RRB-87-54

This manuscript has been prepared for

Toxicity Assessment: An International Quarterly

and the contents are subject to change.

This copy is to provide information

prior to publication.

CERIODAPHNIA RETICULATA SEVEN-DAY SURVIVAL AND REPRODUCTION TEST FOR SCREENING CHRONIC TOXICITY LEVELS OF CONTAMINANTS IN ENVIRONMENTAL SAMPLES

by

S.S. Rao

Rivers Research Branch
National Water Research Institute
Canada Centre for Inland Waters
Burlington, Ontario, L7R 4A6

September 1987
NWRI Contribution #87-163

Abstract

A seven-day, three-brood life cyle test using Ceriodaphnia reticulata for screening sublethal concentrations of contaminants in environmental samples is described.

Management Perspective

This article describes a seven-day, three-brood life cycle test using C. reticulata for assessing the sublethal (chronic) levels of toxicants in environmental samples. This test procedure is now included in Methods Manual as a routine standard procedure for evaluating the chronic levels of toxicants.

Résumé

Description d'un essai de sept jours à cycle de trois générations utilisant la Ceriodaphnia reticulata pour détecter des concentrations subléthales de contaminants dans des échantillons de l'environnement.

Perspective - Gestion

Le présent article décrit un essai de sept jours à cycle de trois générations, basé sur l'utilisation de C. reticulata pour évaluer les concentrations subléthales (chroniques) de contaminants dans des échantillons environnementaux. Ce mode opératoire expérimental est présentement inclu dans le Methods Manual à titre de mode opératoire ordinaire normalisé pour évaluer les concentrations chroniques de substances toxiques.

Ceriodaphnia reticulata Seven-Day Survival and Reproduction Test* for Screening Chronic Toxicity Levels of Contaminants in Environmental Samples

S.S.RAO, National Water Research Institute, P.O. Box 5050, 867 Lakeshore Road, Burlington, Ontario, Canada L7R 4A6

Objective

This method is intended to be used as a screening test for detecting the presence of sublethal levels of toxicants in waters, effluents and sediments.

2.0 SCOPE

This method is applicable to the following types of samples:

(i) Surface and ground waters

(ii) Domestic and industrial wastewaters including sludge and leachates

(iii) Sediments

3.0 PRINCIPLE

3.1 This method employs the cladoceran *Ceriodaphnia reticulata* which has wide distribution and is abundant in reservoirs, streams and lakes. Chronic toxicity test is based on the

---

*This test was originally developed by Norberg and Mount, US EPA, Duluth, Mn. in 1984.

changes in the reproductive success coupled with the long-term survival of the organism.

Normally at 25°C ± 1°C the 3 brood life cycle is completed in 7 days during which period the cumulative number of young produced from all ceriodaphnia from all generations are averaged and the mean number of young produced for each dilution/concentration and control are calculated.

3.2 To perform the test, uniform young Ceriodaphnia reticulata (0-2 h old) are exposed to different log concentrations/ dilutions of the test sample (0.01 to 100%) at 25°C ± 1°C.

3.3 The extent of inhibition of young produced during the 7 day period in the experimental sample compared to control is considered to establish the presence of sublethal levels of toxicants.

4.0 REAGENTS, MATERIALS AND APPARATUS

4.1 Reagents

4.1.1 Reagent grade chemical shall be used in all media preparation. Unless otherwise indicated, water used in this context shall be glass distilled water or prepared by the Milli-Q process.

4.1.2 **Bristol's Medium**

Compound	Stock	Volume of stock to be added to make up to 1 litre of steril glass distilled wate
$NaNO_3$	25 g/L water	10 mL
$CaCl_2.2H_2O$	2.5 g/L	10 mL
$MgSO_4.7H_2O$	7.5 g/L	10 mL
K_2HPO_4	7.5 g/L	10 mL
NaCl	2.5 g/L	10 mL
KH_2PO_4	17.5 g/L	10 mL
KOH	3.1 g/100 mL H_2O	1 mL combined
EDTA	5.0 g/100 mL H_2O	KOH/EDTA stock
$FeSO_4.7H_2O$	0.498 g/L H_2O	10 mL combined
H_2SO_4	0.1 mL/L	$H_2SO_4/FeSO_4.7H_2O$
H_3BO_3	1.142 g/100 mL	1 mL

