Bull. Environm. Contam. Toxicol. 24, 684-691 (1980)

Acute Toxicity of Priority Pollutants to Water Flea (*Daphnia magna*)

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Industrially used chemicals are potential sources of contamination of the aquatic environment either through normal usage, disposal, or accidental spillage. In order to properly evaluate the potential hazards of these materials to the aquatic environment, toxicity tests must be conducted with aquatic organisms. Data derived from toxicity tests are used by the United States Environmental Protection Agency to develop water quality criteria for such chemicals. The purpose of this study was to determine the acute toxicity of selected priority pollutants to the water flea (Daphnia magna). Water flea are commonly used organisms in aquatic toxicity tests, and their susceptibility to contaminants in the aquatic environment tends to be representative of freshwater zooplankton.

MATERIALS AND METHODS

All chemicals tested were purchased from commercial chemical suppliers and had a minimum purity of 80%. The chemicals were tested on an active ingredient basis and concentrations are reported as milligrams (mg) of test material per liter (L) of diluent water.

Daphnia magna (<24 hours old) used in these toxicity tests were from laboratory stocks cultured at EG&G, Bionomics. Water used to culture the organisms used in the initial 15 tests was deionized reconstituted well water having a total hardness of 72 ± 6 mg/L as $CaCO_3$ and a pH of 7.0 ± 0.2. Subsequently, culture water was reconstituted according to U.S. EPA (1975) to a total hardness of 173 ± 13 mg/L as $CaCO_3$ and a pH of 8.0 ± 0.2, to improve conditions for test organisms.

Procedures used in these acute toxicity tests were based on protocols in "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (U.S. EPA 1975). Diluent water used in these tests was of the same quality as previously described for water used to culture these animals. At the initiation of all tests, the dissolved oxygen concentration of diluent water was

0007-4861/80/0024-0684 $01.60

greater than 60% of saturation. Temperature of test solutions or mixtures was maintained at 22 ± 1°C.

Addition of the test material to diluent water varied according to the water solubility of the chemical. A stock solution of the chemical, in distilled water, was prepared and used to provide the desired concentrations for testing if the test material was sufficiently soluble in water. If insoluble at the desired water stock solution concentrations, a stock solution utilizing a co-solvent (triethylene glycol, ethanol, acetone or dimethylformamide, respectively, in order of preference) was prepared. If the chemical was not sufficiently soluble in either distilled water or a co-solvent for the preparation of a stock solution, then the chemical was added directly to diluent water. Five to 8 nominal concentrations of each chemical were tested.

Using one of the methods described above, the chemical to be tested was added to 500 mL of diluent water in 2-L jars to prepare each test solution. If the chemical was soluble in the diluent water, then the 500 mL volume of test solution was divided into three, 150 mL aliquots in 250-mL beakers to provide triplicate exposures. The remaining 50 mL of control, high, middle and low test concentrations were used to measure the 0-hour dissolved oxygen concentration and pH of the solutions. Five daphnids were randomly placed in each 150 mL test solution within 30 minutes of the solution preparation. If the test material was not soluble in the diluent water, the 500 mL test mixtures were not divided into triplicate test vessels but retained in the 2-L mixing jars. Fifteen daphnids were placed directly into the 2-L jars containing diluent water prior to addition of the test material. The tests were also conducted in unreplicated 500 mL solutions containing 15 daphnids if dividing the solution into triplicate test vessels presented a risk of the loss of the test substance through volatilization or if vapors of the substance posed a high health risk to the investigators. In addition, these vessels were covered with plastic wrap secured with an elastic band.

A negative control consisting of the same dilution water, test conditions, and test organisms, but containing no test substance or co-solvent, was maintained concurrently with each test. When appropriate, a positive (solvent) control was also established consisting of the same dilution water, conditions, and a number of test organisms as in the negative control and containing the highest concentration of the co-solvent present in any test vessel.

During these tests, the dissolved oxygen concentration, pH and temperature of test solutions were measured at the initiation and termination of the toxicity tests in the high, middle and low test concentrations and controls. These parameters were measured only at the end of an exposure if a potential loss of the test substance existed due to volatilization or unsolubilized test material adhering to instrument probes. Dissolved oxygen concentration and temperature were measured with a YSI Model 54BP dissolved oxygen meter and combination oxygen-temperature probe. The pH's were measured with an Instrumentation Laboratory pH meter. The total hardness determinations of diluent water were conducted according to APHA et al. (1975).

Observations of test populations were made at 24 and 48 hours of exposure and any mortalities were recorded.

