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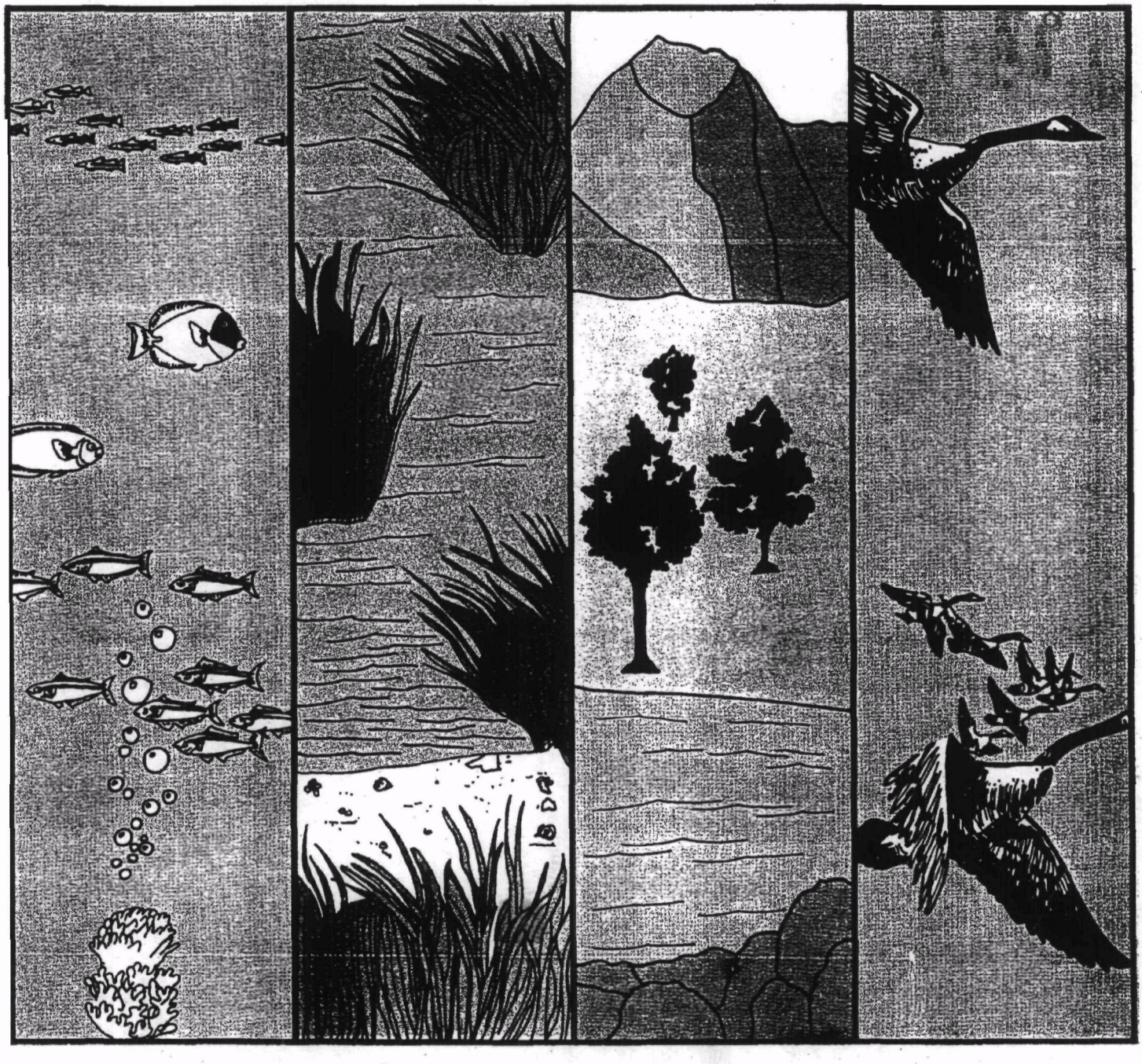
Hazard Evaluation
Standard Evaluation