4.1.3 **Bristol's Trace Elements** — 1 mL combined stock

$ZNSO_4.7H_2O$	0.882 g/100 mL
$MnCl_2.4H_2O$	0.144 g/100 mL
MoO_3	0.071 g/100 mL
$CuSO_4.5H_2O$	0.157 g/100 mL
$Co(NO_3)2.6H_2O$	0.049 g/100 mL

Add the indicated volumes of each stock plus 1 ml of the combined trace elements stock to glass-distilled water for a total volume of 1 litre, while stirring. Adjust pH to 7-8, depending on algal species - indicate pH and date of preparation on label.

NOTE: EDTA, KOH and H_3BO_3 solutions take longest time to dissolve.

4.2 Materials

Dechlorinated water (prepared by aerating or bubbling air through tap water for 24 hrs)

Sediment extract (1:1) in milli Q water or any test effluent or water sample

Glass distilled water and Milli Q water

pH indicator paper

Glass jars (5 or 10 L for growing stock algal culture)

Note: All glassware must be thoroughly washed, rinsed in dechlorinated water and sterilized to avoid unnecessary contamination.

Clear plastic cups 30 ml capacity (polypropylene medicine cup) or small beakers 30 mL capacity

Styrofoam tray made from slab of styrofoam (1"x24"x18") (6-8 rows with holes to hold 6-8 plastic cups in each row) (Any appropriate tray can be used).

Oxford micropipettor with plastic tip, 5 mL and 10 mL capacity to dispense test solution/dilutions and feed stock culture. (Any convenient dispensing device can be used).

Thermometer to monitor temperature in work area.

Eppendorf pipette (100 µl) with tips to feed algal-yeast suspension to test organisms during the test (any pipette with correct capacity can be used).

4.3 Apparatus

Steriomicroscope with light source (Carl Zeiss, Jena, Made in Germany (at least x4 magnification)

5.0 CULTURE MAINTENANCE

Algal culture (Scenedesmus sp.) can be obtained from a biological supply company.

Ceriodaphnia reticulata. This can be obtained from Environmental Research Laboratory, U.S. EPA, Duluth Mn.

Baker's yeast (torula yeast) commercially available in any health food store (Baker's yeast solution is prepared by dissolving 1.5 g in 300 ml dechlorinated water by stirring on a stir plate or putting it in a refrigerator overnight to dissolve.

Note: (One day in advance is useful).

5.1 **Stock Culture Maintenance**

Initial culture of Scenedesmus Sp. can be obtained from a biological supply company. Upon receipt, the organisms are transferred to large clean sterile glass carboys (≈10 L) containing 1/2 strength Bristol's medium. Organisms are grown under a light source, slightly agitating using sterile magnetic stirrer to maintain aerobic conditions, at 25°C ±1 and subcultured weekly. 1 week old growth is used for feeding the animals during experiments.

Upon receipt of Ceriodaphnia reticulata, the organisms are transferred to large clean glass jars (5-10 L) containing dechlorinated water. The organisms are maintained at 25°C ± 1 by feeding every 24 hrs with algal culture (Scenedesmus sp.) (10 cc for 10 L volume and yeast culture 5 cc for 10 L volume every 48 hrs). Organisms can be transferred to new containers every 4 wks.

6.0 **TESTING PROCEDURE**

6.1 Chronic toxicity tests are performed in 30 ml plastic beaker with 10 ml sediment extract or test water sample. The geometry of this combination allows one to view the entire volume of the test sample extract. The surface volume ratio is such that dissolved oxygen will not become a limiting

factor. 4-6 beakers with 1 animal each are used for each test concentration and control. The test concentrations used are 100%, 50%, 10%, 5%, 1.0%, 0.1%, 0.01%.

6.1 Dechlorinated tap water is used as control and for dilution.

6.2 Temperature of the experiment is maintained at 25°C±1. This temperature is used to prevent mortality problems in the control in chronic test.

Note: (Tests should be started with young animals that are as similar in age as possible. (0-2 hr old young are used). If the brood animals are put singly in 30 ml beakers, one can easily keep track of the age of all young and the appropriate beakers can be composited to make the lab or test animals for distribution in the test).