Mortality data from these toxicity tests were used to calculate a median lethal concentration (LC50) and its 95% confidence limits based on the nominal concentrations to which water flea were exposed at the initiation of the test. The LC50's and 95% confidence limits were calculated utilizing a moving average angle method (Stephan, personal communication) when possible. With the moving average angle method, the nominal test concentrations were transformed to logarithms and the corresponding percentage mortalities to angles. Each group of three successive angles was then averaged and the LC50 was estimated by linear interpolation between the successive concentrations whose average angles bracketed 45 degrees. When test data did not meet the moving average angle method requirements, the LC50's were estimated by probit analysis by converting the concentrations to logarithms and percentage mortalities to probits and then calculating a least squares linear regression analysis. Finally, if the data did not permit a probit analysis, then a binomial probability analysis was performed on these data. Calculations were performed with a Hewlett-Packard Model 9815A calculator programmed to scan the data base and to select the most appropriate of the three methods described above for the calculation of an LC50. If no mortality was observed in water flea populations exposed to a nominal concentration of approximately 500 mg/L, no additional concentrations were tested and the LC50 was estimated to be greater than the highest nominal concentration tested (>530 mg/L). LC50's and 95% confidence limits were reported after rounding to 2 significant figures. No correction was made for control mortality.

RESULTS

Measurements of water quality characteristics revealed that dissolved oxygen concentrations ranged from 6.5-9.1 mg/L for all tests during the 48-hour exposure period. Within any one test, the greatest range observed was 6.6-8.1 mg/L. The range of pH values measured for tests conducted in water with a mean hardness of 72 mg/L as $CaCO_3$ was 6.7-8.1 units and in 173 mg/L hard water was 7.4-9.4 units.

A summary of the acute toxicity of the priority pollutants to *D. magna* is presented in Table 1. Mortality among water flea control populations never exceeded 10% in any test. Generally, the chlorinated phenols were more toxic than the chlorinated benzenes, chlorinated ethanes and nitrated phenols. Toxicity trends relating to the degree of chlorine substitution were not evident within any of the chemical groups tested.

TABLE 1

The acute toxicity of priority pollutants to water flea (*Daphnia magna*). Except as noted, all tests were conducted in diluent water with a mean hardness of 173 mg/L as $CaCO_3$.

Test Substance	LC50 (mg/L) 24-hour	LC50 (mg/L) 48-hour	No discernible effect conc. (mg/L)
chlorobenzene[a]	140 (110-180)[b]	86 (64-120)	10
1,2-dichlorobenzene[a]	2.4 (1.9-3.0)	2.4 (1.9-3.0)	0.36
1,3-dichlorobenzene[a]	48 (39-56)	28 (21-34)	6.0
1,4-dichlorobenzene	42 (17-98)	11 (6.6-19)	0.68
1,2,4-trichlorobenzene	110 (32-2,800)	50 (7.2-130)	<2.4
1,2,3,5-tetrachlorobenzene	18 (10-27)	9.7 (6.6-14)	<1.1

TABLE 1 - continued

Test Substance	LC50 (mg/L) 24-hour	LC50 (mg/L) 48-hour	No discernible effect conc. (mg/L)
1,2,4,5-tetrachlorobenzene	>530	>530	320
pentachlorobenzene	17	5.3	1.3
	(12-22)	(4.1-7.2)	
1,2-dichlorethane[a]	250	220	<68
	(190-320)	(160-280)	
1,1,1-trichlorethane[a]	>530	>530	530
1,1,2-trichlorethane	19	18	1.0
	(14-26)	(11-32)	
1,1,2,2-tetrachloroethane	18	9.3	<1.7
	(12-24)	(6.8-13)	
1,1,1,2-tetrachloroethane	27	24	<10
	(22-35)	(17-30)	
pentachloroethane[a]	63	63	46
	(57-68)	(57-68)	
hexachloroethane[a]	26	8.1	0.28
	(13-48)	(4.3-16)	
2-chlorophenol	>22	2.6	1.0
		(2.1-3.2)	
4-chlorophenol	8.8	4.1	1.1
	(6.9-12)	(3.2-5.0)	
2,4-dichlorophenol	>10	2.6	0.46
		(1.7-3.7)	
2,4,5-trichlorophenol	3.8	2.7	0.78
	(3.2-4.7)	(2.3-3.0)	
2,4,6-trichlorophenol	15	6.0	<0.41
	(12-19)	(3.8-8.5)	
2,3,4,6-tetrachlorophenol	>1.0	0.29	0.010
		(0.070-1.2)	
2,3,5,6-tetrachlorophenol	2.5	0.57	0.010
	(1.1-5.1)	(0.28-1.3)	
pentachlorophenol	1.5	0.68	0.32
	(1.1-2.0)	(0.60-0.79)	
4-chloro-6-methylphenol	1.9	0.29	0.028
	(1.5-2.3)	(0.19-0.40)	
2,4-dichloro-6-methylphenol	>1.7	0.43	0.078
		(0.32-0.60)	
4-nitrophenol	24	22	13
	(22-26)	(20-24)	
2,4-dinitrophenol	4.5	4.1	3.1
	(4.1-5.0)	(3.7-4.5)	
2,4,6-trinitrophenol	>220	85	<28
		(67-100)	
2,4-dinitro-6-methylphenol	4.3	3.1	1.5
	(3.9-5.0)	(2.7-3.7)	

