

(21) Zepp R.G., Schlotzhauer P.F., Merritt S.R. "Photosensitized transformations involving electronic energy transfer in natural waters: role of humic substances." *Environmental Science and Technology*, 19:74. (1985).

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Subpart C—Provisional Environmental Effects Guidelines

§ 795.120 Gammarid acute toxicity test.

(a) *Purpose.* This guideline is intended for use in developing data on the acute toxicity of chemical substances and mixtures 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 describes a test to develop data on the acute toxicity of chemicals to gammarids. The United States Environmental Protection Agency (EPA) will use data from this test in assessing the hazard of a chemical to aquatic organisms.

(b) *Definitions.* The definitions in section 3 of TSCA and in part 792 of this chapter, Good Laboratory Practice Standards, apply to this test guideline. The following definitions also apply to this guideline:

Death means the lack of reaction of a test organism to gentle prodding.

Flow-through means a continuous or an intermittent passage of test solution or dilution water through a test chamber or a holding or acclimation tank, with no recycling.

LC50 means the median lethal concentration, i.e., that concentration of a chemical in air or water killing 50 percent of the test batch of organisms within a particular period of exposure (which shall be stated).

Loading means the ratio of the biomass of gammarids (grams, wet weight) to the volume (liters) of test solution in either a test chamber or passing through it in a 24-hour period.

Solvent means a substance (e.g., acetone) which is combined with the test substance to facilitate introduction of the test substance into the dilution water.

Static system means a test chamber in which the test solution is not renewed during the period of the test.

(c) *Test procedures*—(1) *Summary of the test.* In preparation for the test, test chambers are filled with appropriate volumes of dilution water. If a flow-through test is performed, the flow of dilution water through each chamber is adjusted to the rate desired. In a static test, the test substance is introduced into each test chamber. In a flow-through test, the rate in which the test substance is added is adjusted to establish and maintain the desired concentration of test substance in each test chamber. The test is started by randomly introducing gammarids, which have been acclimated to the test conditions, into the test chambers. Gammarids in the test chambers are observed periodically during the test; the dead gammarids are removed and the findings recorded. Dissolved oxygen concentration, pH, temperature, and the concentration of test substance in test chambers are measured at specified intervals. Data collected during the test are used to develop concentration-response curves and LC50 values for the test substance.

(2) [Reserved]

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

(ii) The gammarids shall be exposed to a wide-range of concentrations of the test substance (e.g., 1, 10, 100 mg/l, etc.), usually under static conditions.

(iii) A minimum of five gammarids should be exposed to each concentration of test substance for a period of 96 hours. The exposure period may be shortened if data suitable for determining concentrations in the definitive test can be obtained in less time. Nominal concentrations of the test substance may be acceptable.

(4) *Definitive test.* (i) The purpose of the definitive test is to determine the 24, 48, 72, and 96-hour LC50 values and the concentration-response curves.

(ii) A minimum of 20 gammarids per concentration shall be exposed to five or more concentrations of the test substance chosen in a geometric series in which the ratio is between 1.5 and 2.0 (e.g., 2, 4, 8, 16, 32, 64 mg/L). The range

and number of concentrations to which the organisms are exposed shall be such that in 96 hours there is at least one concentration resulting in mortality greater than 50 and less than 100 percent, and one concentration causing greater than zero and less than 50 percent mortality. An equal number of gammarids may be placed in two or more replicate test chambers. Solvents should be avoided, if possible. If solvents have to be used, a solvent control, as well as a dilution control, shall be tested at the highest solvent concentration employed in the treatments. The solvent should not be toxic or have an effect on the toxicity of the test substance. The concentration of solvent should not exceed 0.1 ml/L.

(iii) Every test shall include a concurrent control using gammarids from the same population or culture container. The control group shall be exposed to the same dilution water, conditions and procedures, except that none of the test substance shall be added to the chamber.

(iv) The dissolved oxygen concentration, temperature and pH of the test solution shall be measured at the beginning of the test and at 24, 48, 72 and 96 hours in at least one replicate each of the control, and the highest, lowest and middle test concentrations.

(v) The test duration is 96 hours. The test is unacceptable if more than 10 percent of the control organisms die during the test.

