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Acute Toxicity of Priority Pollutants to Bluegill (*Lepomis macrochirus*)

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Industrially used chemicals are potential sources of contamination of the aquatic environment either through normal usage, disposal, or accidental spillage. 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 bluegill (Lepomis macrochirus).

MATERIALS AND METHODS

Test chemicals, listed in Table 1, were procured from those commercial sources able to provide the purest grade available. All chemicals tested were ≥80% pure and test results are presented as the active ingredient of the formulation. These static acute toxicity tests followed procedures presented in "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (U.S. EPA 1975).

Test animals utilized were young of the year bluegill (L. macrochirus) obtained from commercial fish suppliers within the continental United States. Wet weight of these fish ranged from 0.32-1.2 g. Upon receipt at Bionomics, each test population was held in a separate tank receiving well-water at a minimum flow rate of 4 volume replacements per day. Chemical and physical characteristics of this well-water were measured weekly, revealing a total hardness of 28-44 mg/L $CaCO_3$, a total alkalinity of 20-30 mg/L $CaCO_3$, a pH of 6.7-7.4, a dissolved oxygen concentration of 5.3-7.0 mg/L, a specific conductance of 95-170 µmhos/cm and a temperature of 20-24°C. All fish were fed ad libitum daily with dry, pelleted food and once weekly with raw ground beef liver. All feeding was discontinued for 48 h prior to testing. No population was used in toxicity tests if the mortality exceeded 3% during this 48-h period.

Test chambers were 19.6-L widemouthed glass jars containing 15 L of test solution. In an effort to control volatilization, the test jars containing a chemical with a high vapor pressure were capped. Chemicals judged to pose a hazard to the investigators

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were also tested in capped jars. Test solution temperatures were controlled by a system designed to maintain the temperature at 22 ± 1°C. Dilution water used to prepare the test solutions was deionized water reconstituted according to recommended procedures (U.S. EPA 1975). The water had a total hardness of 32-48 mg/L $CaCO_3$, a total alkalinity of 28-34 mg/L $CaCO_3$, a pH of 6.7-7.8, a dissolved oxygen concentration of 7.0-8.8 mg/L, a specific conductance as 93-190 umhos/cm.

The water solubility and method for addition of each test chemical to the dilution water were investigated prior to testing. If the chemical appeared to be soluble in water, either a concentrated stock solution was prepared with distilled water or the appropriate amount of the compound was added directly to the diluent in the test jars. If attempts to solubilize a chemical in water were unsuccessful, a stock solution was prepared using, in order of preference, 1,6-hexanediol, acetone or dimethyl formamide as the carrier solvent. This order was based upon the relative acute toxicity of these solvents to bluegill. Ethanol was used as a solvent in two cases where other solvents were inappropriate. If the solvent concentration required to yield a prescribed compound concentration exceeded 500 mg/L in the test dilution water (the maximum allowed under U.S. EPA procedures, 1975), the compound was added directly to the jars.

Ten fish were randomly selected from a test population and added to each test jar within 30 min after the addition of the test chemical or stock solution. If the jars were to be capped, the fish were added within 30 min prior to the addition of the test chemical; jars were capped immediately following addition of the test chemical. Controls and solvent controls were also maintained and capped where appropriate. These controls were maintained under the same conditions as test concentrations, but contained no test chemical. The solvent control also contained the greatest amount of solvent present in the high test chemical concentration for any given test.

The pH and dissolved oxygen (DO) concentration of test solutions were measured at 0, 24, 48 and 96 h of exposure for uncapped jars and at 0 and 96 h for capped jars. All measurements were made in the control(s) low, middle and high test concentrations. Temperature was measured every 24 h in a control jar. Fish mortality, behavioral and physiological responses, and solution appearance were observed and recorded at time 0 and every 24 h during exposure.