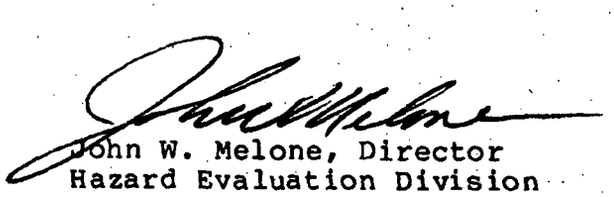
Daphnia Magna Life-Cycle (21-Day Renewal) Chronic Toxicity Test



STANDARD EVALUATION PROCEDURE

PREAMBLE

This Standard Evaluation Procedure (SEP) is one of a set of guidance documents which explain the procedures used to evaluate environmental and human health effects data submitted to the Office of Pesticide Programs. The SEPs are designed to ensure comprehensive and consistent treatment of major scientific topics in these reviews and to provide interpretive policy guidance where appropriate. The Standard Evaluation Procedures will be used in conjunction with the appropriate Pesticide Assessment Guidelines and other Agency Guidelines. While the documents were developed to explain specifically the principles of scientific evaluation within the Office of Pesticide Programs, they may also be used by other offices in the Agency in the evaluation of studies and scientific data. The Standard Evaluation Procedures will also serve as valuable internal reference documents and will inform the public and regulated community of important considerations in the evaluation of test data for determining chemical hazards. I believe the SEPs will improve both the quality of science within EPA and, in conjunction with the Pesticide Assessment Guidelines, will lead to more effective use of both public and private resources.



John W. Melone, Director
Hazard Evaluation Division

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DAPHNIA MAGNA LIFE-CYCLE (21-DAY RENEWAL) CHRONIC TOXICITY TEST

I. INTRODUCTION

A. When Required

The Daphnia magna life-cycle study[1/] is required to support registration of an end-use pesticide product that is applied directly to water or expected to be transported to water from the intended use site, and when any of the following conditions apply:

- If the pesticide is intended for use such that its presence in water is likely to be continuous or recurrent regardless of toxicity, as revealed by studies required by 40 CFR § 158.130;
- If any LC50 or EC50 value determined in testing required by 40 CFR §158.145 [§§ 72-1, -2, or -3] is less than 1 mg/l;
- If the estimated environmental concentration in water is equal to or greater than 0.01 of any EC50 or LC50 determined in acute testing for aquatic organisms required by 40 CFR § 158.145; or
- If the actual or estimated environmental concentration in water resulting from use is less than 0.01 of any EC50 or LC50 determined in acute testing for aquatic organisms required by 40 CFR 158.145 and any of the following conditions exist:
  - Studies of other organisms indicate the reproductive physiology of invertebrates may be affected;
  - The pesticide is persistent in water (e.g., half-life in water greater than 4 days); or
  - Physicochemical properties indicate cumulative effects.

1/ In cases where risk criteria are exceeded for both fish and aquatic invertebrates, the more sensitive organism must be tested in a fish early life-stage or invertebrate life cycle study. Both studies may, however, be required to complete a risk assessment.

B. Purpose

- The Daphnia magna life-cycle study is intended to measure pesticidal effects on daphnid reproduction, survival and growth. Survival, adult length, and the average number of offspring per adult per reproduction day are measured in this study;
- Establish chronic toxicity levels of the active ingredient to daphnids;
- Compare toxicity information with measured or estimated pesticide residues in an aquatic environment in order to assess potential impact to aquatic invertebrates;
- Provide support for precautionary label statements;
- Indicate the need for further laboratory testing or field testing; and
- Renewal tests may not be applicable to chemicals which have high oxygen demand, are highly volatile, are transformed, or sorbed to glass. In these cases, flow-through tests may be more appropriate.

C. Test Material

Testing must be conducted with the technical grade of the active ingredient (a.i.). If more than one active ingredient constitutes a technical product, the technical grade of each active ingredient must be tested separately.

D. Acceptable Protocols

EEB does not endorse any one protocol. It is sometimes necessary and desirable to alter the procedures presented in published protocols to meet the needs of the chemical or test organisms used. However, EEB does recommend some protocols as guidance for developing a daphnid life-cycle test. These protocols include:

Biesinger, K.E. 1974 (a). Procedure for Daphnia magna chronic test in standing system. U.S. EPA, Eviron. Res. Lab., Duluth, MN. Federal Register 40(123): 26902-26903 pp. (June 25, 1975).

