EVALUATION OF THE ACUTE TOXICITY OF THE HEAVY METAL CADMIUM TO NYMPHS OF THE BURROWING MAYFLY, *HEXAGENIA RIGIDA*

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ABSTRACT

Laboratory-cultured, yearling nymphs of the mayfly *Hexagenia rigida* were tested in static, no-replacement 96 h bioassay. The 96 h LC_{50} was estimated to be 1.0 mg/L by a graphical method and 6.2 mg/L by the 0-Trimmed Spearman-Karber calculation. The latter was easier to apply and gave a more precise estimate.

INTRODUCTION

The mayfly, *Hexagenia rigida,* is being investigated as one of the organisms in a toxicological screening system to evaluate the effect of contaminants using a range of sensitive to tolerant organisms and/or stages within their life cycle. In this study, yearling nymphs, from a stock culture of animals, were tested with cadmium, a toxic heavy metal widely distributed in the environment due to increased industrial use. The object of the study was two-fold: to determine the acute toxicity of cadmium (96 h LC_{50}) to yearling nymphs in order to assess their efficacy as a test stage and to compare two popular estimates of LC_{50} used by bioassay researchers; the "routine bioassay method" given in "Standard Methods" (APHA *et al.* 1965) and further described by Sprague (1969), and the trimmed Spearman-Karber calculation (Hamilton *et al.* 1977).

MATERIALS AND METHODS

Nymphs were sieved from stock cultures. These stock cultures had been initiated one year earlier by extruding eggs from fertilized adult females that had been gathered along the Red River at Winnipeg, Manitoba, Canada. The cultures were held at 20°C in approximately 3 cm of silt, from the Red River, covered with 3L of water. The pH of the culture system was 7.0. The animals were fed undefined amounts of a water suspension of Tetramin, fish food flakes, weekly.

Prior to testing, the experimental animals (nymphs 24 mm in length from the tip of the frontal process to the end of the abdomen) were acclimated for 24 h in 1 L pyrex glass beakers (5 animals/beaker) containing 500 mL of water at 18°C. No food substrate or aeration was provided. Light (12 $\mu E/m^2 sec$) was provided by overhead fluorescent light banks for 14 h daily. Winnipeg city water dechlorinated by sodium thiosulphate additions (Table 1) was used in the stock culture maintenance, the acclimation and preparation of dilutions and controls in the toxicity tests.

Exposure system:

The cadmium stock solution was prepared by dissolving 1.0 g/L of the pure metal cadmium in acidified deionized water. The test dilutions of cadmium (mg/L) were as follows: 0.01, 0.1, 1.0, 5.0, 10, 20, and 40. These were made by diluting the stock solution

Table 1. Water chemistry data for thiosulphate-dechlorinated Winnipeg city water (18°C).

Analysis	Units
chloride	4.2 mg/L
sulphate	12.4 mg/L
sodium	3.68 mg/L
potassium	1.30 mg/L
calcium	22.7 mg/L
magnesium	5.44 mg/L
pH	7.96
conductivity	170 µS/cm
dissolved oxygen	8.0 mg/L
hardness (by calculation)	79.1 mg/L

* This sulphate value is an overestimate caused by thiosulphate interference.

one hour prior to the bioassay. Two 100 mL samples were removed from each test container for water chemistry and heavy metal (Table 2) analysis respectively.

The test containers were uncovered, 600 mL pyrex glass beakers containing 500 mL of test solution. There were seven concentrations of cadmium, plus a control and two replicates per concentration. Ten animals were tested at each concentration. The number of mortalities was recorded at eight hour intervals over a 96 h period.

The criteria for determining death were: cessation of respiratory movements of the gills, and no response to abdominal prodding with a Pasteur pipette tip for a period of 60 sec. If no response was visible, the animals were removed with 5 mL of test solution to a 50 mL disposable plastic beaker for further observation at the subsequent mortality check. Confirmed mortalities were discarded.

Statistical analysis:

Mortality data were analysed by two methods. One, using the "routine bioassay method" given in "Standard Methods" (APHA *et al.* 1965). This method yields median tolerance limits (TLM's or LC_{50}'s). For each fixed time, percentage mortalities are plotted against the test concentrations. The concentration lethal to half of the test organisms is estimated by interpolation. Confidence limits have customarily been omitted for LC_{50}'s estimated by this method (Sprague 1969).

Table 2. The concentration of cadmium (mg/L) in the test containers.

Nominal Value	Actual Initial Value*
0 (control)	0.0010
.01	0.0072
0.1	0.036
1.0	0.94
5.0	4.6
10.0	8.5
20.0	18.0
40.0	34.0

* no change after 96 h exposure.

