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Acute toxicity bioassays using rotifers. I. A test for brackish and marine environments with *Brachionus plicatilis*

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A standardized 24-h acute toxicity test for the marine environment using the rotifer *Brachionus plicatilis* is described. Test animals are obtained by hatching cysts, thus eliminating the need for stock cultures. Since animals hatching from cysts are of similar age, genotype and physiologically condition, test variability is greatly reduced. Controlled cyst hatching is achieved by transferring to lower salinity, warmer temperature and light. After 23 h at 25°C and 15 ppt salinity, hatching begins and proceeds rapidly. By 28 h, 90% of the cysts have hatched. A protocol is outlined to collect the neonates and use them in a simple acute toxicity test to calculate a 24 h LC_{50}. A reference test using sodium pentachlorophenate (NaPCP) is described as well as a range-finding test and a definitive test for unknown toxicants. The toxicity of six compounds to *B. plicatilis* was examined with the following decreasing sensitivities: copper > NaPCP > SDS > free NH_3 > cadmium > malathion. For 3 of the 6 compounds tested, salinity increase from 15 to 30 ppt resulted in higher sensitivity, whereas for the other 3 compounds there was no effect. In comparison with current test organisms *B. plicatilis* is either more, equal or less sensitive depending on the compound, confirming the species-chemical specificity of mode of action of toxicants. The repeatability of the rotifer test is 5–6 times better than that reported for *Daphnia* tests and twice as good as the *Artemia* nauplii bioassay. Like the standard brine shrimp nauplii acute test, the cyst-based rotifer test is an important advance in acute toxicity testing since it eliminates stock cultures, is rapid, sensitive, highly repeatable, easy to execute and cost effective.

Key words: Acute toxicity; Bioassay; Rotifers; Dormant eggs; Copper; Cadmium; Free ammonia; SDS; Sodium pentachlorophenate; Malathion

INTRODUCTION

Obtaining invertebrate test animals in good, uniform physiological condition for aquatic toxicity bioassays is a persistent problem in environmental toxicology. In most protocols, test animals are collected from stock cultures which have been main-

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tained in the best possible conditions, but many sources of variability remain. Variations in water quality and food quality are inevitable in long term cultures. Maintenance of environmental conditions within acceptable limits often requires expensive incubators which are not available in many laboratories. The physiological condition of invertebrate test animals is difficult to standardize and acclimation of stock cultures to test conditions takes time. Animals taken from log phase vs stationary phase cultures can have quite different sensitivities to toxicants. Uncontrolled age and sex differences among test animals further increase the variance and reduce the repeatability of toxicity tests. Periodically, unexplained high mortality and low fertility occurs in stock cultures, even among cladocerans which have been cultured in the laboratory for many years (Buikema et al., 1980; DeGraeve and Cooney, 1987). The genetic characteristics of stock cultures are constantly changing as a result of new mutations, selection in the lab environment, and genetic drift during population crashes. The cost of a technician's time to maintain invertebrate stock cultures adds significantly to the cost of aquatic bioassays (Persoone and Van de Vel, 1987). For these reasons, an alternative to stock cultures as a source of invertebrate test animals is highly desireable in aquatic toxicology. Animals collected from the field are not a solution because there is even more uncertainty about their physiological and genetic condition.

An attractive alternative to invertebrate stock cultures as a source of test animals is cryptobiotic or dormant eggs (cysts). Because of their capacity for dormancy, cysts can remain on the shelf until test animals are needed, eliminating the need for stock cultures and their inherent variability. Test animals can be hatched synchronously and the resulting cohort of neonates emerge in a uniform physiological condition. Standard, genetically defined stocks can be used by all laboratories because cysts are easily and economically shipped worldwide. Cysts also can be stockpiled, eliminating the uncertainty about test animal availability. The ease of executing aquatic bioassays with animals hatched from cysts should greatly lower the costs and variability of toxicity tests, while enhancing their repeatability. Development of standardized, repeatable bioassays for the marine environment is an especially critical need (Persoone et al., 1984).