TABLE 1 - continued

Test Substance	LC50 (mg/L) 24-hour	LC50 (mg/L) 48-hour	No discernible effect conc. (mg/L)
1,2-diphenylhydrazine	8.1 (6.5-9.9)	4.1 (2.9-5.6)	0.41
ethylbenzene[a]	77 (57-100)	75 (50-120)	6.8
fluoranthene	1300 (1000-1600)	320 (220-490)	<8.8
4-bromophenyl phenyl ether	0.46 (0.36-0.64)	0.36 (0.28-0.48)	<0.046
isophorone[a]	430 (360-500)	120 (72-170)	15
naphthalene	17 (11-25)	8.6 (5.0-15)	0.60
nitrobenzene	24 (19-30)	27 (22-32)	0.46
N-nitrosodiphenylamine[a]	>46	7.8 (5.8-11)	1.0
phenol	29 (20-39)	12 (7.3-20)	2.2
selenium	0.66 (0.53-0.87)	0.43 (0.35-0.57)	0.22
tetrachloroethylene[a]	18 (17-20)	18 (16-19)	10
thallium	3.6 (2.9-4.2)	2.2 (1.9-2.6)	1.7
toluene[a]	310 (240-420)	310 (240-420)	28
trichloroethylene	22 (16-30)	18 (12-26)	2.2
beryllium	1.9 (1.1-3.3)	1.0 (0.80-1.6)	0.25
bis(2-ethylhexyl)phthalate	>68	11 (7.5-16)	1.1
silver[a]	0.0015 (0.0014-0.0017)	0.0015 (0.0014-0.0017)	0.0011
barium	>530	410 (320-530)	68
bromine	1.5 (1.0-2.2)	1.0 (0.46-2.2)	0.46
camphene	46 (36-60)	22 (13-36)	<13
p-cymene	9.4 (7.9-11)	6.5 (4.3-10)	<4.6
n-decane	23 (18-31)	18 (13-24)	1.3

TABLE 1 - continued

Test Substance	LC50 (mg/L) 24-hour	LC50 (mg/L) 48-hour	No discernible effect conc. (mg/L)
butylbenzylphthalate	>460	92 (74-120)	<36
diethylphthalate	52 (36-74)	52 (36-74)	10
dimethylphthalate	150 (98-220)	33 (16-130)	<1.7
bromoform	56 (44-68)	46 (42-51)	<7.8
dichloromethane	310 (280-340)	220 (140-330)	68
1,1-dichloropropane	30 (20-45)	23 (13-37)	<6.8
1,2-dichloropropane	99 (58-600)	52 (42-68)	<22
1,3-dichloropropane	490 (360-710)	280 (200-390)	68
1,3-dichloropropene	7.2 (5.1-11)	6.2 (4.3-9.0)	0.41
1,1-dichloroethylene	98 (71-130)	79 (62-110)	<2.4
1,2-dichloroethylene (trans)	230 (200-280)	220 (170-290)	<110
1-chloronaphthalene	>3.6<10	1.6 (0.97-2.6)	<0.17
octachloronaphthalene	>530	>530	530
acenaphthene	>280	41 (19-71)	0.60
acrolein	0.23 (0.18-0.30)	0.083 (0.070-0.10)	0.034
acrylonitrile	13 (11-15)	7.6 (6.2-9.2)	0.78
antimony[a]	>530	>530	530
benzene	250 (200-310)	200 (140-320)	<13
carbon tetrachloride	35 (25-47)	35 (25-47)	7.7
bis(2-chloroethyl)ether	340 (290-400)	240 (170-330)	<7.8
chloroform	29 (19-47)	29 (19-47)	<7.8
2,4-dimethylphenol[a]	8.3 (5.9-11)	2.1 (1.8-2.5)	1.0
2,3-dinitrotoluene	>2.8	0.66 (0.42-1.1)	<0.046

TABLE 1 -- continued

Test Substance	LC50 (mg/L)		No discernible effect conc. (mg/L)
	24-hour	48-hour	
diethanolamine	170 (-)[c]	55 (44-68)	<24
n-dibutyl ether	32 (26-36)	26 (21-33)	4.6
diphenyl ether	1.4 (1.1-1.9)	0.67 (0.11-1.1)	0.41
n-docosane	>530	>530	<68
sodium fluoride	680 (-)	340 (280-410)	110
methylethylketone	>520	>520	<70
α-pinene	68 (24-190)	41 (27-62)	8.8
styrene	27 (20-35)	23 (18-29)	<6.8
biphenyl	27 (19-48)	4.7 (3.6-5.9)	<2.2
dibenzofuran	7.5 (4.4-13)	1.7 (1.1-2.7)	0.28

[a] Diluent water had a mean hardness of 72 mg/L as $CaCO_3$.

[b] 95 percent confidence interval.

[c] Confidence interval was not calculatable.

ACKNOWLEDGEMENTS

The author extends sincere appreciation to Donald Surprenant, Brian Robinson, Mitch Ziencina and Mary Hawes for their technical assistance in the completion of this study.

The work upon which this publication is based was performed pursuant to Contract No. 68-01-4646 with the Environmental Protection Agency.

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