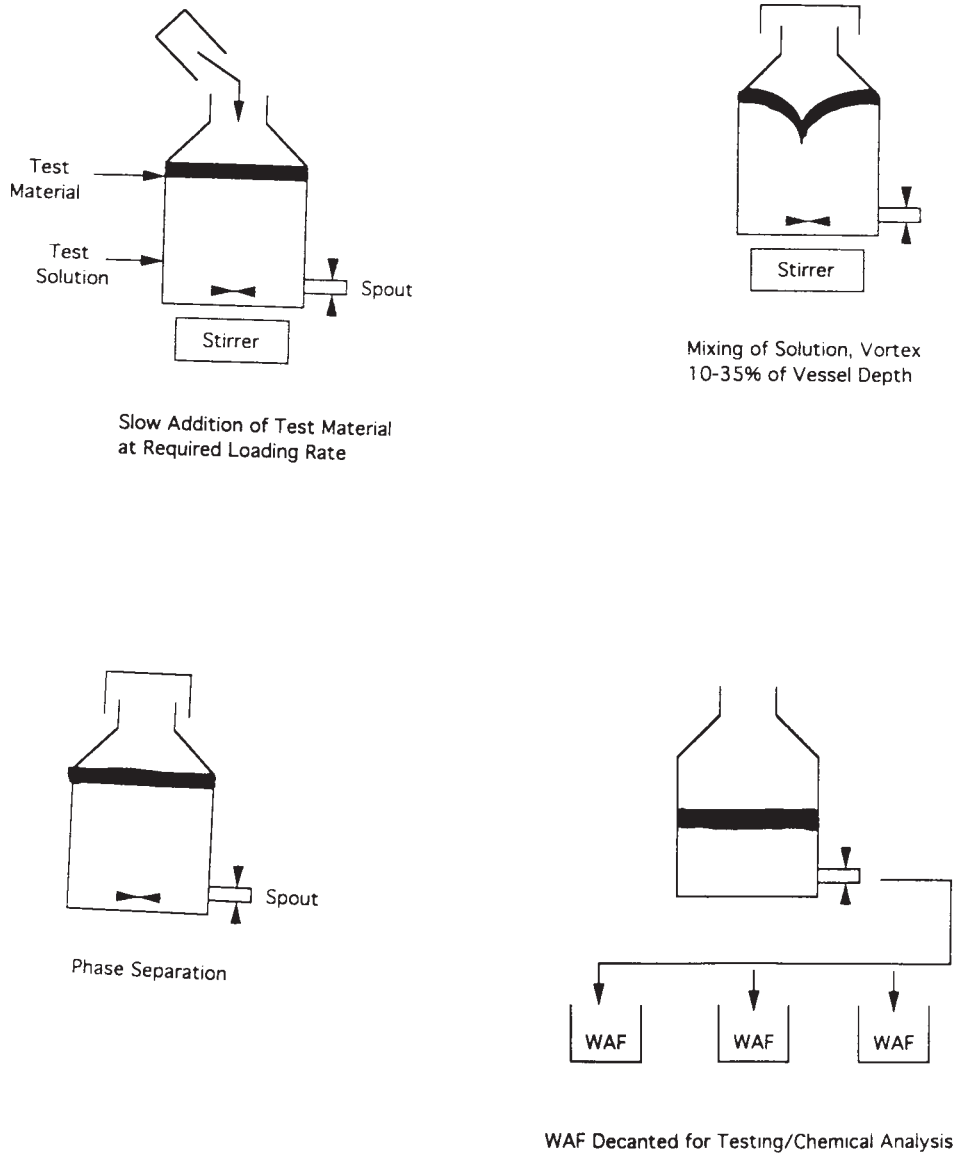


FIG. 1 Water Accommodated Fraction (WAF) Schematic

it should be in place prior to the introduction of the test material. WAF solutions from test materials which are denser than water should be decanted from the top of the vessel. For these dense fluids, a propeller-driven system which stirs the test solution from the top of the vessel may be preferable to a stir plate which stirs the test solution from the bottom of the vessel. In all cases, care should be taken to ensure that non-dispersed test material is not collected with the WAF.

6.4.3 Vessels for WAF preparation should be filled with the appropriate volume of dilution water, and be prepared for stirring as described in 6.4.2. Test material should be slowly added to the top of each vessel after dilution water addition. The vessel should be capped with foil or a non-reactive covering and stirred at the test temperature. For test temperatures different than room temperature, the WAF preparation vessels may need to be housed in water baths or incubators to maintain the desired temperature. If photodecomposition of the test material is likely, the stirring phase should be conducted in

the dark. Preparation vessels should be inspected occasionally to ensure an appropriate vortex is maintained.

