Bull. Environ. Contam. Toxicol. (1987) 38:667–673

Bulletin of Environmental Contamination and Toxicology

Effects of Lowered Dissolved Oxygen Concentration on the Toxicity of 1,2,4-Trichlorobenzene to Fathead Minnows

Anthony R. Carlson

U.S. Environmental Protection Agency, Environmental Research Laboratory-Duluth, 6201 Congdon Boulevard, Duluth, MI 55804

Little experimental information is available on the combined effects of lowered DO concentrations and toxicants on growth and survival during the early life stages of fish. Such toxicity information is needed for predicting safe concentrations of contaminants in fish habitats where diel fluctuations in DO concentrations are normal or are affected by organic pollution.

The primary objective of this study was to determine if the DO concentration affects the toxicity of 1,2,4-trichlorobenzene (1,2,4-TCB) to fathead minnows, Pimephales promelas, exposed during the embryonic-to-larval-juvenile development stage. This stage in the growth of the fathead minnow and several other species of fish has been found to be the most sensitive, or among the most sensitive, to chemical pollutants in life-cycle toxicity tests (McKim 1977). Brungs (1971) also found that the fathead minnow was most sensitive to low DO concentrations at this life stage.

The chemical 1,2,4-TCB has been identified as a "priority pollutant" by the U.S. Environmental Protection Agency. It is used as an intermediate in the synthesis of a number of herbicides and insecticides and has been found as a contaminant in fish (Jan and Malneric 1980). It was selected as the chemical stressor in this study in order to provide information useful to the Agency in deriving water quality criteria for the protection of aquatic life.

MATERIALS AND METHODS

Each glass test chamber was divided into two compartments: a 2-L compartment where water flows containing toxicant and controlled DO concentrations were mixed and a 4.8 L fish-exposure compartment. The chambers were positioned in two rows; each chamber was randomly assigned a DO concentration and toxicant concentration.

Three mean DO concentrations were maintained: 8.1 (7.5-8.6), 5.6 (5.0-6.7), and 4.5 (4.2-5.2) mg/L. Degassed water (Mount 1964) and saturated water, from separate headboxes that

maintained constant hydrostatic heads, were mixed through valves in 4-L glass flasks to obtain the desired DO concentration and then passed through stainless steel coils in a water bath to produce the desired temperature. From the coils the water flowed into three 2.54-cm PVC[1] pipes that were located between and above the chamber rows. Each pipe had 12 outlets to which capillary tubes of similar length and inside diameter were attached by Tygon tubing and used to control the flows, approximately 48 mL/min., into the mixing compartments of the chambers. These flows were monitored daily, and were measured and adjusted if needed on at least every third day of test.

A constant-flow toxicant serial diluter and toxicant-generating system similar to that described by Benoit et al. (1982) were used to obtain five concentrations of 1,2,4-TCB and a control. Each flow was split six ways, and each test chamber mixing compartment received approximately 12 mL/min. The 1,2,4-TCB (97% purity) used to charge the saturator was obtained from the Aldrich Chemical Company, Milwaukee, Wisconsin.

Lake Superior water was used in the tests after filtration through sand and 10μ (nominal) cotton filters. Test water temperature measurements ranged from 25.6 to 25.9 with a mean of 25.7 C.

The total alkalinity, total hardness, and pH of the test water was measured weekly (American Public Health Association et al. 1975) and ranged from 38 to 43 mg/L as $CaCO_3$, 43 to 46 mg/L as $CaCO_3$ and 7.3 to 7.6, respectively. Dissolved oxygen concentrations were measured daily by the unmodified Winkler method (American Public Health Association et al. 1975) and/or a polarographic oxygen meter.

Gas-chromatographic analyses of water and fish tissue samples for 1,2,4-TCB were performed on a 5730A Hewlett-Packard[1] gas chromatograph with an auto sampler and a Hewlett-Packard 3354B lab automation data system. Methodology used was the same as reported for 1,4,-dichlorobenzene (Carlson and Kosian 1987). The detection limit was 5 μg/L.

Water samples (50 mL) for measurement of 1,2,4-TCB concentration were taken from the chambers at similar concentrations at approximately the same time. Samples were extracted with hexane and diluted if necessary before analysis. Because data from test chambers at similar concentrations were not different ($P > 0.05$) when analyzed using the Student t test (Steel and Torrie 1960),

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1 The U.S. Environmental Protection Agency neither recommends nor endorses any commercial product; trade names are used for identification.

the data were combined. The mean ± standard deviation, and range (in parenthesis) of these measurements were 920.0 ± 190 (625-1,210), 500 ± 125 (307-712), 280 ± 55 (185-425), 140 ± 20 (110-160), 75 ± 10 (60-90) and 15 μg/L (control), respectively. The number of measurements at each treatment ranged from 23 to 28. The mean percentage recovery from fortified water samples was 99 percent.

