



Designation: D6081 – 22

Standard Practice for Aquatic Toxicity Testing of Lubricants: Sample Preparation and Results Interpretation¹

This standard is issued under the fixed designation D6081; 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 that 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 that are completely soluble at the intended test treat rates (**1, 2, 3, 4, 5**).²

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 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

¹ This practice is under the jurisdiction of ASTM Committee D02 on Petroleum Products, Liquid Fuels, and Lubricants and is the direct responsibility of Subcommittee D02.12 on Environmental Standards for Lubricants.

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² The boldface epsilon (ϵ) 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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.9 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*³

D1129 Terminology Relating to Water

D1193 Specification for Reagent Water

D4175 Terminology Relating to Petroleum Products, Liquid Fuels, and Lubricants

E729 Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians

E943 Terminology Relating to Biological Effects and Environmental Fate

3. Terminology

3.1 For definitions of terms used in this practice, see Terminologies D1129, D4175, and E943.

3.2 *Definitions:*

3.2.1 *acute toxicity test, n*—a comparative 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.

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

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.

*A Summary of Changes section appears at the end of this standard

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

3.2.4 *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.

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

3.2.5 *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) that has an analog as opposed to a digital measure.

3.2.5.1 *Discussion*—An example of a digital measure would be alive/dead. This terminology (ILXX) should be used instead of the standard ICXX when the test material is not completely soluble at the test treat rates.

3.2.6 *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.

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

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

3.2.8 *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.

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

3.2.10 *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.

3.2.10.1 *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.

3.2.11 *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.

3.3 Definitions of Terms Specific to This Standard:

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

3.3.2 *exposure matrix, n*—the water accommodated fraction (WAF), water soluble fraction (WSF), or mechanical dispersion to which test organisms are exposed for toxicity testing.

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

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 that 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 that 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. Apparatus

6.1 *Stirrer*, magnetic, size sufficient to accommodate vessel (6.2).

6.2 *Vessel*, glass, bottom drain, capacity 10 L to 20 L.

NOTE 1—The vessels shall be of sufficient size to maintain the vortex intensity and optimize surface area contact between the test material and the water.

NOTE 2—Height to diameter ratio for the WAF preparation at initiation should be between 1:1 and 2:1, and solutions shall 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.

NOTE 3—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, it shall be in place prior to the introduction of the test material.