6.4.4 The amount of test material added to each vessel should be determined by the desired nominal exposure load. Nominal exposure loads may be based on direct weight of material added per volume solution (wt/vol) or calculated on the basis of test material density and added on a vol/vol basis.

6.4.5 The stir plates should be run at a sufficient speed to ensure a vortex depth of 10 to 35 % of the test solution height in the WAF preparation vessel. The vortex in all WAF preparation vessels for any one experiment should be the same. Exercise care to ensure that test material is not pulled down to the bottom of the vessel, and that the rate of stirring is not so vigorous as to promote emulsification.

6.4.6 The duration of WAF preparation may depend on the results of an optional equilibrium/stability study (see 6.7). In the absence of this study, a standard 20 to 24-h mixing and 1 to 4-h settling period should be used (6,8).

6.4.7 Upon completion of the WAF mixing and settling period, the aqueous solution should be drained or decanted from below the surface of each preparation vessel. The first 5 to 10 mL of solution decanted from the vessel should be discarded. The remaining solution should be used undiluted in the exposure vessels.

6.5 Preparation of Water-Soluble Fraction (WSF):

6.5.1 Only components dissolved in the water phase are evaluated in a WSF study (6). WSFs are prepared by filtration of WAFs through a 0.45 µm nominal cellulose-acetate filter or by centrifugation to remove undissolved material. Preliminary testing may be necessary to ensure that the filter disc will not remove test material constituents which are dissolved in the water phase. Individual WSFs must be generated for each test exposure load. Serial dilutions of a single WSF are not appropriate due to differential solubility of constituents at low exposure loads. A schematic of the WSF setup is provided in Fig. 2.

6.5.2 The procedures described in 6.4.3-6.4.7 for the WAF preparation are the initial steps of the WSF procedure.

6.5.3 Upon completion of the WAF mixing and settling period, the aqueous solution should be decanted from below the surface of each preparation vessel. The first 5 to 10 mL of solution decanted from the vessel should be discarded, with the remaining solution used to prepare the WSF. Filtration through a 0.45 µm filter or low speed centrifugation (to remove droplets but not to break an emulsion) is performed on the solution to remove undissolved material. The filtrate or centrifugation

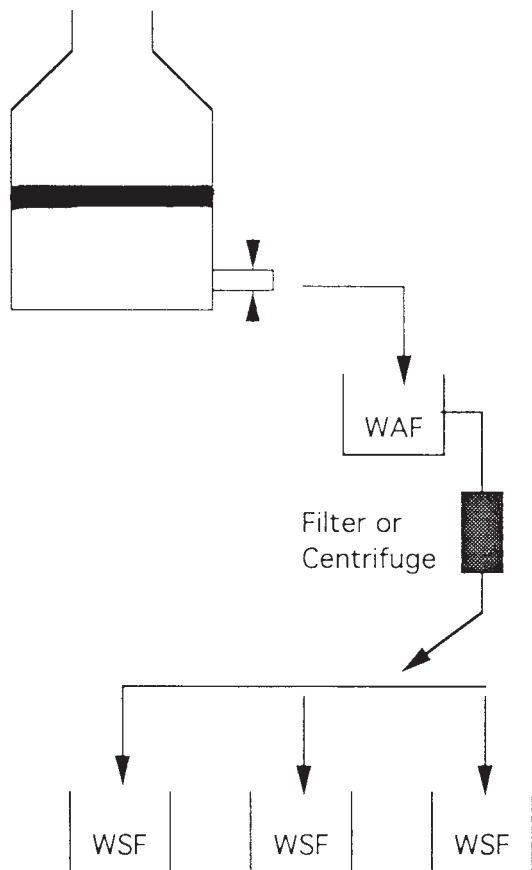


FIG. 2 Water Soluble Fraction (WSF) Schematic

supernatant is the WSF exposure matrix and should be used undiluted in the exposure vessels.

6.6 Preparation of a Mechanical Dispersion:

6.6.1 In the mechanical dispersion technique, a motor-driven propeller assembly is used to continually move test material and dilution water in the test vessel during the study (6,7,10). This procedure should not be used for small-sized, slowly, or poorly-swimming organisms. It is most appropriate for juvenile fish evaluated in acute studies. A schematic of the mechanical dispersion setup is provided in Fig. 3.

6.6.2 Each test vessel is equipped with a central cylinder equipped with a three-bladed propeller. The cylinder should be of appropriate configuration to allow water and test material to spill into the top of the cylinder and be released from small apertures at the bottom of the cylinder. The pitch of the propeller should be between 30 to 45 % and should be the same among test vessels. Screening with a non-reactive material should be used at the top and bottom of the cylinder, as necessary, to prevent organism movement into the cylinder.