Currently, cysts are commercially available for only two aquatic invertebrates: the brine shrimp *Artemia* and rotifers of the genus *Brachionus*. *Artemia* cysts have been used in marine toxicity testing for some time (Vanhaecke et al., 1980; Vanhaecke and Persoone, 1981; Persoone and Wells, 1987), but rotifer cysts are only newly available (Snell and Hoff, 1986). Recent work on rotifer cyst production has identified the physiological and environmental factors that trigger cyst formation (Lubzens et al., 1980; Lubzens, 1981; Pourriot and Snell, 1983; Hino and Hirano, 1984; Hino and Hirano, 1985; Lubzens et al., 1985; Snell and Hoff, 1985; Snell, 1986;) and a method now exists for producing *Brachionus plicatilis* cysts under controlled laboratory conditions. Consequently, the availability of high quality rotifer cysts for aquatic bioassays is assured. In this paper, we outline a protocol for hat-

ching cysts of *B. plicatilis* a cosmopolitan estuarine rotifer, and using the resulting neonates in a definitive 24 h LC_{50} bioassay for brackish and marine environments. The sensitivity of *B. plicatilis* to several reference toxicants is compared to published LC_{50}s for *Daphnia magna, D pulex, Mysidopsis bahia* and *Artemia salina*. In a comparison paper, a similar bioassay is described for freshwater using cysts of the rotifer *Brachionus rubens* (Snell and Persoone, 1988).

ROTIFER CYSTS

Source

B. plicatilis (Muller) has been found on six continents and several strains are being cultured by aquaculturists worldwide. The strain used in the rotifer acute toxicity test is the Russian strain (Snell and Carrillo, 1984), originally collected from salinas near the Azov Sea region. A small sample of cysts was obtained from Dr. Patrick Sorgeloos of the State University of Ghent in 1980 and a rotifer population hatched from these cysts has since been in continuous culture at the University of Tampa. This population has been inbred and selected for cyst production for several generations and consequently is highly homozygous. Rotifer cysts are now commercially available from Florida Aqua Farms, Dade City, FL, USA.

Storage

Cysts are produced under rigorously controlled laboratory conditions (Snell and Hoff 1985; Snell 1986) and stored in 55 ppt salinity synthetic seawater. The best storage conditions are 6°C in the dark, but refrigeration is not essential if cysts are kept in a cool, dark place. We have recorded a 50% hatch from 2-yr-old cysts stored at 6°C and cysts can probably remain viable much longer. Consequently, the shelf life of cysts is more than adequate for aquatic bioassays. The biology of rotifer cysts has been reviewed by Gilbert (1974) and Pourriot and Snell (1983).

Hatching

Cyst hatching is initiated by transferring the cysts to lower salinity, warmer temperature and light. Several hundred cysts are placed in 5 ml of seawater medium in a 20 ml dish, which is a convenient size for neonate collection. The hatching rate and hatching percentage of rotifer cysts has been characterized for a variety of conditions. Higher temperatures accelerate hatching (Figure 1A). The time required for 50% of cysts to hatch (T_{50}) at 20°C is 43.7 h as compared to 19.0 h at 30°C. The Q_{10} for this temperature interval is 2.3, so cysts hatch 2.3 times faster at 30° than at 20°C. Hatching rate appears to increase linearly with temperature over the 20° to 30°C range, but more temperatures need to be examined to verify this observation. Standard hatching conditions for our toxicity tests are 25°C, 15 ppt salinity, pH 7.7 and 1000 lux light intensity from wide spectrum fluorescent tubes. Under these conditions, the time required for 10%, 50% and 90% of the cysts to hatch is 24.3, 25.6,