Data reported were based on the nominal concentration of the active ingredient on each chemical. The LC50's and 95% confidence intervals were calculated, where possible, by the moving average angle method (HARRIS 1959). The nominal test concentrations were transformed to logarithms and corresponding percentage mortalities to angles. Each group of these successive angles was then averaged and the LC50 was estimated by linear interpolation between the successive concentrations whole average angles bracketed 45°. When the test data did not meet Harris' method requirements, the LC50's were calculated by the log probit method, a modification of the LITCHFIELD + WILCOXON (1949) method.

RESULTS

Measurements of water quality characteristics revealed that dissolved oxygen concentrations of all tests were within a range of 9.7 mg/L at the beginning of an exposure to 0.3 mg/L after 96 h exposure. The pH of the test solutions ranged from 7.9-6.5, and temperatures ranged from 21 to 23°C.

The acute toxicity of most of the chemicals tested was at concentrations above their water solubility and therefore, the test material or one or more of its constituents precipitated or formed a slick on the water surface in the jars. Therefore, most of the LC50 values reported, which were calculated based on nominal concentrations, are high and do not reflect he actual concentrations of the chemical which were in solution in the diluent. Table 1 summarizes the solubility observations and the 24 and 96 h LC50's based on nominal concentrations for the chemicals tested. For the four chemical groups with which sufficient data were generated (benzenes, ethanes, ethylenes and phenols), it was determined that the severity of toxicity was significantly ($P \leq 0.01$ for ethanes, ethylenes and phenols, $P \leq 0.05$ for benzenes) correlative to the degree of chlorine substitution. The overall degree of toxicity for these groups were as follows: chlorinated phenols < chlorinated benzenes < chlorinated ethylenes < chlorinated ethanes.

TABLE 1. The chemicals tested, solubility observations and 24- and 96-hour LC50's of 64 chemical compounds tested for acute toxicity to bluegill under static conditions.

Compound (by chemical group)	Solubility observations	LC50 (mg/L)	
		24 h	96 h
<u>Benzenes</u>			
chlorobenzene	undissolved chemical	17	16 (13-20)[a]
1,2-dichlorobenzene	soluble	6.3	5.6 (4.8-6.6)
1,3-dichlorobenzene	undissolved chemical	22	5.0 (3.9-6.2)

Compound (by chemical group)	Solubility observations	LC50 (mg/L)	
		24 h	96 h
1,4-dichlorobenzene	precipitate	4.5	4.3 (3.9-4.8)
ethylbenzene	undissolved chemical	169	150 (130-200)
nitrobenzene[b]	soluble	135	43 (36-49)
1,2,4-trichlorobenzene	undissolved chemical	109	3.4 (2.7-4.1)
1,2,3,5-tetrachlorobenzene	precipitate	59	6.4 (5.2-8.1)
1,2,4,5-tetrachlorobenzene	precipitate	5.7	1.6 (1.3-1.8)
pentachlorobenzene	precipitate	2.3	0.25 (0.18-0.32)
Chlorinated Ethanes			
1,2-dichloroethane[b]	undissolved chemical	>600	430 (230-710)
1,1,1-trichloroethane	undissolved chemical	110	72 (57-90)
1,1,2-trichloroethane[b]	soluble	40	40 (35-47)
1,1,2,2-tetrachloroethane[b]	soluble	21	21 (20-22)
1,1,1,2-tetrachloroethane[b]	soluble	20	20 (16-24)
pentachloroethane	undissolved chemical	8.2	7.2 (6.0-8.4)
hexachloroethane[b]	precipitate	1.8	0.98 (0.85-1.1)
Ethers			
4-bromophenyl phenyl ether[b]	precipitate	51	5.9 (4.0-6.1)
bis(2-chloroethyl)ether[b]	undissolved chemical	>600	600
2-chloroethyl vinyl ether[b]	undissolved chemical	452	350 (300-410)

