Bull. Environ. Contam. Toxicol. (1993) 51:99–106

Environmental Contamination and Toxicology

Toxicity Testing with Coastal Species of Southeastern Brazil. Mysids and Copepods

M. G. Nipper, C. Badaró-Pedroso, V. F. José, and S. L. R. Melo

CETESB (Companhia de Tecnologia de Saneamento Ambiental), Av. Prof. Frederico Hermann Jr., 345, CEP 05489-900 São Paulo, SP, Brazil

Acute toxicity test methods for a variety of freshwater organisms are well developed in Brazil, and are routinely used in industrial effluent quality control in the State of São Paulo. Very little has been done, however, with coastal marine organisms. This study presents the results of research conducted to select suitable species for toxicity testing of marine and estuarine samples, and to establish adequate bioassay conditions. Mysids and copepods were chosen primarily because of their sensitivity to chemicals, suitability for this kind of testing and their eurytopic responses to salinity and temperature. The copepods Acartia lilljeborgi and Temora stylifera and the mysid Mysidopsis juniae were selected as test species, due to their abundance on the coast of the State of São Paulo.

MATERIALS AND METHODS

Temperatures and salinities throughout the acclimation period were 25±2° C and 33.5±1.5°/oo, respectively, which are within the annual range for the São Sebastião Channel where the organisms were collected. Mortality during the acclimation period usually did not exceed 10% for mysids and 15% for copepods.

Toxicity tests were conducted in constant temperature chambers at 25±2° C, with a 12hr light-12hr dark photoperiod. For salinity tolerance tests, with and without toxicants, the animals were transfered directly from the acclimation dishes, at 33.5±1.5°/oo, to the test salinities, which were obtained either by dilution of seawater with distilled water or by addition of highly concentrated brine obtained by freezing seawater

Research conducted at the Centro de Biologia Marinha da Universidade de São Paulo, São Sebastião, SP, Brazil.

Send reprint requests to Marion G. Nipper at the above address.

and retaining the first thawed fraction after its removal from the freezer.

The test solutions, with concentrations following a log scale, were prepared directly in the test dishes for copepod experiments, and in volumetric flasks, with later transference to the test chambers, for mysids. 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 seawater. The salinity of the highest test concentration was monitored to maintain it at no more than $1^{o}/oo$ lower than the control. The concentrations referred to herein are the nominal toxicant concentrations.

Test chambers, as well as mysids and copepods, were randomized prior to beginning the test. The animals were pipetted individually from the acclimation dishes and distributed to each cup, until all the cups had 10 organisms; these were then transfered to the test chambers. Dead organisms were counted under a binocular microscope and removed every 24 hours. Mysids were considered dead when they did not show any appendage movement, and copepods, when they did not move within 30 seconds after being gently touched with a glass rod. Tests were terminated after 48 hours for copepods and 96 hours for mysids. The minimum required control survival rate, to determine test acceptability, was 90% for mysids and 80% for copepods, since the latter seem to be more sensitive to collection and handling.

Tests with M. juniae were 96-hr acute static tests, evaluating mortality after the exposure period. Different tests were performed with animals of different ages, from one day old to adult, to determine age-dependent toxicant sensitivity. Tests with adults involved males only. While the young animals were grown from birth in the laboratory, the adults were collected by hauling a 500µm-mesh net along the sea bottom, at depths of 50 to 100cm. The adults were acclimated for three days to laboratory conditions before use. To obtain juveniles, field-collected females with embryos were kept in 13-L brood chambers for 24 hours, at a density of no more than 20 females per liter. After this period the newborn were harvested, so that only juveniles released within a 24hr period were used. They were then counted and raised for the desired number of days in 13-L aquaria containing 20 animals per liter. Thirty recently hatched brine shrimp nauplii per mysid were added daily as food. Tests were run in 400mL beakers containing 200mL of test solution and 10 mysids, with three

replicates per toxicant concentration. The randomized animals were carefully transfered to the beakers with a minimum amount of water (less than 4mL), so as to not significantly change the toxicant concentrations. As food, 300 brine shrimp nauplii were introduced daily into each chamber.