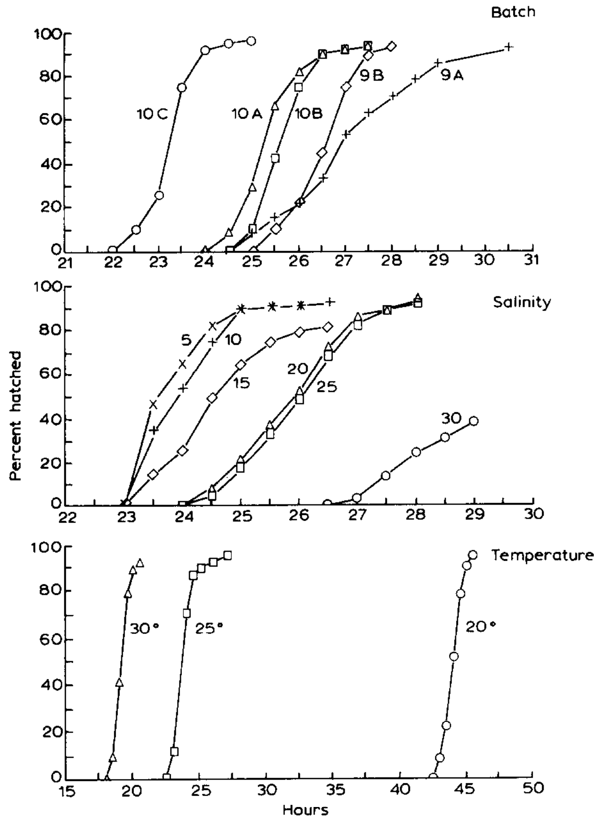

Fig. 1. The effect of temperature, salinity and batches on cyst hatching. Unless otherwise specified, hatching conditions were 25°C, 15 ppt and 1000 lux illumination. Each treatment consisted of 100 cysts. Salinities are in ppt; batches 9A, 9B and 10A, 10B, 10C are F9 and F10 generation cysts, respectively, collected from different cultures.

and 27.5 h, respectively. The time interval between the initiation of hatching and the time when 90% of the cysts have hatched (T_0 to T_{90}) is about 3 h. The effects of salinity on cyst hatching rate can be seen in Fig. 1B. Hatching rate decreases linearly as salinity increases ($Y = 0.22X + 21.7, R^2 = .83, P = .01$), but the range is small with cysts hatching at 30 ppt only 26% slower than at 5 ppt. The time required for 10%, 50%, and 90% of the cysts to hatch at 30 ppt is 27.2, 29.7, 32.2 h, respectively, which is about 15% slower than the hatching rate at 15 ppt. In general, the salinity for cyst hatching should correspond to the salinity of the acute toxicity test so that neonates suffer no osmotic stress when transferred from hatching to testing medium. The hatching rate of different batches of cysts can vary (Fig. 1C). Batches 9A and 9B are F9 cysts produced a few months apart; 10A, 10B and 10C are F_{10} cysts likewise produced in different batches over a few months. The mean T_{50} for these five batches of cysts in standard hatching conditions is 25.7 h with a coefficient of

variation of 6.2%. Based on this variation, the mean T_{50} of any particular batch is predicted to fall between 22–29 h 99% of the time. Light intensity in the 100–5 000 lux range has little effect on cyst hatching rate or percentage. While cyst hatching rate varies considerably with environmental conditions, hatching percentage is very consistent at salinities up to 25 ppt. Hatching percentage averaged 95% with a coefficient of variation of 3.4% in 15 samples. At 30 ppt, a lower hatching percentage was often recorded.

TEST PREPARATION

Procedures for the rotifer acute toxicity bioassay generally follow the recommendations of Standard Methods for the Examination of Water and Wastewater (1985) and USEPA (1985). Modifications have been made where we believe that the changes improve test precision and reliability. A further consideration was to develop a simple protocol that yields consistent results, even when executed by relatively inexperienced personnel.

Medium

Base water was obtained by deioinizing tap water through two high capacity Barnstead D8901 ion exchange cartridges followed by one Lab Flow 1705 adsorber cartridge. The resulting deionized water was used to make all media and stock solutions. Synthetic seawater was prepared by mixing Instant Ocean salts (Aquarium Systems, Mentor, Ohio) with deionized water, stirring for 24 h and filtering through a 0.45-μm filter. Seawater older than 7 days was routinely discarded. Most anomalous results can be traced to problems with the seawater due to inadequate mixing or excessive ageing, so it is very important to monitor seawater preparation closely.

Environmental conditions

Standard environmental conditions for acute toxicity bioassays with *B. plicatilis* are: temperature- 25°C, salinity- 15 or 30 ppt, pH- 7.7, and darkness. Bioassays are conducted in sterile, 24-well polystyrene tissue culture plates (Falcon 3047) which are used once and discarded. One ml of test solution is placed into each well and 10 neonate rotifers introduced. This density of 10 animals/ml gives a loading factor of 2 $\mu g.ml^{-1}$ since each neonate weighs approximately 0.2 μg. Because of the short duration of the bioassay and for simplicity, rotifers are not fed and the medium is not renewed during the acute test. Despite the static conditions and absence of food, control survival is almost always 100% after 24 h. After 48 h, mortality begins to occur, presumably from starvation.