(vi) In addition to death, any abnormal behavior or appearance shall also be reported.

(vii) Gammarids shall be randomly assigned to the test chambers. Test chambers shall be positioned within the testing area in a random manner or in a way in which appropriate statistical analyses can be used to determine whether there is any variation due to placement.

(viii) Gammarids shall be introduced into the test chambers after the test substance has been added.

(ix) Observations on compound solubility shall be recorded. The investigator should record the appearance of surface slicks, precipitates, or material adhering to the sides of the test chambers.

(5) [Reserved]

(6) *Analytical measurements*—(i) *Water quality analysis*. The hardness, acidity, alkalinity, pH, conductivity, TOC or COD, and particulate matter of the dilution water shall be measured at the beginning of each definitive test.

(ii) *Collection of samples for measurement of test substance*. Each sample to be analyzed for the test substance concentrations shall be taken at a location midway between the top, bottom, and sides of the test chamber. Samples should not include any surface scum or material dislodged from the bottom or sides. Samples shall be analyzed immediately or handled and stored in a manner which minimizes loss of test substance through microbial degradation, photogradation, chemical reaction, volatilization, or sorption.

(iii) *Measurement of test substance*. (A) For static tests, the concentration of dissolved test substance (that which passes through a 0.45 micron filter) shall be measured in each test chamber at least at the beginning (zero-hour, before gammarids are added) and at the end of the test. During flow-through tests, the concentration of dissolved test substance shall be measured in each test chamber at least at 0 and 96-hours and in at least one chamber whenever a malfunction of the test substance delivery system is observed.

(B) The analytical methods used to measure the amount of test substance in a sample shall be validated before beginning the test. This involves adding a known amount of the test substance to each of three water samples taken from a chamber containing dilution water and the same number of gammarids as are placed in each test chamber. The nominal concentrations of the test substance in these samples should span the concentration range to be used in the test. Validation of the analytical method should be performed on at least two separate days prior to starting the test.

(C) An analytical method is not acceptable if likely degradation products of the test substance give positive or negative interferences, unless it is shown that such degradation products are not present in the test chambers during the test.

(D) Among replicate test chambers, the measured concentrations shall not

vary more than 20 percent. The measured concentration of the test substance in any chamber during the test shall not vary more than plus or minus 30 percent from the measured concentration in that chamber at zero time.

(E) The mean measured concentration of dissolved test substance shall be used to calculate all LC50's and to plot all concentration-response curves.

(d) *Test conditions for definitive test*—(1) *Test species*—(i) *Selection*. (A) The amphipods, *Gammarus fasciatus*, *G. pseudolimnaeus*, and *G. lacustris* are specified for this test.

(B) Gammarids can be cultured in the laboratory or collected from natural sources. If collected, they must be held in the laboratory for at least 14 days prior to testing.

(C) Gammarids used in a particular test shall be of similar age and/or size and from the same source or culture population.

(ii) *Acclimation*. If the holding water is from the same source as the dilution water, acclimation to the dilution water shall be done gradually over a 48-hour period. The gammarids then shall be held at least 7 days in the dilution water prior to testing. Any changes in water temperature should not exceed 2 °C per day. Gammarids should be held for a minimum of 7 days at the test temperature prior to testing.

(iii) *Care and handling*. Gammarids shall be cultured in dilution water under similar environmental conditions to those used in the test. Organisms shall be handled as little as possible. When handling is necessary it should be done as gently, carefully and quickly as possible. During culturing and acclimation, gammarids shall be observed carefully for signs of stress and mortality. Dead and abnormal individuals shall be discarded.

(iv) *Feeding*. The organisms shall not be fed during testing. During culturing, holding, and acclimation, a sufficient quantity of deciduous leaves, such as maple, aspen, or birch, should be placed in the culture and holding containers to cover the bottom with several layers. These leaves should be aged for at least 30 days in a flow-through system before putting them in aquaria. As these leaves are eaten, more aged

leaves should be added. Pelleted fish food may also be added.