Compound (by chemical group)	Solubility observations	LC50 (mg/L)	
		24 h	96 h
Phenols			
2-chlorophenol[b]	soluble	7.2	6.6 (5.7-8.0)
4-chlorophenol	soluble	4.0	3.8 (3.1-4.8)
2,4-dichlorophenol[b]	soluble	4.7	2.0 (1.6-2.6)
4-nitrophenol	soluble	12	8.3 (6.7-10)
2,4-dinitrophenol	precipitate	2.4	0.62 (0.48-0.81)
2,4,6-trinitrophenol	soluble	193	170 (160-180)
2,4-dinitro-6-methyl-phenol[b]	soluble	0.65	0.23 (0.18-0.28)
2,4,5-trichlorophenol	soluble	0.61	0.45 (0.39-0.54)
2,4,6-trichlorophenol[b]	soluble	0.72	0.32 (0.26-0.37)
2,3,4,6-tetrachlorophenol[b]	soluble	0.19	0.14 (0.11-0.16)
2,3,5,6-tetrachlorophenol	soluble	0.40	0.17 (0.13-0.21)
4-chloro-6-methyl-phenol	precipitate	3.8	2.3 (1.9-2.9)
2,4-dichloro-6-methyl phenol	precipitate	3.4	1.6 (1.4-1.8)
2,4-dimethylphenol[b]	soluble	18	7.8 (6.3-9.6)
Dichloropropanes/propene			
1,1-dichloropropane	soluble	98	98 (88-110)
1,2-dichloropropane	soluble	360	280 (220-340)
1,3-dichloropropane	undissolved chemical	>520	>520

Compound (by chemical group)	Solubility observations	LC50 (mg/L)	
		24 h	96 h
1,3-dichloropropene[b]	soluble	6.8	6.1 (5.1-6.8)
Phthalate esters			
bis(2-ethylhexyl)phthalate	undissolved chemical	>770	>770
butylbenzylphthalate	soluble	62	43 (38-52)
diethylphthalate	undissolved chemical	120	110
dimethylphthalate	undissolved chemical	350	50 (37-69)
di-n-butylphthalate	precipitate	2.1	1.2 (1.0-1.4)
Ethylenes			
1,1-dichloroethylene[b]	soluble	74	74 (57-91)
1,2-dichloroethylene	soluble	165	140 (120-160)
trichloroethylene[b]	undissolved chemical	>68<100	45 (39-54)
tetrachloroethylene	soluble	46	13 (11-15)
Others			
acenaphthene	precipitate	7.2	1.7 (1.3-2.1)
acrolein[b]	soluble	0.10	0.09 (0.08-0.12)
acrylonitrile	undissolved chemical	28	10 (8-12)
antimony trioxide	undissolved chemical	>530	>530
bromoform	soluble	33	29 (24-36)
carbon tetrachloride[b]	precipitate	38	27 (23-33)
1-chloronaphthalene	soluble	3.7	2.3 (2.0-2.7)

Compound (by chemical group)	Solubility observations	LC50 (mg/L)	
		24 h	96 h
2,3-dinitrotoluene	soluble	1.6	0.33 (0.28-0.38)
1,2-diphenylhydrazine	soluble	1.2	0.27 (0.23-0.33)
fluoranthene	precipitate	>32	4.0 (3.1-5.5)
isophorone	undissolved chemical	240	220 (180-250)
dichloromethane	soluble	230	220 (200-250)
n-nitrosodiphenylamine[b]	precipitate	44	5.8 (4.8-7.6)
octachloronaphthalene[b]	undissolved chemical	>600	>600
silver (silver nitrate)	soluble	0.80	0.06 (0.05-0.07)
thallium (thallous sulfate)	soluble	>600	120 (99-160)
toluene	soluble	17	13 (11-15)

[a] 95% confidence interval. [b] Capped jars.

REFERENCES

APHA, AWWA, WPCF: Standard methods for the examination of water and wastewater. 14 Edition, Washington, D.C. 1193 pp. (1975).

HARRIS, E.K.: Confidence limits for the LD50 using the moving average angle method. Biometrics. 4, 457-464. (1950).

LITCHFIELD, J.T., JR. and F. WILCOXON: J. Pharmacol. Exp. Therapeut. 96, 99 (1949).

U.S. EPA: The Committee on Methods for Toxicity Tests with Aquatic Organisms: Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians. Ecological Research Series (EPA-660/3-75-009), 61 pp. (1975).

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