6.6.3 Each test vessel must be filled with a sufficient volume of dilution water to enable operation of the mechanical dispersion apparatus. The stirring speed in each vessel should be adjusted from 1000 to 1500 rpm, creating a vortex within the cylinder of 12 to 20 mm (5 to 10 % of cylinder height). Motor speed should be checked daily and evaporative losses should be replaced with dilution water, as needed, to ensure consistent motor speed.

6.6.3.1 The dimensions and configuration of the test vessels have not been specified. Both circular and rectangular vessels have been used with this technique. The test vessels should be the same for all test treat rates during a study and should be appropriate for the organism being evaluated.

6.6.4 The amount of test material added to each vessel should be determined by the desired nominal exposure load. Nominal exposure loads may be based on direct weight of material added per volume solution (wt/vol) or calculated on the basis of test material density and added on a vol/vol basis.

6.6.5 Test material should be slowly added to the top of the test vessel. The test material may be added directly into the cylinder, but this is not mandatory. For viscous or semi-solid lubricants or their constituents, application of test material to petri dishes, glass plates or TFE-fluorocarbon pads before addition to the test vessels is preferable to direct addition to the

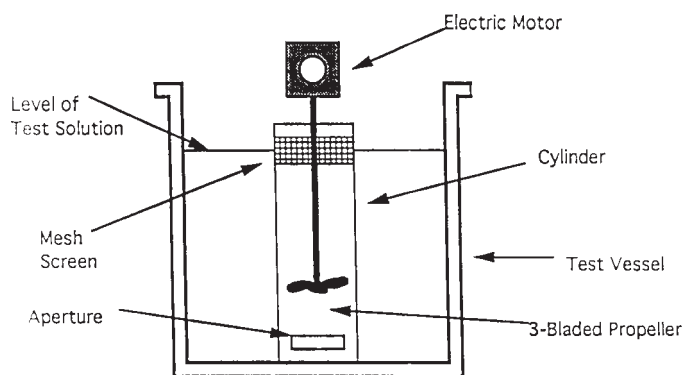


FIG. 3 Mechanical Dispersion Schematic



water, to ensure maximum surface area for test material exposure and reproducible test results.

#### 6.7 Equilibrium/Stability Study (Optional):

6.7.1 The stirring and settling periods used to generate individual WAFs or WSFs are material-sensitive and can be optimized (11). Analytical characterization studies can be done to determine the mixing time which results in maximum test material concentration in the matrix and the duration of matrix stability.

6.7.2 To conduct an equilibrium/stability study, prepare at least one WAF or WSF exposure matrix at the highest anticipated loading rate to be used in the toxicity study for each time interval to be evaluated. The same dilution water and vessel configuration which will be used during the toxicity study should be used during the equilibrium/stability study. Individual WAF or WSF exposure matrices should be sampled at different stirring intervals (that is, 3 h, 6 h, 12 h, 24 h, 36 h) to evaluate test material loading and stability in the exposure matrix. For preparation vessels such as aspirator bottles or other vessels with an outlet port, the matrix can be collected from the port during the equilibrium/stability study. For preparation vessels without an outlet port, the matrix should be sampled near the bottom with a glass siphon. Analyze the matrix solution using an analytical procedure appropriate for the test material (for example, gas chromatography (GC), high pressure liquid chromatography (HPLC), infrared spectrophotometry (IR)).

6.7.3 Based on the analytical characterization results, determine the appropriate stirring and settling periods for WAF or WSF preparation.

### 7. Test Procedure

7.1 Test solutions should not be aerated during the test unless absolutely necessary to maintain adequate dissolved oxygen levels.

7.2 The exposure temperature, organism mass per test solution volume, and test organism size should be appropriate and standard for the species and experimental design as defined in Guide E 729 or applicable regulatory guidelines.

7.3 Test organisms should be placed in the test solutions within 60 min after preparation at study initiation. For the mechanical dispersion technique, test organisms should be added to the test vessels within 60 min prior to test material addition to minimize physical coating of the organisms. The test exposure period begins upon addition of test organisms (or upon addition of test material if organisms are added first). Standard laboratory procedures appropriate to the toxicity test being conducted should be followed during the study. The exposure loads used in the toxicity test are based on the loading rate of test material into the test system (for mechanical dispersion) or into the preparation vessels (for WAF or WSF). The exposure loads will be used to calculate the LLXX, ILXX, or ELXX for the selected test species (9).