The second method, more recently adopted, has four simple steps:

1. Adjust the mortality proportions p_i, ..., pk to form $\tilde{P}_i$'s in a monotone nondecreasing sequence.
2. Plot the $(x_i, \tilde{P}_i)$ points and connect them with straight lines. The polygonal figure formed is an estimate of P(x), the cumulative relative frequency curve for the tolerance distribution
3. Choose α or the % trim desired. Trim off the upper α percent and lower α percent of the polygon. Adjust the ordinate scale appropriately. Where α=0, as herein, no adjustments are required.
4. Calculate the mean associated with the cumulative relative frequency polygon to yield the α% - trimmed Spearman-Karber estimate of μ. The antilog of μ will yield the estimate of LC_{50}.

RESULTS AND DISCUSSION

No mortality was observed at cadmium concentrations of 0 - 0.1 mg/L for the entire 96 h test period. One hundred per cent mortality occurred in the 40.0 mg/L test vessels after 12 h. exposure and in the 20.0 mg/L containers after 30 h. Figure 1 illustrates mean survival time in hours (the calculated time to 50% death in each concentration) as a function of the concentration of cadmium (mg/L) in the test series. The 96 h LC_{50} by interpolation is read as 1.0 mg/L cadmium.

This method is the simplest routine procedure. Increased information may be obtained by fitting a straight line to the data plotted on logarithmic - probability graph paper (Litchfield and Wilcoxon 1949). This refinement is useful when survival percentages are found to change little or erratically with progressive increases of tested concentrations. It has become one of the most widely accepted procedures in calculating and reporting bioassay results (APHA *et al.* 1965). A second popular calculation method is the trimmed Spearman - Karber (Hamilton *et al.* 1977).

Table 3 shows an example of the data set used in the trimmed Spearman - Karber calculation of the LC_{50}. Table 4 demonstrates the application of a 0-trim in the trimmed Spearman-Karber method to estimate μ (the mean of the population tolerance distribution). Using this procedure, the estimate of the LC_{50} is, then, the antilog of the estimate of μ, or, 6.2 mg/L cadmium. Hamilton *et al.* (1977) discuss this procedure in depth and show that this method is a better overall procedure which eliminates the target criticisms of Probit and Logit models; namely, that the maximum likelihood iterative procedure can be very unstable if the data do not conform to the assumed model and that one may not always accurately compare

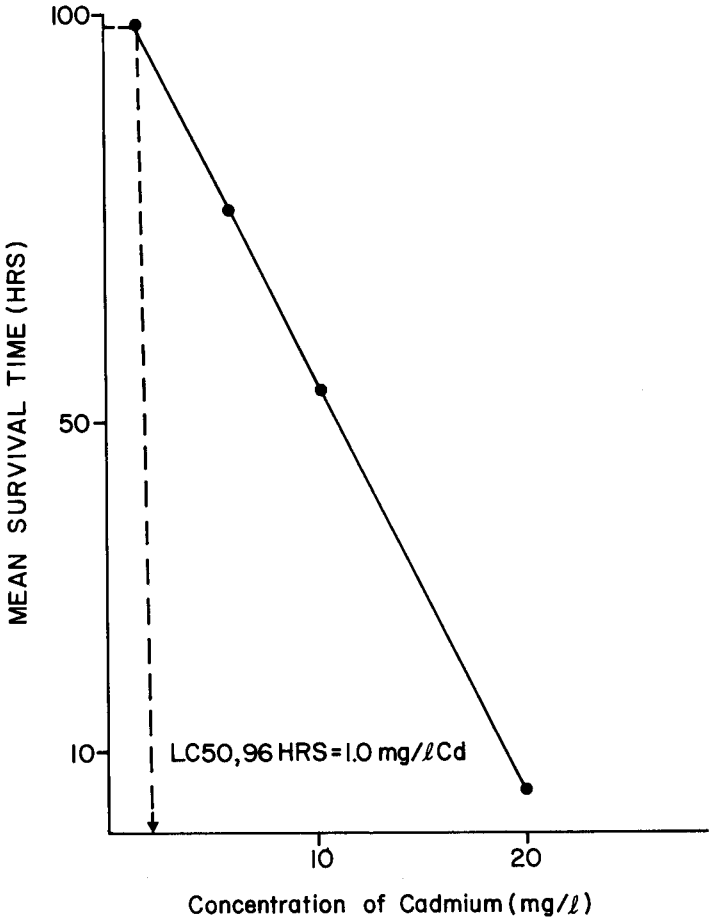

Figure 1. Graphical estimation of the 96 h LC_{50} for cadmium to nymphs of the burrowing mayfly, *Hexagenia rigida*.

results from duplicate experiments.

