Bull. Environ. Contam. Toxicol. (1993) 50:646–652

Environmental Contamination and Toxicology

Toxicity Testing with Coastal Species of Southeastern Brazil. Echinoderm Sperm and Embryos

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Rapid chronic toxicity test methods with echinoderm sperm and embryos have been developed for species from the Northern hemisphere, but little has been done thus far with Southeastern Atlantic species. These methods are useful for a fast toxicity evaluation of liquid effluents that are disposed of in coastal environments, of seawater and sediments, among other purposes. This study presents the research conducted for the establishment of adequate toxicity testing conditions with Lytechinus variegatus Lamarck, 1816, a common echinoderm species on the South American coast and the Caribbean, distributed from Southern Brazil to North Carolina, USA. Sea urchin sperm and embryos were chosen primarily because of their reported sensitivity to chemicals and suitability for bioassay testing (Wells 1984; Dinnel et al. 1988; Weber et al. 1988). Among possible test organisms, the sea urchin Lytechinus variegatus was selected because of its abundance on the coast of the State of São Paulo, and because it is fertile year round. Toxicity tests were conducted with sodium dodecyl sulfate and zinc.

MATERIALS AND METHODS

Temperatures throughout the tests were 25±2° C, which is within the annual range for the São Sebastião Channel where adult sea urchins were collected. Experiments were conducted in constant temperature chambers, with a 12hr light-12hr dark photoperiod for the embryo tests, whereas the sperm tests were run under constant illumination. The acclimation and experimental salinity was 33.5±1.5°/oo.

The test solutions, following a log scale concentration, were prepared in volumetric flasks, and

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then transfered to the test tubes which were randomized prior to beginning the test. Five tubes were prepared for each toxicant concentration and for controls. Initial 100mg/L stock solutions of sodium dodecyl sulfate (SDS) or zinc (as $ZnSO_4.7H_2O$) were prepared in seawater or distilled water, respectively, and afterwards sequentially diluted in 2µm filtered seawater used as dilution water. The salinity of the highest concentration was monitored to maintain it at not more than 1 °/oo lower than the control. The concentrations referred to herein are the nominal toxicant concentrations, and may not directly reflect their activity in seawater.

Individual batches of adult L. variegatus were collected by free diving in shallow areas of the São Sebastião Channel shortly before the test, kept in flowing seawater in the laboratory until use, and supplied macroalga from their collection site as food.

The urchins were spawned by the injection of 5mL 0.5M potassium chloride through the peristomal membrane into the coelomic cavity. Eggs were collected in 400mL beakers containing dilution water, and sperm was collected with a Pasteur pipette and kept on ice until the test was initiated. Each egg batch was observed under a microscope and those which had abnormalities or were overripe were discarded. The selected egg batches were then filtered through a relatively coarse screen to remove debris, and pooled together in a beaker containing 600mL seawater. Eggs were washed three times by decantation, removal of the supernatant, and addition of fresh dilution water before the tests. Pooled sperm and eggs from at least three males and three females were used. A subsample of the egg solution was diluted 100 times and counted under a microscope in a Sedgwick-Rafter cell. The solution volume added to the test tubes for each experiment was calculated according to the desired number of organisms required per vial. This volume did not exceed 100µL. A standard sperm solution was prepared by adding 0.5mL of sperm to 24.5mL of dilution water.

The sperm cell test using L. variegatus is a 60-minute short-term chronic test run under static conditions, and evaluating sperm viability after the exposure period. It is performed in test tubes containing 5mL of test solution. Five replicates were prepared per concentration. To start the test, 100µL of standard sperm solution were added to each tube containing the toxicant solutions, at 15 second intervals. This amount of sperm solution, instead of a standard amount of sperm per egg, was adopted because the minimum amount of sperm causing 70% fertilization in the

control varied greatly in tests with different sperm batches. Thus we decided to use an excess of sperm, despite the consequent decrease in sensitivity. After 60 minutes of exposure, 1000 eggs (200 eggs/mL) were added to each vial, also at 15 second intervals, and maintaining the same order. The test was ended 20 minutes later, by adding the tube contents into a 6-mL glass flask containing 0.1mL of borax-buffered formalin. A 100-times diluted subsample of the standard sperm solution was preserved with borax-buffered formalin and counted for evaluation of the sperm amount per egg. The lowest ratio used was 18,700 sperm/egg, and the highest, 58,200. Test sensitivity did not show significant variation due to the different sperm amounts. Fertilization rate in a 100-egg subsample per replicate was analyzed under a microscope. Sperm viability was evaluated by its ability to fertilize the eggs at the end of the exposure period. Tests were disregarded if the control fertilization rate was lower than 70%.

While Dinnel et al. (1982) considered eggs unfertilized if they had partially formed membranes, we considered them fertilized since the test endpoint is the evaluation of sperm viability. Partial membranes indicated the existence of enough viable sperm to fertilize the eggs, in spite of the eggs' inability to raise the whole fertilization membrane.

The embryo test using L. variegatus is a 24-hr short-term chronic test, evaluating the retardation of embryonic development during the exposure period. It was conducted under static conditions, performed in test tubes containing 10mL of solution and 300 fertilized eggs, with five replicates per concentration. Egg fertilization was promoted by the addition of one to two mL standard sperm solution to a beaker containing egg solution. A 100-times diluted subsample of this solution was counted after ten minutes to evaluate fertilization success. The solution was only used if at least 80% of the eggs were fertilized. The solution amount containing 300 fertilized eggs was calculated. This volume was added to the test vials (30 eggs/mL), at 15 second intervals. The test was ended after 24 hours, the minimum time necessary to reach the pluteus stage, under test conditions. Each tube's content was added to 12-mL glass vials containing 0.5mL of borax-buffered formalin.

