Bull. Environ. Contam. Toxicol. 31, 309-314 (1983)

Acute Toxicity of Chlorine and Bromine to Fathead Minnows and Bluegills

E. W. Wilde,[1] R. J. Soracco,[2] L. A. Mayack,[2] R. L. Shealy,[3] and T. L. Broadwell[3]

[1]*Savannah River Laboratory, E. I. du Pont de Nemours & Co., Aiken, SC 29808;* [2]*Rensselaer Polytechnic Institute, Troy, NY 12181; and* [3]*Enwright Laboratories, Inc., Greenville, SC 29606*

The environmental acceptability of using chlorine as an antifouling agent in industrial cooling systems is a subject of increasing concern. Several alternatives to chlorination have recently been proposed including the use of a bromine-based biocide, 1-bromo-3-chloro-5,5-dimethylhydantoin (BCDMH). This compound has reportedly outperformed chlorine in several cooling tower applications (MACCHIAROLO, et al. 1980; MATSON & CHARACKLIS 1982). However, information on the use of BCDMH in once-through cooling systems and the relative environmental acceptability of BCDMH in comparison to chlorine are lacking.

The objective of this study was to compare the relative toxicity of chlorine and BCDMH to three types of fish in a freshwater system used for cooling a nuclear reactor.

METHODS AND MATERIALS

Flow-through 96-h toxicity tests were conducted in March 1982 using a mobile laboratory located adjacent to Par Pond, a reactor cooling reservoir at the Savannah River Plant (SRP) near Aiken, S. C. Two solenoid-activated proportional, flow-through dilutor systems as described by PELTIER (1978) were used for simultaneous testing of oxidant residual toxicity resulting from chlorine and BCDMH additions. Each system delivered approximately 100%, 75%, 60%, 35%, 20%, 2%, and 0% of stock solution of the appropriate biocide to duplicate test chambers using Par Pond water as diluent.

A Hydrolab 8000 system was used to make daily measurements of: dissolved oxygen, temperature, conductivity, and pH in the test chambers receiving 100%, 35%, 2%, and 0% biocide solutions. Alkalinity was measured by APHA standard methods (APHA 1980).

Juvenile (six-week-old) and yearling fathead minnows (Pimephales promelas) and young-of-the-year bluegills (Lepomis macrochirus) were used as test organisms. The fish were acclimated to Par Pond water for ten days prior to the tests. Juvenile minnows were fed daily during the acclimation and test periods. Ten of each of the three types of fish were placed in each of the duplicate chambers (a loading rate of <2.0 g fish/L of water). Each test chamber was 30.0 cm deep and had a capacity of 15 L. Juvenile fathead minnows were placed in small glass holding chambers (8.2 cm x 20.0 cm x 15.5 cm) which were suspended in the test chambers. These holding chambers had a 3.0 cm x 8.2 cm Nitex screen (#0 mesh, 0.505 mm aperature) at each end to allow circulation through the chamber and prevent predation by the other test organisms.

Two 80 L stock solutions of each biocide were prepared daily. Chlorine stock solutions were prepared by adding reagent grade 5% sodium hypochlorite. BCDMH solutions were prepared by adding Bromicide® (Great Lakes Chemical Corp., Lafayette, Indiana). Stock biocide solutions were added to their respective dilutor systems for 1 h/day, 0, 24, 48, and 72 h after testing began.

Water samples (ca. 20 ml) were collected from each of the test chambers and tanks containing stock solutions at 10-minute intervals during the periods (ca. 2 h/day) that biocide residuals were measurable in the test chambers. Levels of total and free residual chlorine (TRC and FRC) were determined by the DPD spectrophotometric method (APHA 1980). Oxidant residuals resulting from BCDMH treatment were also routinely measured as chlorine by the DPD method; however, the DPD differentiation method (using glycine) was performed on a few representative samples to distinguish between bromine and chlorine residuals (WHITE 1978).

Median lethal concentrations (96-h LC_{50}'s) were determined by the probit, moving average, and binomial methods (STEPHAN, 1977). Log transformations of dose values were not used because a better goodness of fit was obtained in the majority of cases by using actual dose values. Biocide dosages were calculated as follows:

(1) 96-h peak = the single highest biocide residual detected during the four days of testing.

(2) 96-h mean maximum = the average maximum biocide residual detected during the four days of testing.

(3) 96-h intermittent exposure mean = the mean biocide residual level during the four ~2-h exposure periods.

