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Acute Toxicity of Malathion, Tetrabromobisphenol-A, and Tributyltin Chloride to Mysids (*Mysidopsis bahia*) of Three Ages

Larry R. Goodman,[1] Geraldine M. Cripe,[1] Paul H. Moody,[2] and Darrel G. Halsell[3]

[1]U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida 32561; [2]Computer Sciences Corp., c/o U.S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Florida 32561, and [3]Faculty of Biology, Division of Environmental Biology, University of West Florida, Pensacola, Florida 32514

In the approximately 10 yr since an estuarine mysid (Mysidopsis bahia) was recommended for use in toxicity testing (Nimmo et al. 1977), use of the species has been incorporated into several acute toxicity test protocols (US EPA/CE 1977; US EPA 1978; ASTM 1980; Reider 1985; US EPA 1985 a,b,c). Age of animals recommended at test initiation varies depending on the method. Borthwick (1978) recommended mysids $\leq$ 24-h-old. The Toxic Substances Control Act Test Guidelines (US EPA 1985a) recommended both <24-h-old and 5- to 6-d-old mysids be used in range-finding tests and that the most sensitive of the two ages should be used in the definitive test. One- to five-day-old mysids are recommended for effluent tests (US EPA 1985b) and 3- to 6-d-old mysids are recommended for drilling fluids tests (US EPA 1985c). Immature organisms are recommended in the ASTM (1980) acute method, and juveniles are recommended by Reider (1985). Since mysids emerge from the marsupium at an early juvenile stage (Mauchline 1980), and M. bahia attains sexual maturity in 10 to 12 days at 20 C in laboratory tests (Gentile et al. 1982), methods that recommend juveniles or immature animals allow considerably more latitude in age of test animals.

Little published comparative information is available on effect of age on acute sensitivity of M. bahia to toxic compounds. Nimmo et al. (1981) reported that in general, juveniles were more acutely sensitive to the pesticides they tested than were adults. However, diazinon and EPN were more toxic to adults than to juveniles but only by factors less than two or three. Schimmel (1981) reported results of static acute toxicity tests with 2-d-old and 6-to 8-d old M. bahia exposed to silver nitrate and endosulfan. Ninety-six-hour LC50 values for mysids exposed to silver nitrate were 181 µg/L for 2-d-olds and 203 µg/L for 6-to 8-d-olds; those for endosulfan were 0.29 µg/L for 2-d-olds and 0.24 µg/L for 6- to 8-d-olds.

The purpose of our studies was to obtain additional information on the relative sensitivity of M. bahia of different ages and to ob-

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Send reprint requests to L. Goodman at the above address (Gulf Breeze Contribution No. 598).

tain toxicity data on malathion [S-(1,2-dicarbethoxyethyl)-0, 0-dimethyldithiophosphate], tetrabromobisphenol-A [2,2-bis(3,5-dibromo-4-hydroxy phenyl) propane], and tributyltin chloride (TBTC). Malathion is an organophosphorous insecticide used for control of mosquitos and numerous other insects. In static tests with nominal malathion concentrations, 96-h LC50 values derived for three saltwater decapod crustaceans ranged from 33 to 83 µg/L (Eisler 1969). Tetrabromobisphenol-A (TBBPA) is a flame retardant used in the manufacture of epoxy and polyester resins and has been detected at 22 to 140 µg/kg (dry wt.) in river sediments in Japan (Watanabe et al. 1983). TBBPA has also been found in stream sediments near two unidentified manufacturing sites and in stream water near one of the sites (Zweidenger et al. 1979). Very little aquatic toxicity data are available for this compound. In 72-h tests with two marine algal species, average EC50 values for Skeletonema costatum tested in five growth media ranged from 0.09 to 0.89 mg TBBPA/L and those for Thalassiosira pseudonana tested in six growth media ranged from 0.13 to 1.0 mg TBBPA/L (Walsh et al. 1987). TBTC is a major speciation product of tributyltin oxide in seawater (Laughlin et al. 1986). Tributyltin oxide is used in antifouling paints to prevent growth of organisms on the hulls of boats and ships and is toxic to marine organisms with 48- to 96-h LC50 values $\leq$ 2.0 µg/L for several saltwater crustaceans and molluscs (Cardwell and Sheldon 1986).