American Public Health Association, American Water Works Association and Water Pollution Control Federation. 1985. Standard Methods for the Examination of Water and Wastewater. Sixteenth Edition. Publication Office: American Public Health Association, 1015 18th Street, N.W., Washington, D.C. 20036. 765 pp.

ASTM Standard E 729-80, Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. American Society for Testing and Materials, 1916 Race street, Philadelphia, PA 19103.

II. MATERIALS, METHODS AND REPORTING REQUIREMENTS

A. Biological System

1. Test Organisms

Daphnia[1/] are used in this test because they represent an important aquatic phyla, they are members of a trophic level of primary consumers, and they are a sensitive test organism for pesticide evaluation. The use of these organisms is also advantageous because of their short life-cycle, ease of culture, and requirements for limited space and water volume.

2. Source

Daphnia can be obtained from laboratory, commercial, or wild stocks. All test organisms must be produced from a laboratory reared culture that has been maintained for at least 21 days at test conditions in dilution water with renewal of the culture medium at least three times a week. The identity of organisms must be verified regardless of any information that comes with the organisms.

3. Food

A variety of foods appear to be adequate for culturing daphnids. These include: 1) synthetic foods (trout chow); 2) synthetic foods in combination with alfalfa yeast and algae; and 3) algae including Ankistrodesmus falcatus, Chlamydamonas reinhardtii, and Selenastrum capricornutum. Other food mixtures may also be acceptable.

4. Beginning the Test

Prior to starting a test, daphnids which are at least 10-12 days old (those that have had at least one brood) should be separated from the culture, put in a separate culture container and maintained for at least 21 days to insure that good health and conditions are present.

1/ Registrants wishing to use other test species for this study may do so, provided those species are deemed acceptable by the Agency.

Young daphnids $\leq$ 24 hours old are obtained from this subculture and are used to start the test. Ten 250 ml beakers (200 mls of test solution) are used for each toxicant concentration: a) seven beakers at each concentration will contain one daphnid each for collection of data on survival, growth, and reproduction; b) three beakers at each concentration will contain five daphnids each for collection of data on survival only (not reproduction or growth). Assignment of daphnids should be randomized. A test begins when test organisms are first placed in the test solution.

5. Renewal

A renewal schedule (i.e., Monday, Wednesday, Friday) must be set-up for counting live and dead daphnids. Parent daphnids in all beakers are counted and transferred to beakers containing the same toxicant concentration as that from which they were removed. In the seven beakers containing one parent daphnid each, the offspring, both live and dead, are counted and discarded.

6. Duration

Testing is concluded on the 21st day. On this final day, the first generation (parent) daphnids are counted and individually measured to the nearest 0.01 mm from the apex of the helmet to the base of the spine. The number of young, both alive and dead, in each beaker are counted.

7. Test Rejection

A test is rejected if the following occurs:

- 30 percent of specimens in the controls (including solvent control) die;
- Daphnids in either control do not produce at least 40 young after 21 days;
- Production of ephippia by any of the controls. These "resting eggs" are capable of withstanding adverse environmental conditions;
- Temperature deviation from 20°C exceeds 5°C for more than 48 hours;
- Dissolved oxygen drops below 50 percent of saturation for more than 48 hours; or
- pH deviates by more than one unit for more than 48 hours.

8. Data Endpoints

A report of the results of a test must include data on the survival of first generation daphnids, production of young by first generation daphnids at various times for each treatment, and the length of first generation daphnids at the end of test.

B. Physical System

1. Test Water

Test water can be supplied from a well or spring provided that the source is not polluted. Water must be tested for pesticides, heavy metals, and other possible contaminants. Hardness of 160 to 180 mg/L as $CaCO_3$ and pH of 7.6 to 8.0 is recommended. If reconstituted water is used, detailed descriptions of acceptable procedures for preparing dilutent are found in the protocols by the American Society of Testing Materials (1980).

2. Temperature

Life-cycle tests with daphnids should be conducted at 20 $\pm$ 2°C.

3. Photoperiod

A 16-hour light and 8-hour dark photoperiod should be provided. Light intensity should be 400 to 800 Lux (37 to 74 footcandles at the surface of the test solutions) and be provided by wide-spectrum fluorescent lamps.