Neonate collection

Neonate hatchlings from rotifer cysts are always females so sex determination is

not a problem. Neonate females average 164 μm in length and 147 μm in width which is about 0.25 the size of newly hatched *Daphnia pulex*. Manipulation of rotifers is easy with small bore micropipettes which can be made by heating and drawing out 5 mm O.D., 3 mm I.D. glass tubing. Care should be taken to make the bore large enough so that rotifers can enter and exit without injury. A piece of rubber tubing with one end closed can serve as the bulb to provide suction. Rotifers can be easily observed under a dissecting microscope at 10–12 × magnification. After hatching, neonates can be collected with a micropipette and transferred directly into the test solutions. The volume of medium transferred with the rotifers should be minimized to avoid dilution of test solutions. Entry of the rotifers into the test wells should be observed under the microscope to confirm the transfer. Test animals need to be $<$ 3 h old, so neonate collection should be completed 3 h after hatching begins. We have found no difference in the LC_{50}s of early and late hatching neonates in this 3-h interval. Six hours after hatching increased variation in neonate sensitivity has been observed, influencing the precision of the test.

TEST PROTOCOL

Reference test

In order to confirm the suitability of the laboratory environment, the good physiological condition of the test animals and the sensitivity of the rotifers to known toxicants, a reference test should be carried out simultaneously with the bioassay (Davis & Hoos, 1975; Standard Practice, 1980; Lee, 1980; USEPA, 1985). Since reference tests also facilitate comparison of results between laboratories and results obtained at different times from a single laboratory, they can be regarded as quality control measures for the rotifer bioassay.

Lee (1980) discussed several reference toxicants and considered their suitability as controls in aquatic bioassays. The use of sodium pentachlorophenate (NaPCP) was recommended because it is fast acting, nonselective in its toxicity to invertebrates and fish and it is an environmental contaminant (Adelman and Smith, 1976; Lee, 1980; USEPA, 1985). The disadvantage of NaPCP is that it is very toxic, even to humans, and its toxicity is pH dependent. At low pH NaPCP is less ionized resulting in increased toxicity (Crandall and Goodnight, 1959). Thus, it is necessary to control carefully the pH of the seawater medium, which should be between 7.5 and 8.0.

To establish the range of 24 h LC_{50}s for *B. plicatilis* using NaPCP as a reference toxicant, several tests with different batches of cysts were carried out at different times at 15 ppt salinity under standard conditions. A stock solution of NaPCP (Aldrich, 17130-1) containing 1 $g \cdot l^1$ was prepared and diluted to 0.7, 1.0, 1.3, 1.8 and 2.4 $mg \cdot l^{-1}$ with seawater medium. These concentrations were used in a 24 h LC_{50} bioassay, along with a control lacking NaPCP, to produce a mortality curve from which an LC_{50} could be calculated. Approximately 500 cysts were incubated in standard conditions and the resulting neonates randomly assigned to the six concen-

trations. A 24-well plate has six columns across and 4 rows down, so a single plate can hold all six concentrations for one acute toxicity test. Ten neonates were placed in one ml in each of 4 replicate wells in a column, for a total of 40 animals per concentration (column). The six concentrations in this design therefore require 240 neonates to complete one toxicity test. Bioassay plates were placed in an incubator under standard conditions and the percent dead in each treatment was recorded after 24 h. Rotifer death is defined as the absence of internal or external movement for 10 seconds. Dead rotifers also change from translucent to opaque which further aids the discrimination between live and dead animals. An LC_{50} was calculated by linear regression of log concentration vs mortality probit values. Desiccation in the plates during the test was avoided by placing dishes of water in the incubator to maintain high humidity. The mean LC_{50} value for NaPCP was 1.36 $mg \cdot l^{-1}$ with a coefficient of variation of 7.4% (Table 1). The no observed effect concentration (NOEC) was 1.0 $mg \cdot l^{-1}$ and the mean slope of the probit lines was 6.0 with 95% confidence limits of 4.8–7.2. The 99% confidence limits for NaPCP LC_{50}s under standard conditions were 1.22 and 1.50, which is within 10% of the mean and thus better than the precision recommended in Standard Methods (1985). The most extreme LC_{50}s recorded were within 92% and 115% of the mean.