A. lilljeborgi or T. stylifera were used in 48-hr acute static tests, evaluating mortality after the exposure period. Adult copepods were exposed in 60mm in diameter x 30mm deep dishes containing 50mL of test solution, with three replicates per toxicant concentration. Copepods were obtained from plankton hauls, usually at depths of 15 to 25m, in the vicinity of the laboratory. Adult organisms of the desired species were sorted under a binocular microscope from freshly collected samples and acclimated for 24 hours to laboratory conditions in 3-L crystalizing dishes containing no more than 150 copepods per liter. No attempts were made to identify the exact life stage (instar) of the organisms, and adults were selected by their approximate size. They were fed 2×10^4 Tetraselmis gracilis cells per mL. The algae were cultured in f/2 Guillard medium, centrifuged, and then resuspended in seawater before use. To initiate the tests, the randomized copepods with 10mL of seawater were carefully transfered to the test dishes containing the necessary volumes of dilution water and toxicant stock solution, so that the desired final test concentrations would be reached. Food (Tetraselmis gracilis) was added at the beginning of the test.

The LC50 values for mysid and copepod tests were calculated by the trimmed Spearman-Karber method (Hamilton et al. 1977), except for zinc toxicity tests with reduced salinities using copepods. In that case, the data distribution was not suitable for the analysis, and the LC50 values were derived using the probit method.

RESULTS AND DISCUSSION

The lowest survival rate (mean of three replicates) for M. juniae at salinities ranging from 24 to 45 °/oo was 92%, dropping to 84% at 21 °/oo and to 0% at 18 °/oo (Table 1). A. lilljeborgi presented at least 86% survival in salinities above 24 °/oo. T. stylifera had a higher control survival variability in the different salinities, indicating a higher sensitivity of the species to this environmental factor variation, or perhaps to stress due to collection and handling in the laboratory (Table 1).

The effect of zinc on copepods did not vary

Table 1. Effect of zinc (48-hr LC50, in mg/L) on copepods at reduced salinities, with 95% confidence intervals in brackets.

S^{o}/oo	A. lilljeborgi		T. stylifera		Mj*
	LC50(CI)	%S	LC50(CI)	%S	%S
18	NT	70	NT	NT	0
21	NT	90	NT	70	84
23	0.80(0.31-0.96)	76	0.004(0.0-0.018)	76	NT
24	NT	90	NT	83	100
25	0.70(0.01-0.94)	86	0.030(0.023-0.035)	86	NT
27	NT	93	NT	67	96
28	0.55(0.0-0.86)	90	0.023(0.004-0.036)	83	NT
30	NT	87	NT	80	100
32	0.89(0.60-1.020)	90	0.031(0.010-0.046)	97	NT
34	NT	NT	NT	NT	92
37	NT	NT	NT	NT	96
40	NT	NT	NT	NT	92
45	NT	NT	NT	NT	96

*Mj=Mysidopsis juniae; NT=not tested; S^{o}/oo=salinity; %S=%survival of copepods (48-hr) and mysids (96-hr) in non contaminated water.

significantly at salinities between 25 and 32^{o}/oo (Table 1). At 23^{o}/oo salinity the control survival rate for both species was lower than the acceptable value for toxicity tests, but the zinc LC50 for A. lilljeborgi was not significantly affected by this salinity reduction. Data were considered significantly different if the confidence intervals did not overlap. Zinc toxicity was considerably greater to T. stylifera in 23^{o}/oo than in higher salinities, showing an increased deleterious effect of the toxicant. The influence of salinity variation on zinc toxicity to M. juniae was not tested.

It has been reported by different authors that increased sensitivity of crustaceans to metals with salinity decrease may be due to any of the following factors: interaction between metal and salts in seawater, forming a nontoxic precipitate, or causing complexation of the metal by chloride, in proportion to the salinity; direction of the osmotic gradient in higher salinities; disruption of the osmoregulatory mechanism. In the present study zinc toxicity changed with salinity for only one of the test species, so that we would expect it to be related primarily with changes in osmoregulatory ability, depending on each species' physiological characteristics.

At salinities of 33.5 ± 1.5^{o}/oo, SDS and zinc were similar in toxicity to M. juniae of different ages, as SDS was to adult A. lilljeborgi and T. stylifera as well.

Table 2. Effect of SDS and zinc (LC50 values) on M. juniae (MJ) at different ages (days), and on adult A. lilljeborgi (AL) and T. stylifera (TS). 95% confidence interval in brackets.