(4) 96-h accumulative exposure = the total 96-h biocide exposure in mg/L residual x minutes of exposure (area under a time-concentration curve).

LC_{50}'s for TRC and TRB (total residual bromine measured as chlorine) exposure were statistically compared. Differences were considered significant if: greater LC_{50}/smaller LC_{50} > 1.96 $\underline{SE_{Diff}}$ (APHA, 1980).

RESULTS AND DISCUSSION

The DPD differentiation measurements showed that > 95% of the oxidant residual resulting from BCDMH treatment was bromine.

Therefore, residuals from this treatment were considered total and free residual bromine (TRB and FRB).

LC_{50} values computed by the three methods (probit, binomial and moving average) were similar. Values obtained by the moving average method are presented in Table 1. Juvenile fathead minnows were significantly more tolerant of TRB than TRC. TRC and TRB 96-h LC_{50} values for adult fathead minnows were not significantly different. Bluegills were significantly more tolerant of TRC than TRB.

Table 1. Comparative toxicity (LC_{50}'s and 95% confidence intervals) of intermittent (ca. 2 h/day) exposure to residual chlorine or bromine

Type of biocide dose computation and fish	Biocide	
	TRC	TRB
96-h peak (mg/L)		
Juv. fatheads	0.44(0.22-0.62)	1.21(0.96-1.42)*
Adult fatheads	1.56(1.34-1.79)	1.83(1.59-2.10)
Bluegills	2.48(2.20-2.64)	2.35(1.92-2.73)
96-h mean max. (mg/L)		
Juv. fatheads	0.39(0.21-0.53)	0.81(0.65-0.94)*
Adult fatheads	1.37(1.19-1.55)	1.17(1.03-1.31)
Bluegills	2.13(1.93-2.34)	1.43(1.28-1.62)*
96-h int. exp. mean (mg/L)		
Juv. fatheads	0.18(0.11-0.24)	0.35(0.28-0.41)*
Adult fatheads	0.58(0.51-0.65)	0.51(0.46-0.57)
Bluegills	0.88(0.82-0.98)	0.63(0.56-0.71)*
96-h accum. (mg/L x min)		
Juv. fatheads	85(48-113)	164(132-194)*
Adult fatheads	274(240-308)	248(221-276)
Bluegills	421(387-465)	301(271-338)*

* LC_{50}'s for TRC and TRB significantly different at 0.05 level.

Bluegills were significantly more tolerant of both biocides than were fathead minnows. Adult minnows were significantly more tolerant of both biocides than juvenile minnows.

Water quality data are summarized in Table 2. All values for parameters monitored during the tests were within acceptable testing limits (PELTIER, 1978), and variability over time or between test chambers was not substantial.

The percentage of total residual oxidant (TRO) consisting of free residual oxidant (FRO) was greatest at times of maximum exposure

Table 2. Summary of water quality measurements made in conjunction with the toxicity tests.

Parameter	Mean ± SE	Range
Temp (°C)	21.1 ± 0.1	19.9 - 22.9
pH	7.0 ± 0.1	6.7 - 7.1
DO (mg/L)	7.8 ± 0.1	6.5 - 9.1
Cond (μmhos/cm)	66.6 ± 0.1	63 - 71
Alkalinity (mg/L)	15.3 ± 0.1	14 - 16

in tests with both biocides. The FRC and FRB contributions to TRC and TRB in test chambers receiving 100% stock biocide solutions averaged 83.2% and 62.2%, respectively during the 30 min periods of maximum exposure. FRC and FRB contributed 68.8% and 50.4%, respectively to TRC and TRB during all exposure periods in the test chambers receiving 100% biocide stock solution.

This was the second of two studies comparing effluent toxicity with chlorination and chlorination alternatives in an SRP reactor heat exchanger cooling system. The first study (WILDE et al. 1983) showed that oxidant residuals from chlorine dioxide were 2-4 times more toxic to three types of fish than those from chlorination. The present study showed that on the basis of 96-h LC_{50} values for halogen residuals, chlorine and BCDMH additions resulted in similar overall toxicity to fish. Compared to chlorine, BCDMH was more toxic to bluegills and less toxic to juvenile fathead minnows.

