
40 C.F.R. § 797.1330

Daphnid chronic toxicity test.

(a) *Purpose.* This guideline is intended for use in developing data on the chronic toxicity of chemical substances and mixtures (“chemicals”) subject to environmental effects test regulations under the Toxic Substances Control Act (TSCA) (Pub. L. 94-469, 90 Stat. 2003, 15 U.S.C. 2601 *et seq.*). This guideline prescribes a chronic toxicity test in which daphnids are exposed to a chemical in a renewal or a flow-through system. The United States Environmental Protection Agency will use data from this test in assessing the hazard a chemical may present to the aquatic environment.

(b) *Definitions.* The definitions in section 3 of the Toxic Substances Control Act (TSCA), and the definitions in part 792 *Good Laboratory Practice Standards* of this chapter apply to this test guideline. In addition, the following definitions apply to this guideline:

- (1) *Brood stock* means the animals which are cultured to produce test organisms through reproduction.
- (2) *Chronic toxicity test* means a method used to determine the concentration of a substance in water that produces an adverse effect on a test organism over an extended period of time. In this test guideline, mortality and reproduction (and optionally, growth) are the criteria of toxicity.
- (3) *EC₅₀* means that experimentally derived concentration of test substance in dilution water that is calculated to affect 50 percent of a test population during continuous exposure over a specified period of time. In this guideline, the effect measured is immobilization.
- (4) *Ephippium* means a resting egg which develops under the carapace in response to stress conditions in daphnids.
- (5) *Flow-through* means a continuous or intermittent passage of test solution or dilution water through a test chamber or culture tank with no recycling.
- (6) *Immobilization* means the lack of movement by daphnids except for minor activity of the appendages.
- (7) *Loading* means the ratio of daphnid biomass (grams, wet weight) to the volume (liters) of test solution in a test chamber at a point in time or passing through the test chamber during a specific interval.
- (8) *MATC (Maximum Acceptable Toxicant Concentration)* means the maximum concentration at which a chemical can be present and not be toxic to the test organism.
- (9) *Renewal system* means the technique in which test organisms are periodically transferred to fresh test solution of the same composition.

(c) *Test procedures—(1) Summary of the test.* (i) Test chambers are filled with appropriate volumes of dilution water. In the flow-through test the flow of dilution water through each chamber is then adjusted to the

rate desired. The test substance is introduced into each test chamber. The addition of test substance in the flow-through system is done at a rate which is sufficient to establish and maintain the desired concentration of test substance in the test chamber.

(ii) The test is started within 30 minutes after the test substance has been added and uniformly distributed in the test chambers in the renewal test or after the concentration of test substance in each test chamber of the flow-through test system reaches the prescribed level and remains stable. At the initiation of the test, daphnids which have been cultured or acclimated in accordance with the test design, are randomly placed into the test chambers. Daphnids in the test chambers are observed periodically during the test, immobile adults and offspring produced are counted and removed, and the findings are recorded. Dissolved oxygen concentration, pH, temperature, the concentration of test substance, and other water quality parameters are measured at specified intervals in selected test chambers. Data are collected during the test to determine any significant differences ($p \leq 0.05$) in immobilization and reproduction as compared to the control.

(2) [Reserved]

(3) *Range-finding test.* (i) A range-finding test should be conducted to establish test solution concentrations for the definitive test.

(ii) The daphnids should be exposed to a series of widely spaced concentrations of the test substance (e.g., 1, 10, 100 mg/l), usually under static conditions.

(iii) A minimum of five daphnids should be exposed to each concentration of test substance for a period of time which allows estimation of appropriate chronic test concentrations. No replicates are required and nominal concentrations of the chemical are acceptable.

(4) *Definitive test.* (i) The purpose of the definitive test is to determine concentration-response curves, EC_{50} values and effects of a chemical on immobilization and reproduction during chronic exposure.

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