Range-finding test

When testing an unknown toxicant, a range-finding test is usually carried out to determine the range of effect concentrations that will produce a mortality curve from which can LC_{50} can be calculated. The basic procedures for this test are similar to the reference test described above, but only two wells of 10 neonates each are used per concentration. Test concentrations are a logarithmic series including a control, 0.1, 1, 10, 100, and 1000 $mg \cdot l^{-1}$ for chemicals, and a control, 0.01%, 0.1%, 1%, 10%, and 100% for effluent or water samples. After 24 h, each concentration is scored for mortality. The highest concentration with no mortality and the lowest concentration with 100% mortality are chosen as the limits of the definitive test.

Definitive test

For the definitive test, five toxicant concentrations are chosen covering the 0 and 100% mortality concentration range determined in the range-finding test according to conventional procedures. The time between preparation of the toxicant solutions and introduction of the test rotifers should not exceed one hour so that changes in toxicant concentration after preparation are minimized. Forty test animals (10 per well, 4 replicates) are assigned to the control and each of the five concentrations in the same design as the reference test. After 24 h incubation under standard conditions, mortality is recorded and an LC_{50} calculated.

If effluents or water samples are used for the definitive test, sampling and handling of the effluent prior to the bioassay should follow USEPA (1985) guidelines. Effluents should be diluted to the desired concentrations with synthetic seawater of

the appropriate salinity. Usually effluent concentrations of 0, 6.25, 12.5, 25, 50 and 100% yield a mortality curve appropriate for LC_{50} calculation. Dissolved oxygen in the sample should be at least 40% of saturation, if not the sample should be aerated through a 1 ml pipette at no more than 100 bubbles per minute. The effluent sample should be brought to the experimental temperature and the pH adjusted to 7.5–8.0 with NaOH or HCl prior to dilution. If effluent salinity needs to be increased, add Instant Ocean salts. If the salinity of the sample is too high, it can be lowered by dilution with deionized water.

Validity of test

The rotifer acute toxicity test is considered valid if the following two conditions are met: mortality in the controls is less than 10% and the NaPCP LC_{50} from the reference test falls within the 99% confidence limits of 1.22 and 1.50 $mg \cdot l^{-1}$.

COMPARATIVE SENSITIVITY

Compounds tested

The toxicity of the following six compounds to *B. plicatilis* at 15 and 30 ppt was examined under standard conditions: sodium pentachlorophenate (NaPCP), sodium dodecyl sulfate (SDS), malathion, free ammonia (NH_3), copper sulfate ($CuSO_4$), and cadmium chloride ($CdCl_2$). All toxicant concentrations reported here represent calculated as opposed to measured concentrations. The source of NaPCP and the concentration range tested was described in the reference test. SDS was obtained from Aldrich (85192-2) and concentrations from 4 to 7 $mg \cdot l^{-1}$ were examined in a definitive test. The organophosphate insecticide Malathion 50 was produced by Chevron Chemical Company and contains 50% malathion in a xylene solvent. 80 $mg \cdot l^{-1}$ xylene was therefore added to the controls for these experiments, corresponding to the xylene concentration in the highest malathion treatment. Malathion concentrations of 40 to 80 $mg \cdot l^{-1}$ were tested in definitive tests. Free ammonia toxicity was investigated by adding NH_4Cl to synthetic seawater to produce free ammonia concentrations ranging from 14 to 29 $mg \cdot l^{-1}$ in definitive toxicity tests. Ammonia is only 33.7% of the mass of NH_4Cl and the percentage free ammonia was calculated from the Henderson-Hasselbalch equation for 25°C, pH 7.5 and the ionic concentration of seawater (Cameron, 1986). Heavy metal toxicity was examined using $CuSO_4$ $5H_2O$ and $CdCl_2$. Copper and cadmium are 25.4% and 61.3% of the mass of their respective molecules and these proportions were used to calculate the metal ion concentrations in test solutions. The range of copper tested was from 0.064 to 0.19 $mg \cdot l^{-1}$ and cadmium from 40 to 80 $mg \cdot l^{-1}$ in definitive tests.