The LC_{50} values for TRC were substantially higher in this study than in the previous SRP study where nearly identical methods were used. However, the pattern of relative TRC toxicity with regard to fish type was the same; juvenile fathead minnows were least tolerant, and bluegills were most tolerant. Two factors which probably contributed to the greater tolerance to TRC in the present tests compared to previous tests were: 1) a longer acclimation period to the test water (10 days compared to 3 days), and 2) a lower average water temperature (21.1°C compared to 27.7°C). HEATH (1977) and BASS and HEATH (1977) concluded that water temperature did not significantly influence the 96-h LC_{50} values for bluegills intermittently exposed to chlorine. However, toxicity testing of several fish species by BROOKS and SEEGERT (1977) and SEEGERT and BROOKS (1978) clearly demonstrated the existence of a direct relationship between chlorine toxicity and temperature. DICKSON et al. (1977) also showed that chlorine was more toxic to goldfish at higher temperatures.

Although all fish toxicity studies are somewhat site and time specific, the intermittent biocide regime and relatively high percentage of FRO in the TRO make the present results more applicable to power plant cooling systems than to domestic wastewater treatment systems. BROOKS et al. (1982) have recently demonstrated significantly different toxicities among various forms of residual chlorine to three species of fish. MATTICE et al. (1981) determined that hypochlorous acid was about four times as toxic as hypochlorite ion to mosquitofish. Other workers (ZILLICH, 1972; BRUNGS, 1973; BASS & HEATH, 1977) previously concluded that free chlorine is more toxic to fish than combined chlorine.

There are no previous studies comparing BCDMH toxicity with chlorine toxicity. Bromine chloride has been shown to be similar or slightly less toxic than chlorine (BURTON & MARGREY, 1979; DEGRAEVE & WARD, 1977; LIDEN et al., 1980). These results along with those of the current study indicate that chlorine and bromine produce similar residual halogen toxicity to fish.

Acknowledgements. The information contained in this article was developed during the course of work under Contract No. DE-AC09-76SR00001 with the U.S. Department of Energy.

REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION: Standard methods for the examination of water and wastewater. 15th ed. APHA, Washington, D.C. (1980).

BASS, M. L., and A. G. HEATH: Bull. Environ. Contam. Toxicol. 17, 416 (1977).

BROOKS, A. S., and G. L. SEEGERT: Trans. Am. Fish. Soc. 106, 278 (1977).

BROOKS, A. S., J. M. BARTOS, and P. T. DANOS: The effects of chlorine on freshwater fish under various time and chemical conditions. Electric Power Research Institue Report EA-2481 (1982).

BRUNGS, W. A.: J. Water Pollut. Control Fed. 45, 134 (1973).

BURTON, D. T., and S. L. MARGREY: Environ. Sci. Technol. 13, 684 (1979).

DEGRAEVE, G. M., and R. W. WARD: J. Water Pollut. Control Fed. 10, 2172 (1977).

DICKSON, K. L., J. Jr. CAIRNS, B. C. GREGG, D. I. MESSENGER, J. L. PLAFKIN, and W. H. VAN DER SHALTE: J. Water Pollut. Control Fed. 49, 35 (1977).

HEATH, A. G.: Hydrobiologia 56, 39 (1977).

LIDEN, L. H., D. T. BURTON, L. H. BONGERS, and A. F. HOLLAND: J. Water Pollut. Control Fed. 52, 173 (1980).

MACCHIAROLO, N. T., C. H. JOHNSON, and R. S. HORNACK: Cooling Tower Institute Paper TP-219A, Houston, Texas (1980).

MATSON, J. V., and W. G. CHARACHLIS: Cooling Tower Institute Paper TP-250A, Houston, Texas (1982).

MATTICE, J. S., S. C. TSAI, and M. B. BURCH: Trans. Am. Fish. Soc. 110, 519 (1981).

PELTIER, W.: US EPA Ecol. Res. Ser. EPA-600/4-78-012 (1978).
SEEGERT, G. L., and A. S. BROOKS: Trans. Am. Fish. Soc. 107, 346 (1978).
STEPHAN, C. E.: Methods for calculating an LC_{50}. ASTM, Aquatic Toxicology and Hazard Evaluation, ASTM STP 634. F. L. Mayer and J. L. Hamelink, (eds.) pp. 65-84 (1977).
WHITE, C. F.: Disinfection of wastewater and water for reuse. New York: Van Nostrand Reinbold. (1978).
WILDE, E. W., R. J. SORACCO, L. A. MAYACK, R. L. SHEALY, T. L. BROADWELL, and R. F. STEFFEN: Water Res. (in press).
ZILLICH, J. A.: J. Water Pollut. Control Fed. 44, 212 (1972).

Accepted May 4, 1983.