(2) *Facilities*—(i) *Apparatus*—(A) Facilities needed to perform this test include:

(1) Containers for culturing, acclimating and testing gammarids;

(2) Containers for aging leaves under flow-through conditions;

(3) A mechanism for controlling and maintaining the water temperature during the culturing, acclimation and test periods;

(4) Apparatus for straining particulate matter, removing gas bubbles, or aerating the dilution water, as necessary; and

(5) An apparatus for providing a 16-hour light and 8-hour dark photoperiod with a 15- to 30-minute transition period.

(B) Facilities should be well ventilated and free of fumes and disturbances that may affect the test organism.

(C) Test chambers shall be covered loosely to reduce the loss of test solution or dilution water due to evaporation and to minimize the entry of dust or other particulates into the solutions.

(ii) *Construction materials*. Construction materials and equipment that may contact the stock solution, test solution or dilution water should not contain substances that can be leached or dissolved into aqueous solutions in quantities that can alter the test results. Materials and equipment that contact stock or test solutions should be chosen to minimize sorption of test substances. Glass, stainless steel, and perfluorocarbon plastic should be used wherever possible. Concrete, fiberglass, or plastic (e.g., PVC) may be used for holding tanks, acclimation tanks, and water supply systems, but they should be aged prior to use. Rubber, copper, brass, galvanized metal, and lead should not come in contact with the dilution water, stock solution, or test solution.

(iii) *Test substance delivery system*. In flow-through tests, diluters, metering pump systems or other suitable devices shall be used to deliver the test substance to the test chambers. The system used shall be calibrated before each test. The general operation of the

test substance delivery system shall be checked twice daily during a test. The 24-hour flow shall be equal to at least five times the volume of the test chamber. During a test, the flow rates should not vary more than 10 percent from one test chamber to another.

(iv) *Test chambers.* Test chambers shall contain at least one liter of test solution. Test chambers made of stainless steel should be welded, not soldered. Test chambers made of glass should be glued using clear silicone adhesive. As little adhesive as possible should be left exposed in the interior of the chamber. A substrate, such as a bent piece of stainless steel screen, should be placed on the bottom of each test chamber to provide cover for the gammarids.

(v) *Cleaning of test system.* Test substance delivery systems and test chambers should be cleaned before each test. They should be washed with detergent and then rinsed sequentially with clean water, pesticide-free acetone, clean water, and 5-percent nitric acid, followed by two or more changes of dilution water.

(vi) *Dilution water.* (A) Clean surface or ground water, reconstituted water, or dechlorinated tap water is acceptable as dilution water if gammarids will survive in it for the duration of the culturing, acclimating, and testing periods without showing signs of stress. The quality of the dilution water should be constant enough that the month-to-month variation in hardness, acidity, alkalinity, conductivity, TOC or COD, and particulate matter is not more than 10 percent. The pH should be constant within 0.4 unit. In addition, the dilution water should meet the following specifications measured at least twice a year:

Substance	Maximum concentration
Particulate matter	20 mg/L
Total organic carbon (TOC) or	2 mg/L
chemical oxygen demand (COD)	5 mg/L
Boron, fluoride	100 ug/L
Un-ionized ammonia	1 ug/L
Aluminum, arsenic, chromium, cobalt, copper, iron, lead, nickel, zinc.	1 ug/L
Residual chlorine	3 ug/L
Cadmium, mercury, silver	100 ng/L
Total organophosphorus pesticides	50 ng/L
Total organochlorine pesticides plus:	
polychlorinated biphenyls (PCBs) or	50 ng/L
organic chlorine	25 ng/L

(B) If the dilution water is from a ground or surface water source, conductivity and total organic carbon (TOC) or chemical oxygen demand (COD) shall be measured. Reconstituted water can be made by adding specific amounts of reagent-grade chemicals to deionized or distilled water. Glass-distilled or carbon-filtered deionized water with a conductivity less than 1 micromho/cm is acceptable as the diluent for making reconstituted water.

(C) The concentration of dissolved oxygen in the dilution water shall be between 90 and 100 percent saturation. If necessary, the dilution water can be aerated before the addition of the test substance. All reconstituted water should be aerated before use.