Composite whole-fish samples were homogenized with 70 g of cold anhydrous Na_2SO_4. The powdered homogenate was transferred to a 300-mL chromatographic column and eluted with 250-mL of redistilled hexane. The eluate was appropriately diluted and analyzed. The mean recovery from fortified samples was 92 percent. All tissue samples were corrected for percentage recovery.

The test was begun by placing an incubation basket, containing 50 embryos 14 to 26 h. old, into each test chamber. These embryos were obtained from parental stock cultured in Lake Superior water at the U.S. EPA Environmental Research Laboratory, Duluth, MN. Water flows from the mixing compartments were directed through the incubation baskets which were partially emersed in the exposure compartments of the test chambers. Larvae were removed from the baskets and counted on the fifth day of testing, and 30 were returned to their respective chambers and reared to the end of the test, 28 days later. Larvae from three baskets that had escaped into the chambers were also reared to the end of the test. Data obtained from these three chambers were used only in the bioconcentration comparisons. Live brine shrimp nauplii, Artemia salina, were fed to the fish two or three times daily. Similar amounts by volume were placed in each chamber at each feeding. The fish were initially offered food on the fourth day of the test and were not fed the last 24 h. of the test. For details of rearing procedures used see Benoit et al. (1982). The percentage of normal larvae hatching, survival of those returned to the test chambers, final live weight, and bioconcentration (content in water divided by content in fish) were used as endpoints to assess toxicity. Survival and growth data were analyzed with balanced or unbalanced analysis of variance procedures. Dunnett's procedure for comparing all treatments with a control ($P = 0.95$) was used to identify significant differences in survival and growth at each DO treatment. Percentage survival data were transformed to arcsins for analysis of variance tests (Steel and Torrie 1960).

A 96 h. flow-through acute toxicity test, exposing 30-day-old fathead minnow (mean weight 110 mg), was completed. Standard procedures as described by the Committee for Toxicity Tests with Aquatic Organisms (1975) were followed. A 96-h. LC50 (estimate of the concentration lethal to 50% of the test organisms) was estimated by using the trimmed Spearman-Karber Method (Hamilton et al. 1977).

RESULTS AND DISCUSSION

For fish chronically exposed to the mean 1,2,4-TCB concentration of 920 μg/L at 8.1 mg/L DO, mean survival was 37.5% less and mean weight was 28.7% less when compared to the 8.1 mg/L DO control (Table 1). At the comparable toxicant concentration at 5.6 mg/L DO, the mean percentage survival and mean weight of test fish were not statistically different ($P = 0.95$) when compared to the 5.6 mg/L DO control. At the comparable mean 1,2,4-TCB concentration of 920 μg/L at 4.5 mg/L DO, more marked effects on survival and growth occurred; mean survival was reduced by 88.5% and mean weight by 60.5% when compared to the 4.5 mg/L DO control. No effects of 1,2,4-TCB on survival, growth, and bioconcentration were evident at mean concentrations of 500 μg/L or lower. Also, no direct effects of lowered DO concentrations were evident.

Effects on fathead minnow survival and growth were not evident at the 920 μg/L 1.2.4-TCB concentration and 5.6 mg/L DO because of high variability between some replicates; however, at this treatment means of survival and weight measurements were at least 18% less when compared to the control. Although these reductions are not statistically different, significant effects on survival and growth obtained at the 920 μg/L 1,2,4-TCB exposures at the higher and lower DO concentrations indicate that exposure under natural conditions at this 1,2,4-TCB concentration may have significant biological implications.

The results show that exposure to the mean concentration of 920 μg/L 1,2,4-TCB at the 4.5 mg/L DO concentration resulted in increased susceptibility of the fish to the toxicant. Chapman and Shumway (1978) found similar increased susceptibility to pentachlorophenate for trout larvae reared at low DO concentrations. Such increased susceptibility to toxic stress indicates that the DO concentrations in natural habitats of larval fish should be relatively high when toxicant concentrations are near effect levels.

The estimated 1,2,4-TCB 96-h. LC50 for juvenile fathead minnows was 2,760 μg/L. Nineteen of the twenty test fish died at the 4,340 μg/L concentration during the 96-h. test period. All fish survived at concentrations of 1,670 μg/L or less. These data indicate that the early life stage test concentration of 920 μg/L, where subtle effects of 1,2,4-TCB on growth and survival were evident, is near the lethal threshold for this species.