4. Test Vessels

Any container made of glass, No. 316 stainless steel, or perfluorocarbon plastics which hold 200 mls of test solution can be used. The 250 ml borosilicate glass beakers have been found convenient to use. Test vessels should be covered with glass plates to prevent evaporation of test solutions.

5. Aeration

Dilution water should be aerated vigorously insuring that dissolved oxygen concentration will be at or near 90 to 100 percent saturation. Tests tanks chambers should not be aerated.

C. Chemical System

1. Concentrations

A minimum of five concentrations of toxicant and a control (all duplicated) are used in this chronic test. A solvent control is added if a solvent is utilized. The recommended concentration of food to be used is 5 mg/L (dry weight) if trout food or synthetic diet is used, and 10^8 algal cells per liter if an algae diet is used. For each concentration, an aliquote of toxicant is added to the dilutions water/food mixture and the solution is well mixed. Test solutions should be made up less than four hours before the test begins.

2. Measurement of Other Variables

Dissolved oxygen must be measured at each concentration at least once a week. Freshwater parameters in a control and one concentration must be analyzed once a week. These parameters should include pH, alkalinity, hardness, and conductance.

3. Solvents

If solvents other than water are necessary, they should be used sparingly and not to exceed 0.5 mL/L in a static system.

The following solvents are acceptable:

dimethylformamide
triethylene glycol
methanol
acetone
ethanol

D. Calculations

Data from these toxicity studies are of two types, continuous (e.g., length) or dichotomous (e.g., number hatching or surviving). In general, continuous data will be analyzed using an appropriate analysis of variance (ANOVA) technique followed by an appropriate multiple comparison test. Dichotomous data will be analyzed using a 2 x 2 contingency table. All test results must be accompanied by copies of the original (raw) data for the reviewer's evaluation. Transcripts of original raw data are acceptable if they provide all of the information in the original data set, including comments or notes provided by the investigator.

III. REVIEWER'S EVALUATION

The reviewer should identify each aspect of the reported procedures and determine if there is any inconsistency with recommended methodologies. The number of deviations and their severity will determine the validity of the study and the interpretation of the results.

A. Verification of Statistical Analysis

The reviewer should ensure that an MATC has been properly derived by recalculating the reported results. If the recalculated results differ substantially from the submitted results, the reviewer should note this and attempt to explain the differences.

B. Conclusions

The significance of inconsistencies in the test procedures must be determined by the reviewer so that the results of the test can be categorized as to whether they fulfill Part 158 regulations and are useful in performing a risk assessment. Categories are described as:

- Core: All essential information was reported and the study was performed according to recommended protocols. Minor inconsistencies with standard methodologies may be apparent; however, the deviations do not detract from the study's soundness or intent. Studies within this category fulfill the basic requirements of current guidelines and are acceptable for use in a risk assessment.

- Supplemental: Studies in this category are scientifically sound; however, they were performed under conditions that deviated substantially from recommended protocols. Results do not meet guideline requirements; however, the information may be useful in a risk assessment.

  Some of the conditions that may place a study in a supplemental category include:

  - Unacceptable test species;
  - Inappropriate test material; or
  - Deviations from recommended test solution characteristics (variations in DO, temperature, hardness, and pH can affect toxicological response).

- Invalid: These studies provide no useful information. They may be scientifically unsound, or they were performed under conditions that deviated so significantly from recommended protocols that the results will not be useful in a risk assessment.

Examples of studies placed in this category commonly include those where the test system was aerated, test vessels were constructed from materials other than glass, or there were problems of solubility of volatility of the test material. Unless acceptable chemical analyses of actual toxicant concentrations were performed in studies such as these, the reviewer cannot be sure that test organisms were actually exposed to nominally designated concentrations. A study where the test material was not properly identified can also be invalidated.

1. Rationale

Identify what makes the study supplemental or invalid. While all deviations from recommended protocol should be noted, the reviewer is expected to exercise judgment in the area of study categorization.

2. Reparability

Indicate whether the study may be upgraded or given a higher validation category if certain conditions are met. Usually this would involve the registrant submitting more data about the study.

3. Descriptive Conclusions

The reviewer should indicate what the results were and how much information can be drawn from them. These results are useful in a risk assessment.