Rotifer acute toxicity testing began with a range-finding test, then at least six replicate definitive tests were completed. No more than two LC_{50} values for a compound were determined on the same day and the experiments spanned over three months. Consequently, the data reported are a good estimate of the mean and

variance to be expected from rotifer acute toxicity tests in a single laboratory. LC_{50} values were calculated by linear regression of log toxin concentration on mortality probit values using Statview 512™ on a Macintosh Plus computer. Only regressions yielding significant F values were used to calculate LC_{50} values.

Comparative toxicity

As can be expected from the different modes of action of the compounds, *B. plicatilis* displayed a range of sensitivities to the toxicants tested (Table I). LC_{50} values ranged from 0.12 $mg \cdot l^{-1}$ for copper to 59.5 $mg \cdot l^{-1}$ for malathion, rankings from most to least toxic being copper > NaPCP > SDS > NH_3 > cadmium > malathion. In comparison to other test species currently used in aquatic toxicology such as freshwater *Daphnia magna* (DM) or *D. pulex* (DP), the estuarine mysid shrimp *Mysidopsis bahia* (MB), and the euryhaline anostracan *Artemia salina* (AS), *B. plicatilis* (BP) is in some cases more sensitive and in others less sensitive, depending on the compound and species compared. As can be seen in Table I, the LC_{50} ratios of BP to either DM or DP can range as low as 0.11 for free ammonia to 30000–60000 for the organophosphate pesticide malathion (to which crustaceans and insects are extremely sensitive). For the two heavy metals tested, it is interesting to note that whereas BP roughly displays the same high sensitivity to copper as DM and DP, the rotifer is 100 to 500 times more resistant to cadmium than the two daphnids. BP, in contrast, is 6 times more sensitive to cadmium than AS.

We compared the LC_{50} values of BP at 15 and 30 ppt salinity by an analysis of variance. No significant differences in LC_{50} values were detected between these salinities for NaPCP, copper and cadmium. LC_{50} values at 30 ppt were 18.5% lower for SDS ($F = 33.8$, $p < .01$), 13.2% lower for free NH_3 ($F = 11.5$, $p < .01$), and 23.5% lower for malathion ($F = 20.7$, $p < .01$). An increased sensitivity at higher salinities suggests that osmoregulation plays a role in determining the effect of exposure to toxicants, but this must only be regarded as an hypothesis that bears further investigation.

NOEC values calculated ranged from 0.02 for copper to 36.2 $mg \cdot l^{-1}$ for cadmium (Table I). For several compounds NOEC levels were quite close to the LC_{50}, NOEC/LC_{50} ratios being as high as 0.8. Only for the lowest LC_{50} (copper) was the NOEC/LC_{50} ratio lower than 0.2.

Test precision

The precision (repeatability) of acute toxicity tests using aquatic animals and conducted in single laboratories was reported by the USEPA (1985). For the reference toxicants SDS, NaPCP and cadmium, the average coefficients of variation for fathead minnows, *D. pulex* and *D. magna* were 49.3%, 33.3% and 37.0%, respectively. The average *B. plicatilis* coefficient of variation for these same toxicants is 6.6%, indicating that the single laboratory precision of the rotifer test is 5–6 times better that of *Daphnia*. The repeatability of the *B. plicatilis* test for SDS is also two

TABLE I

Mean *B. plicatilis* LC_{50} values (X) for six chemicals and comparative sensitivities with other species.