Dissolved oxygen concentration did not demonstrably affect uptake of 1,2,4-TCB by the fish. The range of the bioconcentration factors between no-effect toxicant treatments at the three DO concentrations were similar (Table 1). Whole tissue concentrations of 1,2,4-TCB at the highest no-effect concentration (500 μg/L) ranged from 140 to 180 μg/g. At

Table 1. Survival, growth, and bioconcentration data for fathead minnows exposed for 32 days to similar concentrations of 1,2,4-trichlorobenzene (1,2,4-TCB) at three dissolved oxygen (DO) concentrations.

Mean 1,2,4-TCB concentration (μg/L)	Percentage survival to hatch	Percentage survival to test end	Mean wet weight (mg)	1,2,4-TCB residue (μg/g)	Mean Bioconcentration Factor
		8.1 mg/L DO			
15 (Control)	88 ; 92	93.3 ; 90.0	101.0 ; 88.1	5 ; ___	___
75	_ ; 84	_ ; 83.3	_ ; 92.0	32 ; 30	410
140	88 ; 100	93.3 ; 90.0	92.1 ; 85.3	65 ; 70	480
280	96 ; 90	90.0 ; 93.3	85.2 ; 85.0	125 ; 130	450
500	88 ; 98	90.0 ; 86.7	80.5 ; 90.0	150 ; 170	320
920	70 ; 88	56.6 ; 66.7[a]	63.1 ; 71.8[a]	465 ; 490	520
		5.6 mg/L DO			
15 (Control)	88 ; 90	100.0 ; 83.3	77.9 ; 89.4	5 ; 5	___
75	96 ; 88	100.0 ; 86.7	83.9 ; 92.4	28 ; 35	420
140	86 ; 90	96.7 ; 100	86.8 ; 77.9	50 ; 35	300
280	96 ; 80	96.7 ; 93.3	77.1 ; 85.6	130 ; 110	430
500	90 ; _	96.7 ; _	73.9 ; _	140 ; 130	270
920	92 ; 88	73.3 ; 76.7	78.3 ; 61.1	355 ; 340	380
		4.5 mg/L DO			
15 (Control)	_ ; 86	_ ; 76.6	_ ; 79.9	5 ; 5	___
75	76 ; 92	83.3 ; 90	85.4 ; 81.3	25 ; 35	400
140	38[b] ; 96	73.3 ; 86.7	99.0 ; 80.3	65 ; 65	460
280	82 ; 88	73.3 ; 66.7	78.0 ; 77.7	150 ; 135	510
500	88 ; 90	86.7 ; 90.0	64.9 ; 73.9	180 ; 140	320
920	92 ; 94	23.3 ; 0.0[a]	29.1 ; _[a]	220 ;	290

[a] Mean significantly less than the controls ($P < 0.05$). [b] High mortality due to fungus infection.

the effect concentration (920 μg/L) tissue concentrations ranged from 270 to 490 μg/g. These tissue residues are several orders of magnitude higher than residues reported for fish taken from natural habitats. For example, the highest mean no-effect tissue residue, converted to a μg/g of lipid based on mean lipid content of 3.1% for fathead minnows of similar weight and reared under similar conditions, (Carlson and Kosian, 1987) is 5200 μg/g, whereas, several species of fish taken from rivers in a highly populated region of Yugoslavia (Jan and Malneric 1980) contained only 0.005 μg/g on a lipid basis for edible tissues. These low background residues may be reflective of exposure to high water concentrations from chemical spills and rapid metabolism of 1,2,4-TCB (Barrows et al. 1980, reported an equal to or less than three day half-life for 1,2,4-TCB in fish tissue) or a more constant exposure to relatively low concentrations of 1,2,4-TCB in food and/or in the water column resulting from multiple inputs from air and water sources. Assuming that monitoring data are reflective of this latter source of 1,2,4-TCB contamination of aquatic environments, the high concentrations in the water and tissues that were needed to produce subtle effects on survival and growth in this study indicated that similar direct effects of 1,2,4-TCB on fishes in natural habitats are remote possibilities associated with slug doses from industry discharges or spills.

The presence of detectable (5 μg/g whole tissue) 1,2,4-TCB tissue residue in the control fish indicate slight contamination from volatilization. This exposure is thought not sufficient to affect the results of this experiment because total weight and percentage survival of the control fish at 8.1 mg/L were similar to fish reared under similar conditions as controls in many other experiments at this laboratory.

Acknowledgments. I thank Kristine Olson for assisting in the maintenance of the test; and Patricia Kosian and Dr. Gilman Veith for 1,2,4-TCB analyses.

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Received May 21, 1986; accepted September 19, 1986