(3) *Test parameters.* Environmental parameters during the test shall be maintained as specified below:

- (i) Water temperature of 18 ± 1 °C.
- (ii) Dissolved oxygen concentration between 60 and 105 percent saturation.
- (iii) The number of gammarids placed in a test chamber shall not be so great as to affect the results of the test. Ten gammarids per liter is the recommended level of loading for the static test. Loading requirements for the flow-through test will vary depending on the flow rate of dilution water. The loading should not cause the dissolved oxygen concentration to fall below the recommended levels.

(iv) Photoperiod of 16 hours light and 8 hours darkness.

(e) *Reporting.* The sponsor shall submit to the EPA all data developed by the test that are suggestive or predictive of toxicity. In addition, the test report shall include, but not necessarily be limited to, the following information:

- (1) Name and address of the facility performing the study and the dates on which the study was initiated and completed.
- (2) Objectives and procedures stated in the approved protocol, including any changes in the original protocol.
- (3) Statistical methods employed for analyzing the data.
- (4) The test substance identified by name, Chemical Abstracts (CAS) number or code number, source, lot or batch number, strength, purity, and

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composition, or other appropriate characteristics.

(5) Stability of the test substance under the conditions of the test.

(6) A description of the methods used, including:

(i) The source of the dilution water, its chemical characteristics (e.g., hardness, pH, etc.) and a description of any pretreatment.

(ii) A description of the test substance delivery system, test chambers, the depth and volume of solution in the chamber, the way the test was begun (e.g., test substance addition), the loading, the lighting, and the flow rate.

(iii) Frequency and methods of measurements and observations.

(7) The scientific name, weight, length, source, and history of the organisms used, and the acclimation procedures and food used.

(8) The concentrations tested, the number of gammarids and replicates per test concentration. The reported results should include:

(i) The results of dissolved oxygen, pH and temperature measurements.

(ii) If solvents are used, the name and source of the solvent, the nominal concentration of the test substance in the stock solution, the highest solvent concentration in the test solution and a description of the solubility determination in water and solvents.

(iii) The measured concentration of the test substance in each test chamber just before the start of the test and at all subsequent sampling periods.

(iv) In each test chamber at each observation period, the number of dead and live test organisms, the percentage of organisms that died, and the number of test organisms that showed any abnormal effects in each test chamber at each observation period.

(v) The 48, 72 and 96-hour LC50's and their 95 percent confidence limits. When sufficient data have been generated, the 24-hour LC50 value also. These calculations should be made using the mean measured test substance concentrations.

(vi) The observed no-effect concentration (the highest concentration tested at which there were no mortalities or abnormal behavioral or physiological effects), if any.

(vii) Methods and data for all chemical analyses of water quality and test substance concentrations, including method validations and reagent blanks.

(9) A description of all circumstances that may have affected the quality or integrity of the data.

(10) The names of the sponsor, study director, principal investigator, names of other scientists or professionals, and the names of all supervisory personnel involved in the study.

(11) A description of the transformations, calculations, or operations performed on the data, a summary and analysis of the data, and a statement of the conclusions drawn from the analysis. Results of the analysis of data should include the calculated LC50 value, 95 percent confidence limits, slope of the transformed concentration-response line, and the results of a goodness-of-fit test (e.g., chi-square test).

(12) The signed and dated reports prepared by any individual scientist or other professional involved in the study, including each person who, at the request or direction of the testing facility or sponsor, conducted an analysis or evaluation of data or specimens from the study after data generation was completed.

(13) The locations where all specimens, raw data, and the final report are stored.

(14) The statement prepared and signed by the quality assurance unit.

[52 FR 24462, July 1, 1987]

Subpart D—Provisional Health Effects Guidelines

§ 795.225 Dermal pharmacokinetics of DGBE and DGBA.

(a) *Purpose.* The purpose of these studies is to determine:

(1) The absorption of diethylene glycol butyl ether (DGBE) after administration by the dermal route.

(2) The biotransformation of DGBE administered dermally.

(3) The dermal absorption of DGBE and diethylene glycol butyl ether acetate (DGBA).

(b) *Test procedures*—(1) *Animal selection*—(i) *Species.* The species utilized for investigating DGBE and DGBA shall be the rat, a species for which historical