Sp.	SDS(mg/L) Test nº	Age	LC50(CI)	Zinc(mg/L) Test nº	Age	LC50(CI)
MJ	1	4d	2.2(2.0-2.4)	1	1d	0.35(0.32-0.38)
	2	10d	2.3(2.1-2.5)	2	1d	0.34(0.30-0.40)
	3	adult	2.3(2.1-2.5)	3	2d	0.37(NC)
				4	3d	0.36(0.31-0.41)
				5	3d	0.38(0.36-0.40)
				6	5d	0.35(0.31-0.39)
AL	1	adult	2.6(1.7-3.9)	1	adult	0.32(0.11-0.94)
	2	"	1.8(1.0-2.8)	2	"	0.42(0.31-0.56)
	3	"	1.4(1.0-2.0)			
TS	1	adult	2.64(2.21-3.15)	1	adult	0.031(0.01-0.046)
	2	"	3.00(1.80-5.20)	2	"	0.040(0.02-0.080)
	3	"	2.57(2.19-2.99)	3	"	0.014(0.005-0.04)
	4	"	2.31(1.91-2.70)	4	"	0.090(0.06-0.120)

NC=not calculable

Adult T. stylifera were significantly more sensitive to zinc than either the mysid or A. lilljeborgi (Table 2).

The sensitivity of the North American west and east coast mysids, Holmesimysis costata and Mysidopsis bahia, respectively, to a variety of compounds, was not significantly affected by the age of the animals, 3 to 9 days old for the first species (Martin et al. 1989), and 1 to 10 days old for the second (Goodman et al. 1988). Our data show M. juniae as having a sensitivity to zinc and SDS within the range shown by North American Atlantic and Pacific species (Table 3), with no significant effect of age on the species sensitivity to these toxicants (Table 2).

The toxicity of zinc and SDS to a variety of mysids and copepods from the Northern hemisphere (Table 3) was generally similar to that at species occurring on the Brazilian coast, in spite of differences in specific test conditions which usually reflect the physiological needs of organisms inhabiting different regions. The toxicity to different species of the same group did not vary more than one order of magnitude, except for copepod tests with zinc. This might have been due to sensitivity variability among species, as was certainly the case for A. lilljeborgi and T. stylifera which were submitted to identical test conditions, or to test duration and temperature of experiments conducted by other individuals. However, neither the temperature nor

Table 3. SDS and zinc toxicity test results for different species of mysids and copepods.

Sp.[a]	T°C	S°/oo	Test time (hr)	LC50 (mg/L)	CV%	Reference
				SDS		
MJ	25±2	32-35	96	2.3	2.6	this study
MB	26-27	20-30	168	---	39.6	Schimmel et al.1989
MB	22±.5	20±.5	96	6.6	10.7	Roberts et al. 1982
NA	22±.5	20±.5	96	7.2	30.2	Roberts et al. 1982
TS	25±2	32-35	48	2.6	10.8	this study
AL	25±2	32-35	48	1.9	31.6	this study
AT	22	10	96	0.55	----	Roberts et al. 1982
EA	22	10	96	2.6	----	Roberts et al. 1982
				ZINC		
MJ	25±2	32-35	96	0.360	4.1	this study
MB	20-25	30±2	96	0.499	----	Lussier et al. 1985
HC	13-16	34-40	96	0.097	23.0	Martin et al. 1989
TS	25±2	32-35	48	0.044	74.7	this study
AL	25±2	32-35	48	0.370	19.1	this study
AS	17±1	35±.5	24	1.860	----	Arnott et al. 1979
PP	17±1	35±.5	24	1.380	----	Arnott et al. 1979
Ssp	17±1	35±.5	24	1.090	----	Arnott et al. 1979

[a]AL=*Acartia lilljeborgi*; AS=*A. simplex*; AT=*A. tonsa*; EA=*Eurytemora affinis*; HC=*Holmesimysis costata*; MB=*Mysidopsis bahia*; MJ=*M. juniae*; NA=*Neomysis americana*; PP=*Paracalanus parvus*; Ssp=*Scutellidium* sp; TS=*Temora stylifera*

time variations of tests conducted by others, nor specific differences, seemed to substantially affect the sensitivity of mysids to the studied toxicants.

A comparison of mean LC50 values and their coefficient of variation (CV%) with other referenced data for mysid and copepod tests with zinc and SDS (Table 3) shows that *M. juniae* had the lowest sensitivity variability among tests, for both toxicants, as represented by the low CV% values. The highest CV% value of 74.7% was obtained with zinc toxicity tests with *T. stylifera*.