MATERIALS AND METHODS

Mysids of three ages were exposed in the same aquaria during flow-through 96-h acute tests with each of the three test compounds. The age of mysids used were $\leq$ 1, 5(greater than four but less than or equal to five), and 10 (greater than nine but less than or equal to ten) days old at test initiation. They were obtained by isolating laboratory-reared females with young in their brood pouch and collecting young released over an interval $\leq$ 24 h on the appropriate day preceding test initiation. Juveniles were reared on the same food and at approximately the same salinity, temperature, and photoperiod as used during testing. Twenty mysids of each age were exposed per treatment, except that only 18 10-day-old mysids per treatment were used in the TBTC test. Mysids of each age group in each treatment were distributed equally between two exposure cups constructed by attaching a 12.5-cm-high tube of 363-µm nylon mesh to the inside walls of a 14 cm (I.D.) glass petri dish with silicone adhesive. The cups were placed in glass aquaria (100 x 31.5 x 22 cm high) equipped with self-starting overflow siphons that varied the water depth between 9.0 and 11.5 cm. Seawater for the toxicity tests was pumped from Santa Rosa Sound, Florida, through a sand filter and polypropylene filters ($\leq$ 20 µm) and diluted to a nominal 20 °/oo using freshwater from a chlorinated municipal supply. Prior to use in dilution of the seawater, the municipal freshwater was filtered, using both polypropylene filters ($\leq$ 20 µm) and charcoal filters, and aerated. The filtered and salinity adjusted seawater was further aerated and delivered to each aquarium at 13 L/h via a siphon from a constant-head trough. Aquaria were immersed in a freshwater bath

that maintained test water temperature at 25 ± 1 C. Mysids were fed live Artemia nauplii twice daily during testing. The photoperiod was 14L:10D.

Test material was diluted with solvent and infused into the seawater in a mixing tube located below each siphon using Harvard[4] syringe pumps (Harvard Apparatus Co., Inc., Millis, Massachusetts) equipped with Hamilton gas-tight syringes (Hamilton Co., Reno, Nevada) and Teflon® (registered trademark, Dupont, Corporation, Wilmington, Delaware) tubing. Carrier solvents were 32 µL triethylene glycol/L for the TBTC test and a mixture of 90% triethylene glycol and 10% acetone at 32 µL/L for the malathion test and at 96 µL/L for the TBBPA test. Control treatments with and without the carrier solvent were utilized in all tests. Within tests, all treatments except the seawater control received the same carrier solvent concentration.

Salinity of the seawater used in testing was monitored continuously and the pH and dissolved oxygen content of test water was determined at least twice during each toxicity test. Test conditions are summarized in Table 1.

Table 1. Test conditions during 96-h acute toxicity tests with three age groups of mysids (Mysidopsis bahia) exposed to malathion, tetrabromobisphenol-A (TBBPA) and tributyltin chloride (TBTC). Mean salinity was derived by averaging daily maximum and minimum values.

Test Compound	Salinity (°/oo)		pH (Range)	Dissolved Oxygen (mg/L)	
	Mean	Range		Mean	Range
Malathion	20.7	20.3-21.4	7.90-8.01	7.4	7.2-7.6
TBBPA	20.6	18.4-21.3	7.96-8.16	6.9	6.2-7.3
TBTC	19.9	19.0-22.3	7.98-8.01	6.8	6.7-7.0

Seawater samples (1 L) were taken from each treatment on 2 days during each toxicity test and analyzed to determine the concentration of test material. Samples for malathion analyses were extracted with two 100-mL portions of petroleum ether and the combined extracts were concentrated on a steam table to appropriate volumes for analysis with a gas chromatograph equipped with a nitrogen phosphorus detector and a glass column packed with 2% SP 2100 on Gas Chrom Q. Concentrations of malathion were determined

[4]Mention of trade names or commercial products does not constitute endorsement by the U.S. Environmental Protection Agency.

with standards obtained from EPA's pesticide repository. Average recovery of malathion fortified in seawater was 84% (n=3) for 50 µg malathion/L, and 103% (n=3) for 500 µg malathion/L. The detection limit was 0.10 µg malathion/L; malathion concentrations were not corrected for percentage recovery. The purity of the malathion used was 71.5%.