Note: (The entire test is designed to fit into the normal 5 day work week to reduce the cost of the test due to the week end work).

6.3 Test is set up on Monday (Day 1).

6.4 Place one young animal in each 10 ml of test solution.

6.5 Place one young animal in a 10 ml dechlorinated water as control.

6.6 Appropriate number of beakers with one animal each are used for each test sample dilution or concentration and control. Feed the animals every other day with a 100 µL mixture of algal culture (having an O.D. 0.4 at 700 nm) and yeast culture (1:1).

6.7 Determine the number of young produced in the experimental and control test solutions every 24 hrs for 7 days.
Move the adult into new test solutions of the same concentrations and count the young and discard the young on Wednesday (Day 3) and Friday (Day 5).

6.8 Terminate the test on the following Monday (Day 7) by counting the number of young produced altogether in the final sample.

6.9 Test can also be started on Friday (changes are made on Monday and Wednesday) and terminated the following Friday.

6.10 Record pH at the beginning and at the end of the test (pH may be determined using pH indicator paper).

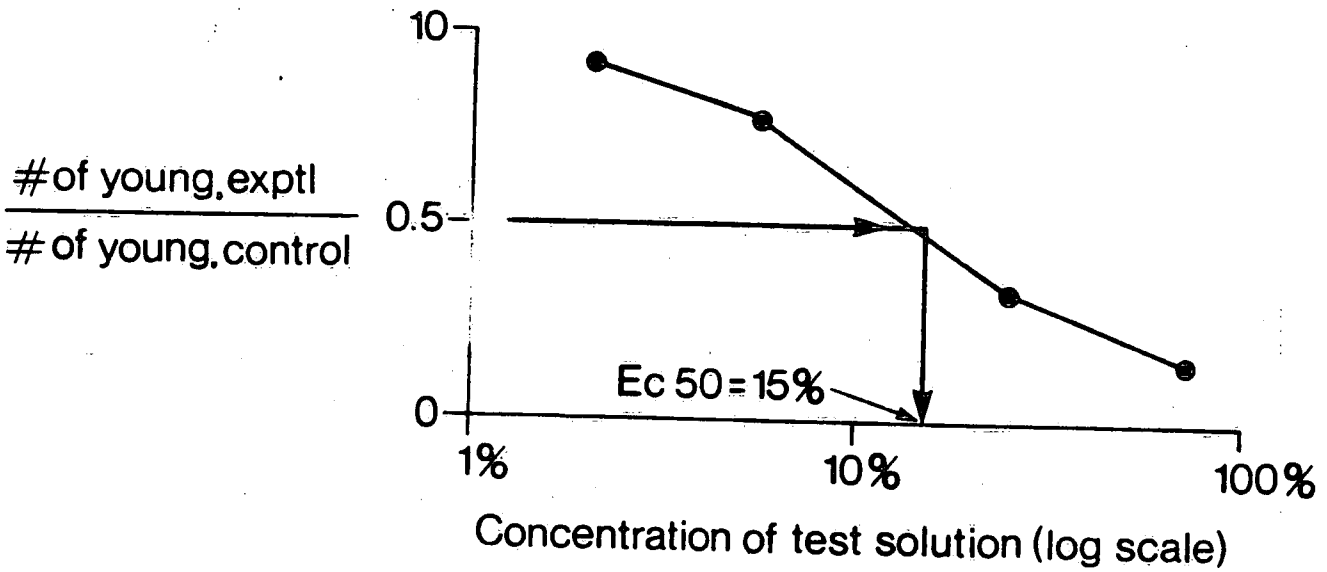
7.0 EXPRESSION OF RESULTS

Ch EC_{50} (effective concentration) is calculated as concentration or dilution at which there is a 50% reduction of young production

compared to the control. Plot # young produced/# young produced in control against (log) test concentration, and extrapolate to concentration at which relative # young is 0.5 (see figure). Log concentrations of test solution/extract may vary from 0.01% to 100% depending on the nature of the test sample.

Ch = Chronic toxicity

EC_{50} = Effective concentration



8.0 PERSONAL REMARKS

8.1 This test is highly recommended because the space requirements are very minor and test can be performed with reasonable equipment cost.

8.2 This test is specifically useful when the volume of the test sample and/or dilution water available is small

8.3 The organisms are easily cultured and monitored.