



Designation: D 1345 - 59 (Reapproved 1977)

Standard Test Methods for EVALUATING ACUTE TOXICITY OF WATER TO FRESH-WATER FISHES¹

This Standard is issued under the fixed designation D 1345; 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.

1. Scope and Application

1.1 This bio-assay procedure² is intended as a nonreferee batch method (Note 1) for evaluating acute toxicity of wastes and of other water pollutants to fresh-water fishes. The test provides information on the relative acute toxicity (Note 2) of the substance tested under prescribed experimental conditions.

NOTE 1—Tests may be performed using a standard reference water and reference chemicals (6.2 and 6.3). The reference water is recommended for obtaining basic comparative research data. Test chemicals are recommended for standardizing the lot of fish under test.

NOTE 2—Chronic or cumulative toxicity can interfere seriously with the growth and reproduction of organisms and may eventually cause death. This method detects only acute toxicity.

1.2 The test is applicable to industrial waste water and to the determination of the toxicity of pure chemical compounds in water solution. Wastes having volatile or readily oxidizable constituents (Note 3) and excessive biochemical or other oxygen demand (Note 4) may also be tested by this method.

NOTE 3—The concentration of highly volatile and unstable toxicants may decline throughout the test. In the case of highly volatile toxicants, it may be possible to obtain more reliable data by substituting initial oxygenation of the diluent in place of the standard controlled artificial oxygenation procedure. In the case of readily oxidizable constituents, it may be advisable to use test solution renewal techniques.

NOTE 4—A distinction is made between death due to deficiency of dissolved oxygen in polluted water and death due to toxicity. In order to detect and evaluate the direct lethality of test material, it is necessary to maintain adequate dissolved oxygen concentrations during toxicity tests.

1.3 The results of the test data are reported in terms of median tolerance limits (2.2). The critical concentration range may be reported, also, as supplementary information (2.3).

1.4 The test data is applicable for use as a

guide in estimating "safe concentrations" of the discharge of industrial wastes (see Appendix A3).

2. Definitions

2.1 The terms *acute toxicity*, *median tolerance limit* (TL_m), and *critical concentration range* in this method are defined in accordance with ASTM Definitions D 1129, Terms Relating to Water,³ as follows:

2.1.1 *acute toxicity*—any direct lethal action of pollution to fresh-water fishes that is demonstrable within 96 h or less.

NOTE 5—The lethal action includes both internal and external effects, but excludes indirect action such as depletion of dissolved oxygen through chemical or biochemical oxidation of the test material.

2.1.2 *median tolerance limit* (TL_m)—the concentration of pollutants at which 50 percent of the test animals are able to survive for a specified period of exposure.

NOTE 6—The exposure period may be 24, 48, or 96 h. The 24-h TL_m and the 48-h TL_m generally should be determined whenever the toxicity is sufficiently pronounced to permit their determination.

2.1.3 *critical concentration range*—the in-

¹ This method is under the jurisdiction of ASTM Committee D-19 on Water.

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² This method is based in large part on the recommendations of the Committee on Research of the Federation of Sewage and Industrial Wastes Associations, and it is essentially the same as the first one of the so-called "Auxiliary Methods" described in the report of that committee: Doudoroff, *et al.*, "Bio-Assay Methods For The Evaluation of Acute Toxicity of Industrial Wastes To Fish," *Sewage and Industrial Wastes*, SIWAA, Vol 23, No. 11, 1951, pp. 1380-1397. Many of the recommendations of that committee, in turn, derive directly from an earlier, more detailed and comprehensive publication by Hart, Doudoroff, and Greenbank, "The Evaluation of the Toxicity of Industrial Wastes, Chemicals and Other Substances to Fresh-Water Fishes," The Atlantic-Richfield Co., Philadelphia, Pa., 1945, p. 317. (Out of print.)

³ *Annual Book of ASTM Standards*, Part 31.