The developmental stage of the control organisms, pluteus larvae, was later observed under the microscope and compared to the embryos in the toxicant concentrations. The number of embryos showing retarded

or abnormal development was recorded. Tests were disregarded when more than 20% of the embryos in the control vials did not reach the pluteus stage.

The test results were expressed as EC50, instead of NOEC and LOEC, because the purpose of this study was methods development, and establishment of sensitivity to reference toxicants and analysis of variability of the test results. EC50 values suited this purpose, since they are obtained through dose-effect curves which allow the calculation of the coefficient of variability among tests (CV). The EC values were calculated by the trimmed Spearman-Karber method (Hamilton et al. 1977).

RESULTS AND DISCUSSION

Several tests were run with the toxicants, and the individual results are presented in Table 1. The data show a similar sensitivity of both test types to SDS, as well as to zinc.

Table 1. SDS and zinc 60-min and 24-hr EC50 for L. variegatus sperm and embryos, respectively. Confidence intervals in brackets.

	SDS (mg/L)		Zinc (mg/L)	
	Test nº	EC50 (CI)	Test nº	EC50 (CI)
Sperm	1	3.06 (2.94-3.17)	1	0.078 (0.07-0.09)
	2	3.03 (2.92-3.15)	2	0.130 (0.12-0.14)
	3	2.33 (2.28-2.38)	3	0.046 (0.04-0.05)
	4	3.61 (3.51-3.72)	4	0.047 (0.04-0.05)
	5	2.60 (NC)	5	0.050 (NC)
	6	1.94 (1.92-1.96)	6	0.054 (0.03-0.09)
	7	3.46 (3.33-3.59)		
Embryo	1	1.96 (1.92-2.00)	1	0.065 (0.06-0.07)
	2	3.52 (3.45-3.60)	2	0.130 (0.11-0.14)
			3	0.040 (0.03-0.05)
			4	0.061 (NC)

NC=not calculable

The mean EC50 values, their coefficient of variation (CV%), together with reference to other cited data for different urchin species sensitivity are presented in Table 2. The sensitivity of L. variegatus sperm and embryos to SDS and zinc is in the same range of that of other species from the Northern hemisphere, in spite of temperature and time variations. These factors did not seem to considerably affect the toxicants' effect on different echinoderm species. The different exposure times for embryo tests must have been due to slower development at lower temperatures, which reduces

Table 2. SDS and zinc toxicity test results for different echinoid species.

Sp[a]	T°C	S°/oo	Test time (hr)	EC50 (mg/L)	CV%	References
				SDS		
Sperm tests						
LV	25±2	32-35	1	2.9	21.2	this study
AP	20±1	30±2	1	3.0	30.6	Weber et al. 1988
AP	20±1	30±2	1	---	26.1	Schimmel et al. 1989
Embryo tests						
LV	25±2	32-35	24	2.7	40.3	this study
				ZINC		
Sperm tests						
LV	25±2	32-35	1	0.068	48.6	this study
SP	12	30	1	0.262*	----	Dinnel et al. 1989
SD	12	30	1	0.383*	----	Dinnel et al. 1989
SF	12	30	1	0.313*	----	Dinnel et al. 1989
DE	12	30	1	0.028*	----	Dinnel et al. 1989
Embryo tests						
LV	25±2	32-35	24	0.074	52.6	this study
SP	8.2-8.4	30	96	0.023*	----	Dinnel et al. 1989
SD	8.2-8.4	30	96	>0.027,<0.051*		Dinnel et al. 1989
DE	12.5-13	30	96	>0.58,<0.82*		Dinnel et al. 1989

[a]AP=Arbacia punctulata; DE=Dendraster excentricus; LV=Lytechinus variegatus; SD=Strongylocentrotus droebachiensis; SF=S. franciscanus; SP=S. purpuratus
*No CV% reported

metabolic rates and, perhaps, the toxic action of chemicals, therefore making data comparable. The CV% of L. variegatus sperm tests with SDS was lower than some reported by other individuals for other species (Table 2).

Schimmel et al. (1989) obtained CV% values of 26.1 for urchin sperm tests with SDS, and 55.9 for copper sulfate, in an analysis of the results of a minimum of five replicate toxicity tests in an intralaboratory comparison. Based on these results, they considered that the methods provided highly reproducible toxicity data. The EC50 data presented by Weber et al. (1988) for five Arbacia punctulata sperm tests with SDS and five with copper were used to calculate their CV%. The results showed values of 30.6 and 48.0, respectively. These variability data were higher or equal to those observed in the present study for L. variegatus sperm tests with SDS and zinc.

The sensitivity of L. variegatus sperm to zinc was

similar to that of embryos in 24-hr tests, and one order of magnitude higher than the sensitivity of acute tests with the copepod Acartia lilljeborgi and the mysid Mysidopsis juniae (Nipper et al in press). Similar results were observed by Dinnel et al. (1982) for silver and endosulfan on sea urchin and sand dollar sperm and embryos, when compared to each other and to many acute tests with other aquatic animals. The different test endpoints, however, must be kept in mind, i.e., sublethal for urchin sperm and embryos, and lethal for the other species. On the other hand, tests with L. variegatus sperm and embryos, juveniles of M. juniae, and adults of the copepods A. lilljeborgi and Temora stylifera showed similar sensitivity to SDS (Nipper et al. in press).

The data show that the urchin tests may serve the purpose of a rapid screening for chronic toxicity evaluation of different kinds of samples.

Acknowledgments. We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico for the fellowships granted to VA Prósperi and AJ Zamboni, and the staff of the Centro de Biologia Marinha-USP, where this work was developed.

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Received May 21, 1992; accepted December 2, 1992.