		BP 15 ppt		BP 30 ppt		DM	DP	MB	AS		
Compound		LC_{50}	NOEC	LC_{50}	NOEC	LC_{50}	LC_{50}	LC_{50}	LC_{50}	BP/DM	BP/DP
NaPCP	X	1.36	1.0	1.36	1.0	0.50[2]	0.52[2]	–	0.51[7]	2.72	2.62
	CV	7.4%	22.6%	10.4%	26.8%	32.1%	33.5%				
	95% CL	1.2–1.5		1.0–1.7							
	N	7	7	6	6						
SDS	X	5.42	4.47	4.42	2.58	17.4[2]	15.02[2]	7–18.3[6]	19.1[7]	0.31	0.36
	CV	6.4%	12.8%	5.4%	79.7%	39.6%	36.5%				
	95% CL	5.0–5.8		3.8–5.1							
	N	6	6	6	6						
Free NH_3	X	20.4	11.5	17.7	15.3	189[1]	187[1]	3–4[5]	–	0.11	0.11
	CV	10.8%	62.4%	1.6%	6.3%	–	–	–			
	95% CL	15.2–25.6		17–18.4							
	N	8	8	8	8						
Malathion	X	59.5	11.4	45.5	38.5	0.001[3]	0.002[3]	–	–	59,000	30,000
	CV	9.1%	171%	11.8%	12.3%	–	–				
	95% CL	54.3–64.7		41.9–49.3							
	N	7	7	10	10						
Copper	X	0.12	0.02	0.13	0.05	0.05[1]	0.05[1]	0.18[4]	4.9[7]	2.22	2.26
	CV	2.9%	138%	18.8%	81.9%	–	–	–			
	95% CL	0.11–0.13		0.07–0.19							
	N	8	8	6	6						
Cadmium	X	54.9	36.3	56.8	46.7	0.13[2]	0.48[2]	0.11[4]	–	422	114
	CV	6.0%	19.2%	5.1%	17.5%	81.8%	45.0%	–			
	95% CL	52–58		51.3–62.3							
	N	8	8	6	6						

Table entries are LC_{50} or NOEC values in $mg \cdot l^{-1}$. CV is the coefficient of variation, CL the confidence limits and N the number of replicates. The ratios BP/DM and BP/DP indicate the difference in sensitivity of these species to the chemicals. BP, *B. plicatilis* DM- *D. magna*, DP- *D. pulex* MB- *M. bahia*, AS- *A. salina* A11 LC_{50}s are 24 h tests' except MB which is 96 h.

[1] Mount & Norberg 1984.

times better than the standard *Artemia* nauplii bioassay which has a coeficient of 14.5% (Vanhaecke and Persoone, 1987). The multi-laboratory reproducibility of the rotifer test needs to be determined, but it is likely that this precision also will equal or surpass that of existing acute aquatic toxicity tests.

DISCUSSION

Interest is growing in rotifes as animals for aquatic toxicity testing (Halbach, 1984). Over the past several years, the response of rotifers to a variety of toxicants has been characterized in both natural and laboratory populations. The effect of various insecticides and herbicides on natural rotifer populations has been investigated (Hurlbert, et al. 1972; Martin and Yeates, 1975; Kaushik et al., 1985; Richard et al., 1985; Stephenson, et al., 1986), as well as copper (Kallqvist and Meadows, 1978) and wastewater (Toman and Rejic, 1985). Sladecek (1983) has suggested that rotifers serve as general indicators of water quality. Three studies have examined the uptake of toxicants by rotifers, chlorinated hydrocarbons by Scura and Thielacker (1977), benzene by Echeverria (1980) and selenium by Bennett et al. (1986). The use of population dynamics in laboratory populations as sensitive indicators of toxicity has been promoted by Halbach (Halbach, et al., 1981; Halbach, et al., 1983; Halbach, 1984), with others following this lead (Hirata, et al., 1984; Rao and Sarma, 1986). A multispecies approach to toxicity assessment with a laboratory microcosm consisting primarily of rotifers has also been attempted (Jenkins and Buikema, 1985). Several short-term acute toxicity tests with a variety of substances have been conducted using rotifers: insecticides (Dad and Kant Pandya, 1982; Serrano, et al., 1986), heavy metals (Schaffer and Pipes, 1973; Buikema, et al., 1974; Borgmann and Ralph, 1984; Gvozdov, 1986; Couillard, et al., 1987; Couillard, et al., 1988), crude oil and petrochemicals (Erben, 1978; Rogerson et al., 1982), free chlorine and chloramines (Capuzzo, 1979a & b), free ammonia (Yu and Hirayama, 1986), sodium dodecyl sulfate (Persoone, et al., 1987), and diterpenoid toxins from octocorals (Yuh Lee and Macko, 1981). Methodology varied considerably among these short-term toxicity studies in the species used, test animal age, physiological condition, strain, and duration of exposure. The results are therefore not directly comparable among themselves or with the *B. plicatilis* test that we propose. The contribution of the present paper is that it provides a mechanism for standardizing rotifer bioassay protocols by using cysts as the source of test animals.