7.4 The test solutions may be renewed either to maintain adequate loading levels of the test material (loss may be due to test material instability) or to maintain adequate levels of dissolved oxygen. Renewal may not be common with the mechanical dispersion technique, but is sometimes prompted by dissolved oxygen or material stability requirements. The

loading rate may change if significant material remains adhering to the cylinder and test vessel when using this technique. Acute and chronic studies may be renewed at 24 or 48 h intervals with subsequent and separate WAF/WSF preparations or solution addition (mechanical dispersion technique). Renewals should take place as rapidly as possible after test solution preparation. Some understanding of material composition is necessary to determine the appropriate frequency of renewal. A minimum of 75 % of the test solution should be replaced with each renewal.

### 8. Characterization

8.1 The exposure matrix may be analyzed either to demonstrate the qualitative maintenance of exposure conditions or to measure the quantitative concentrations of individual constituents. The analytical procedure used should be appropriate for the test material.

8.1.1 If the test material is uniformly dispersed throughout the test vessel, samples for analytical characterization of the exposure matrix are taken from the midpoint of the vessel.

8.1.2 If the test material is not uniformly distributed throughout the test vessel as may occur during the WAF or mechanical dispersion procedures, multiple samples or larger volumes (greater than 100 mL) may be necessary for analytical characterization.

8.1.3 If the exposure matrix samples cannot be analyzed immediately, they should be minimally handled, extracted or preserved, or both, as appropriate for stabilizing the test material constituents, and stored in the dark under reduced temperature (4°C) to minimize loss of test material by microbial degradation, hydrolysis, photolysis, reduction, sorption, and volatilization. If the exposure matrix will be stored at reduced temperature, the entire volume, including vessel walls, should be extracted to minimize the effects of partitioning.

8.1.4 The precision and bias of the analytical method used to measure test material constituents should be determined in dilution water or a substance comparable to the dilution water.

### 9. Interferences/Complexities

9.1 Limitations to the methods described in this practice might arise and thereby influence aquatic toxicity test results and complicate data interpretation. The following factors should be considered when testing poorly water-soluble lubricants or lubricant constituents.

9.1.1 Test material may be altered by storage beyond the acceptable shelf life or under inappropriate environmental conditions.

9.1.2 Volatility of one or more of the components in the neat test material can occur at various stages in the course of the study.

9.1.2.1 Improper storage of materials with volatile components can lead to a change in the composition of the test material.

9.1.2.2 Preparation of the exposure matrix for a material with a volatile component in an open vessel or a vessel with a large headspace can lead to a change in the matrix composition over time.

9.1.2.3 Organism exposure to the exposure matrix of a material with a volatile component in an open vessel or a vessel

with a large headspace can lead to a change in the matrix composition over time.

9.1.2.4 Improper sampling or storage, or both, of matrix samples which contain a volatile component prior to characterization or analysis can lead to a change in the matrix composition over time.

9.1.3 Test materials with chemically different components may show compartmentalization of some of the components.

9.1.3.1 Materials/components that have low water solubility may compartmentalize out of the water phase (for example, floating on surface, clumping on the vessel walls).

9.1.3.2 Materials/components that are ionic may bind at active sites on glass and plastic vessels and so be removed from the water phase.

9.1.3.3 Materials/components with large log  $K_{ow}$  (octanol-water partition coefficients) values may adsorb onto non-glass vessels.

9.1.3.4 Materials/components may be lost by absorption on the filter disc during preparation of a WSF exposure matrix.

9.1.4 Physical characteristics of the test material may make the addition of the material to the test system difficult.

9.1.4.1 Quantification of highly viscous materials during material addition is often difficult due to retention of some of the material on the measuring/transport container.

9.1.5 The ion composition of the dilution water used for aquatic toxicity testing may influence the integrity of the test material composition.

9.1.5.1 Materials/components may react/chelate with ions in the dilution water with a resulting change in characteristics (for example, precipitation of components, altered solubility, increased bioavailability).

9.1.5.2 The ionic strength of the aqueous phase may affect the partitioning (or lack thereof) of non-aqueous hydrophobic components into that phase.

9.1.6 Any alterations in the mixing energy or mixing vessel shape can influence the form of the material in the exposure matrix.

9.1.6.1 Mixing energy that is too great may create an emulsion, while mixing energy that is too low may not optimize the distribution of the test material in the exposure matrix.

9.1.6.2 Using mixing vessels of different shapes or volumes to prepare the exposure matrices for the same test can produce different mixing levels and subsequently different exposure loads for some test materials.

9.1.6.3 Vessels used for preparing the exposure matrix should provide maximum surface area for contact between the test material and dilution water. Vessels that are narrow at the opening (for example, volumetric flasks, Erlenmeyer flasks) should not be used if the contact zone is in the restricted portion of the vessel.