Seawater samples for TBBPA analyses were acidified with 4 mL of concentrated hydrochloric acid and extracted twice with 100 mL aliquots of petroleum ether. The extracts were combined and concentrated on a steam table to approximately 10 mL and, if necessary, further concentrated with a gentle stream of nitrogen at 35 C. The TBBPA (lot #051450, no purity listed) was obtained from Great Lakes Chemical Corp., West Lafayette, Indiana. The primary standard (1.0 mg/mL) was prepared in acetone; working standard (2.5 ng/µL) in hexane. Quantitation was performed with on-column capillary gas chromatography using an electron-capture detector. The response of the detector was linear from 0.50 ng to 5.0 ng ($r = 0.996$). The detection limit was 1.0 µg/L. Recovery of TBBPA fortified at 535 µg/L was 99%, at 445 µg/L was 104 $\pm$ 1.4% (n=2), and at 84 µg/L was 93 $\pm$ 11.5% (n=3). TBBPA concentrations were not corrected for percentage recovery.

Analyses for TBTC were performed according to the methods of Matthias and Bellama (1986) except that 20 mL of 4% aqueous sodium borohydride solution were added to each 1-L sample and samples were extracted with one 30-mL portion of methylene chloride followed by two extractions with 13 mL. Quantitations were performed with a tributyltin hydride (Organometallics, Inc., East Hampstead, New Hampshire) external standard method. Concentrations of tributyltin chloride were determined by correcting the quantity of tributyltin hydride as calculated from gas chromatographic analysis by adjusting the molecular weight from hydride to chloride, and correcting for percentage extraction efficiency using the overall average recovery of 68% (n=11) for samples fortified at 100 ng TBTC/L. The detection limit was 0.030 µg TBTC/L. The TBTC used (96% purity) was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin.

Mortality data were analyzed by the moving average method (Harris 1959), the binomial test (Siegel 1956), or the probit method with graphical analysis to estimate 96-h LC50 values and associated 95% confidence intervals (moving average method) or bounds (binomial method).

RESULTS AND DISCUSSION

The mysid age groups selected for testing encompassed the entire juvenile stage of *M. bahia*. At the end of the 96-h tests, those $\leq$ 1-d-old at test initiation were approximately 5-d-old, the initial 5-d-olds were 9-d-old, and the initial 10-d-olds were 14-d-old adults (young were in the brood pouches of some females). Survival of mysids in control treatments in all tests was $\geq$ 94%.

Malathion, TBBPA, and TBTC were acutely toxic to all three age groups of mysids at the concentrations tested (Table 2). However, all data sets were not suitable for analysis with the same statistical method.

Table 2. Ninety-six hour LC50 values, based on mean measured exposure concentrations, for three age groups of mysids (Mysidopsis bahia) exposed to malathion, tetrabromobisphenol-A (TBBPA), and tributyltin chloride (TBTC) in flowing seawater. Ninety-five percent confidence intervals (for moving average method) or bounds (for binomial method) are in parentheses.