Thus, the 96 h LC_{50} for the nymphs of the burrowing mayfly, *Hexagenia rigida* lies between 1.0 and 6.2 mg/L cadmium when tested in thiosulphate dechlorinated water and using two estimators. These estimates may be high since tests conducted using thiosulphate water as diluent may yield higher estimates of the LC_{50} than those conducted using ultraviolet dechlorinated water or a reconstituted medium as diluent. This elevation of LC_{50} in thiosulphate dilutions was observed by Friesen (personal communication) in defining the

Table 3. Example of Trimmed Spearman-Karber calculations. Data set at 96 h exposure.

Concentration	mg/L	0	.01	0.1	1.0	5	10	20	40
Log_e conc.	x_i	0	-4.605	-2.302	0	1.609	2.302	2.996	3.689
No. of nymphs	n_i	10	10	10	10	10	10	10	10
No. of mortalities	r_i	0	0	0	5	7	7	10	10
Mortality proportion	p_i	0.0	0.0	0.0	0.5	0.7	0.7	1.00	1.00
Adjusted mortality proportion	$\tilde{p}_i$	not necessary; $\tilde{p}_i$'s are in a monotone nondecreasing sequence							

Table 4. Calculation of 0-Trimmed Spearman-Karber estimates of μ (the mean (and median) of the population tolerance distribution).

(1)	Log_e of conc. interval: (x_{j-1}, x_j)	0 to -4.605	-4.605 to -2.302	-2.302 to 0	0 to 1.609	1.609 to 2.302	2.302 to 2.996	2.996 to 3.689
(2)	Relative frequency: $P_j - P_{j-1}$	0	0	.5	.2	0	.3	0
(3)	Midpoint of interval: $(x_{j-1} + x_j)/2$	-2.302	-3.454	-1.151	.804	1.955	2.649	3.342
(4)	(2) x (3)	0	0	-0.575	1.608	0	.7947	0

The estimate of μ is 1.8277 and the estimate of LC_{50} is thus $e^{1.8277}$ = 6.2 mg/L

incipient LC_{50} for early (1 mg/L) and mid-stage eggs (3.5 mg/L) of *Hexagenia rigida* exposed to cadmium. Apparently, the yearling nymphs of *Hexagenia rigida* are not any more sensitive to cadmium exposure than the developing eggs.

In this study, care was taken not to select late instar nymphs (identified by dark wing pads), since Fremling (1975) reported that physiological stresses involved in the transformation to the adult stage may produce atypical results in tests done with such animals. Further studies should be designed to assess the sensitivity of newly hatched (hours to days old) nymphs with the continuing aim of identifying the most vulnerable stage in the life cycle of this mayfly.

CONCLUSIONS

The 96 h LC_{50} for cadmium exposure with *Hexagenia rigida* yearling nymphs lies between 1.0 and 6.2 mg/L depending on the estimator used.

The yearling nymphs of this species do not appear to be any more sensitive than the developing eggs in determining the acute toxicity of cadmium. Further assessments should be made on newly hatched nymphs.

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RESUME

Développées en milieu de culture, des nymphes d'éphéméroptères d'un an appartenant à l'espèce *Hexagenia rigida* ont été soumises à des tests biologiques statiques et sans modification d'éléments d'une durée de 96 heures. La LC_{50}, après 96 h, a été évaluée à 1.0 mg/L selon une méthode graphique et à 6.2 mg/L selon la méthode de calcul dite "O-Trimmed" de Spearman-Karber. Cette dernière s'est révélée d'application plus simple et a permis une estimation plus précise.

ZUSSAMENFASSUNG

Im Labor gezuchtete einjährige Nymphen der Eintagsfliege *Hexagenia rigida* wurden in statischen 96 Stunden Bioassayas ohne Neuzufuhr getestet. Die 96 Stunden LC_{50} wurde an Hand einer graphischer Methode auf 1.0 mg/L geschatzt. Die O-Trimmed Spearman-Karber Kalkulation ergab den Schätzwert von 6 2 mg/L. Letztere war leichter anzuwenden und ergab präzisere Schätzwerte.

REFERENCES

APHA *et al.* 1965. Standard Methods for the Examination of Water and Wastewater including Bottom Sediments and Sludges. Am. Pub. Health Assoc., New York, 12th edition.

Fremling, C.R. 1975. Acute toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) to nymphs of mayflies *(Hexagenia* sp.). *U.S. Fish. Wildl. Serv. Invest. Fish Control* 58: 1-8.

Hamilton, M.A., R.C. Russo, and R.V. Thurston. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. *Environ. Sci. Technol.* 11(7): 714-719.

Litchfield, J.T., Jr. and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther.* 96: 99-113.

Sprague, J.B. 1969. Measurement of pollutant toxicity to fish I. Bioassay methods for acute toxicity. *Water Res.* 3: 793-821.