The nutritional condition of the organisms and other factors, such as differences between ambient and experimental temperatures, may have affected the present results, since the copepods were collected on the day before the test. *Acartia tonsa* field-collected populations are reported to be significantly more sensitive to cadmium and copper than filial populations from laboratory stocks (Sosnowski and Gentile 1978).

Some animals may be more thermally stressed than

others, and in some cases the temperature acclimation may not be complete at the start of an experiment (Arnott and Ahsanullah 1979).

Coefficient of variation values reported by various authors testing different species of mysids and copepods, in inter and intra laboratory comparisons, involving many compounds, varied considerably. While Jop (1989) affirmed that toxicity tests with a reference toxicant shcuild achieve a coefficient of variation not greater than 40%, other authors have considered tests with CV% up to 64% reliable, with acceptable precision (Gentile et al. 1984; Schimmel et al. 1989). While most of the data herein presented show CV% within the range considered acceptable by Jop (1989), tests evaluating zinc toxicity to the copepod T. stylifera had a higher variability. The causes for it shall be analyzed in future research.

The data obtained in this study and reported in literature show that the estimation of copepod field populations' sensitivity, in comparison to laboratory populations, should help establish realistic toxicant disposal limits protective to populations from coastal environments. On the other hand, it is advisable to maintain laboratory populations to verify whether the variability among tests really depends on thermal history, nutritional state and age, or if it is natural for some species and categories of toxicants.

The LC50 values observed for copepods and mysids and their similarity to those obtained by others using related species show the suitability of the chosen species for toxicity testing with chemicals and possibly with other kinds of samples as, for instance, industrial effluents disposed of in coastal environments.

Acknowledgments. We thank the CNPq for the fellowships (process nº 824163/88-5) to VF José, C Badaró-Pedroso and SLR Melo, Drs TKS Bjornberg and JM Reis for the identification of copepods and mysids, Dr EA Aragão for the algal inoculum, and the Centro de Biologia Marinha-USP, for the help and use of facilities.

REFERENCES

Arnott GH, Ahsanullah M (1979) Acute toxicity of copper, cadmium and zinc to three species of marine copepod. Aust J Mar Freshwater Res 30:63-71

Gentile JH, Johns DM, Cardin JA, Heltsche JF (1984) Marine ecotoxicological testing with crustaceans. In: Persoone G, Jaspers E, Claus C (eds) Ecotoxicological Testing for the Marine Environment. State University

of Ghent and Institute for Marine Scientific Research, Bredene, Belgium, pp 479-502
Goodman LG, Cripe GM, Moody PH,Halsell DH (1988) Acute toxicity of malathion, tetrabromobisphenol-A, and tributyltin chloride to mysids (Mysidopsis bahia) of three ages. Bull Environ Contam Toxicol 41:746-753
Hamilton MA, Russo RC, Thurston RV (1977) Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ Sci Technol 11:714-719 Correction 12:417 (1978)
Jop KM (1989) Acute and rapid-chronic toxicity of hexavalent chromium to five marine species. In: Cowgill UM, Williams LR (eds) Aquatic Toxicology and Hazard Assessment: 12th Volume, ASTM STP 1027, American Society for Testing and Materials, Philadelphia, pp 251-260
Lussier SM, Gentile JH, Walker J (1985) Acute and chronic effects of heavy metals and cyanide on Mysidopsis bahia (Crustacea: Mysidacea). Aquat Toxicol 7:25-35
Martin M, Hunt JW, Anderson BS, Turpen SL, Palmer FH (1989) Experimental evaluation of the mysid Holmesimysis costata as a test organism for effluent toxicity testing. Environ Toxicol Chem 8:1003-1012
Roberts Jr MH, Warinner JE, Tsai C-F, Wright D, Cronin LE (1982) Comparison of estuarine species sensitivities to three toxicants. Arch Environ Contam Toxicol 11:681-692
Schimmel SC, Morrison GE, Heber MA (1989) Marine complex effluent toxicity program: test sensitivity, repeatability and relevance to receiving water toxicity. Environ Toxicol Chem 8:739-746
Sosnowski SL, Gentile JH (1978) Toxicological comparison of natural and cultured populations of Acartia tonsa to cadmium, copper, and mercury. J Fish Res Board Can 35:1366-1369

Received July 30, 1992; accepted January 15, 1993.