Compound	96-h LC50 (µg/L)		
	≤ 1-d-old[a]	5-d-old[a]	10-d-old[a]
Malathion[b]	3.0 (2.6-4.0)	3.1 (2.8-3.5)	2.6 (2.3-2.9)
TBBPA	860[b] (670-1200)	1100[c]	1200[c]
TBTC[d]	1.1 (0.68-1.4)	2.0 (1.4-2.6)	2.2 (1.4-2.6)

[a] Age at beginning of test
[b] Moving Average method used in calculating LC50 values
[c] LC50 estimated using probit method with graphical analysis; 55% mortality of 5-d-old mysids and 45% mortality of 10-d-old mysids at measured 1150 µg/ℓ.
[d] Binomial method used in calculating LC50 values

Of the compounds tested, TBTC was more toxic, with 96-h LC50 values of 1.1 µg/L for ≤ 1-d-old mysids, 2.0 µg/L for 5-d-old mysids, and 2.2 µg/L for 10-d-old animals. Although the LC50 value for ≤ 1-d-old mysids is approximately 1/2 that for the two older groups, the confidence bounds overlap. Davidson et al. (1986) exposed juveniles of another genus of mysid (Acanthomysis sculpta) to tributyltin (TBT) leachate from an antifouling paint and obtained a 96-h LC50 of 0.42 µg TBT/L.

Sensitivities of the three age groups of mysids to malathion were the same statistically. The 96-h LC50 values (µg/L) were 3.0 for ≤ 1-d-olds, 3.1 for 5-d-olds, and 2.6 for 10-d-olds. The 95% confidence intervals for all three age groups overlapped. TBBPA was the least toxic of the three compounds tested, with 96-h LC50 values of 860 µg/L for ≤ 1-d-old mysids, 1100 µg/L for 5-d-old mysids and 1200 µg/L for 10-d olds (Table 2). The 95% confidence interval for the ≤ 1-d-olds encompassed the estimated LC50 value for the 5- and 10-d-old animals. Only 55% of the 5-d-old mysids

and 45% of the 10-d-olds died during exposure to 1150 µg/L, the highest concentration tested. Solubility problems were encountered in concentrations higher than those reported so further testing to obtain more definitive LC50 values for the 5- and 10-d-old age groups was not conducted.

Our data and that reported by Schimmel (1981) demonstrate that age was not a large factor in the acute sensitivity of juvenile M. bahia to the compounds tested. The 96-h LC50 values of 2- and 6- to 8-d-old mysids differed by factors $\leq$ 1.2 for endosulfan and silver nitrate (Schimmel 1981). In our tests with $\leq$ 1-, 5-, and 10-d-old mysids exposed to malathion and TBTC, 96-h LC50 values for the three age groups were within a factor of two and confidence intervals or bounds overlapped. Our data for TBBPA also suggest that the sensitivities of three age groups tested ($\leq$1-, 5- and 10-d-old) may be within a factor of approximately two. If it is necessary to to determine the acute toxicity of a particular compound to M. bahia at their most sensitive age, multiple age groups, including adults, must be tested. However, if toxicity to juveniles need only be determined within a factor of two or three, the age of juvenile M. bahia tested would appear to be relatively unimportant for representative chemicals from several classes of compounds.

We found no monitoring data for TBBPA from estuarine waters but TBTC is a known estuarine contaminant and potential for malathion contamination of estuarine waters exists through drift from insect control activities. From the studies reviewed, concentrations of TBTC in open estuarine waters are generally well below the 96-h LC50 values for M. bahia (1.1-2.2 µg/L). However, TBTC concentrations in some vessel mooring and repair facilities tend to be higher than in open waters (Grovhoug et al. 1986). Valkirs et al. (1986) reported mean TBTC concentrations that ranged from 0.24 to 0.79 µg/L in unfiltered seawater samples from a yacht basin in San Diego Bay, California. The highest mean concentration they reported is within the 95% confidence bounds (0.68-1.4 µg/L) of the 96-h LC50 for the most sensitive age group of M. bahia (Table 2). In field studies to investigate possible worst case accidental drift and over-spray of mosquito control pesticides, Tucker et al. (1987) reported peak malathion concentrations of 0.09 to 5.0 µg/L in estuarine ditches following ultra low volume applications by truck and by aircraft. Although the malathion concentrations of 5.0 µg/L was greater than our 96-h LC50 values for mysids (2.6-3.1 µg/L), the concentration decreased to less than 1 µg/L within approximately 8 h.

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