9.1.7 Exposure matrix samples that are collected for characterization can vary in composition during the preparation phase and the exposure phase of the test.

9.1.7.1 Samples collected at different times from the same mixing vessel may not be comparable.

9.1.7.2 The physical characteristics of the test material may make some sampling methods inappropriate.

9.1.7.3 The physical characteristics of the test material may make some preservation methods inappropriate.

9.1.8 Test materials evaluated using the exposure methods recommended in this practice may affect the bioassay test organisms independently of their inherent toxicity.

9.1.8.1 Materials/components that move to the surface of the exposure vessel may reduce oxygen transfer and cause a reduction in the dissolved oxygen.

9.1.8.2 Suspended test material may adhere to the test organisms and cause injury independent of the inherent toxicity of the test material (for example, fish suffocating due to test material covering on gills, organisms adhering to the sides of the test vessel, and so forth).

9.1.9 The characteristics of the exposure matrix (for example, suspended test material) or the design of the exposure vessel (for example, no head space) can make the observation and enumeration of test organisms difficult, particularly when using the mechanical dispersion technique or toxicity test vessels with lids.

## 10. Calculation or Interpretation of Results

10.1 The LLXX, ILXX, or ELXX and its 95 % confidence limits shall be calculated on the basis of nominal loading rates.

10.2 Standard statistical programs can be used to calculate the LLXX, ILXX, or ELXX. The method used should appropriately take into account the number of test vessels per treatment and the number of test organisms per vessel.

## 11. Keywords

11.1 aquatic toxicity; ecotoxicity; effect load; incident load; lethal load; lubricant; mechanical dispersion; water accommodated fraction (WAF); water soluble fraction (WSF)

**REFERENCES**

- (1) EPA, "Guidelines and Support Documents for Environmental Effects Testing," *EPA 560/6-82-002*, Sections EG-9, ES-6, 1982.
- (2) EPA, "Toxic Substances Control Act (TSCA)—Environmental Effects Testing Guidelines," *40 CFR Part 797.1400 and 797.1440*, 1993.
- (3) OECD, "203: Fish Acute Toxicity Test," *OECD Guidelines for Testing of Chemicals*, Section 2: Effects on Biotic Systems, 1993.
- (4) EU, "C.1. Acute Toxicity for Fish," *Official Journal of the European Communities*, No. L383A/163-171, 1992.
- (5) Environment Canada, "Biological Test Method: Acute Lethality Test Using Rainbow Trout," *Environment Canada Environmental Protection Series*, Report EPS 1/RM/9, 1990.
- (6) Girling, A. E., "Preparation of Aqueous Media for Aquatic Toxicity Testing of Oils and Oil-Based Products: A Review of the Published Literature," *Chemosphere*, 19(10-11): 1635-1641, 1989.
- (7) Concawe, "Ecotoxicological Testing of Petroleum Products: Test Methodology," Report No. 92/56, 1993.
- (8) Anderson, J. W., Neff, J. M., Cox, B. A., Tatem, H. E., Hightower, G. M., "Characteristics of Dispersions and Water-Soluble Extracts of Crude and Refined Oils and Their Toxicity to Estuarine Crustaceans and Fish," *Marine Biology*, 27: 75-88, 1974.
- (9) Girling, A. E., Markarian, R. K., Bennett, D., "Aquatic Toxicity Testing of Oil Products—Some Recommendations," *Chemosphere*, 24(10): 1469-1472, 1992.
- (10) Blackman, R. A. A., Franklin, F. L., Norton, M. G., Wilson, K. W., "New Procedures for the Toxicity Testing of Oil Slick Dispersants," *Fisheries Research Technical Report*, No. 39, 1980.
- (11) Bennett, D., Girling, A. E., Bounds, A., "Ecotoxicology of Oil Products: Preparation and Characterisation of Aqueous Test Media," *Chemosphere*, 21(4-5): 659-669, 1990.

*The American Society for Testing and Materials takes no position respecting the validity of any patent rights asserted in connection with any item mentioned in this standard. Users of this standard are expressly advised that determination of the validity of any such patent rights, and the risk of infringement of such rights, are entirely their own responsibility.*

*This standard is subject to revision at any time by the responsible technical committee and must be reviewed every five years and if not revised, either reapproved or withdrawn. Your comments are invited either for revision of this standard or for additional standards and should be addressed to ASTM Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend. If you feel that your comments have not received a fair hearing you should make your views known to the ASTM Committee on Standards, 100 Barr Harbor Drive, West Conshohocken, PA 19428.*