Several median lethal concentrations have been reported in the literature for *B. plicatilis*, revealing a range of sensitivities to different toxicants. Capuzzo (1979a) found *B. plicatilis* to be one of the most sensitive of 7 marine organisms to free chlorine and chloramine. After a 30 min exposure at 25°C, LC_{50} values of 0.09 and <0.01 mg·l^{-1} were recorded for chlorine and chloramine, respectively, and are still among the lowest *B. plicatilis* LC_{50} values reported for any chemicals. Gvozdov (1986) found the 24 h LC_{50} values for *B. plicatilis* exposed to $HgCl_2$, $CuSO_4$ and

$CdSO_4$ to be 0.045, 0.40 and 35.0 mg · l^{-1}, respectively. These data support our findings that *B. plicatilis* is two orders of magnitude more sensitive to copper than to cadmium. Yu and Hirayama (1986) reported a 24 h LC_{50} for un-ionized ammonia of 17.0 mg · l^{-1} at 23°C and 23 ppt salinity which is quite similar to our values of 20.4 and 17.7 at 15 and 30 ppt, respectively. The 24 h LC_{50} values for five organophosphate and one organochlorine pesticides ranged from 0.9 to 150 mg · l^{-1} for three different *B. plicatilis* strains (Serrano et al., 1986). These authors observed that pesticide resistance of this rotifer species is about 1000 times greater than that reported for other aquatic organisms. They further found a correlation between a strain's osmoregulatory capability and its pesticide resistance. The malathion LC_{50} and 59.5 mg · l^{-1} for our *B. plicatilis* strain also supports the idea that rotifers are generally resistant to organophosphate and organochlorine pesticides. Persoone, et al. (1987) investigated how salinity and temperature affects *B. plicatilis*' resistance to potassium dichromate and sodium dodecyl sulfate (SDS). Potassium dichromate LC_{50} values decrease as temperature increases from 10 to 31°C, but *P. plicatilis* is quite resistant with LC_{50} values ranging from 130 to 690 mg · l^{-1}. LC_{50} values increase as salinity increases from 5 to 45 ppt, but at 65 ppt lower LC_{50} values were recorded. With SDS, lower LC_{50} values were also recorded at higher temperatures and LC_{50} values decreased with salinity from 5 to 45 ppt. These data indicate that *B. plicatilis* sensitivity to chromium and SDS varies with environmental conditions, resistance generally being higher at lower temperatures. The SDS LC_{50} value of 5.42 mg · l^{-1} that we found for *B. plicatilis* is considerable lower than those reported by Persoone et al. (1987) which ranged from 10.9 to 29.4 mg · l^{-1}. We do not have a clear explanation for this difference except that the protocols for the two toxicity tests were somewhat different and the chemicals of different origin. This latter fact underscores the necessity of standardizing routine toxicity protocols for comparison of data.

While the protocol set forth here establishes a standardized procedure for acute toxicity testing with *B. plicatilis*, rotifer bioassays would benefit by further investigation into a variety of areas. The multiple laboratory precision of the *B. plicatilis* test needs to be evaluated by round robin testing. The effect of environmental conditions on rotifer sensitivity to a wider variety of toxicants is needed. The role of osmoregulation in rotifer sensitivity to chemicals needs clarification and any work on the detoxification capability of rotifers would be helpful. More compounds need to be assayed for comparative analysis of LC_{50} values especially heavy metals where the range of toxicity is several hundred fold. Nothing is known about the interactive effects of mixtures of toxicants on rotifers, although this more closely reflects the real world. Finally, a chronic test for rotifers needs to be devised so that the effects of long-term, low level exposure can be compared to acute effects. Rotifers have been grown in chemostats (Droop and Scott, 1978; Boraas, 1983; Rothhaupt, 1985), which provide a mechanism for both chronic exposure and continuous monitoring of toxicity.

In conclusion, we believe that the cyst-based *B. plicatilis* short-term for assessing acute toxicity in brackish and marine environments is a welcome addition to the standardized, cyst-based *Artemia* nauplii test (Vanhaecke and Persoone, 1981). Eliminating stock cultures as the source of test animals substantially improves the convenience, reliability and cost-effectiveness of aquatic invertebrate bioassays. The rotifer test has been shown to be rapid, sensitive and highly repeatable. Its ease of execution, even by inexperienced technicians, should make its adoption for routine toxicity screening attractive.

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