Bull. Environm. Contam. Toxicol. 29, 184–189 (1982)

Acute Toxicity of Silver to Selected Fish and Invertebrates

Ann R. Lima, Carolanne Curtis, Dean E. Hammermeister, Daniel J. Call, and Taryl A. Felhaber[1]

Center for Lake Superior Environmental Studies, University of Wisconsin—Superior, Superior, WI 54880

Silver is one of the most toxic metals to freshwater aquatic organisms. DAVIES et al. (1978) reported the silver Maximum Acceptable Toxicant Concentration (MATC) for rainbow trout (Salmo gairdneri) to be 0.09-0.17 μg/L. This value represents one of the lowest MATC's of the heavy metals. BIRGE et al. (1978) found silver to be among the most toxic of metals to largemouth bass (Micropterus salmoides) and rainbow trout.

Silver is used in jewelry, silverware, ink, electroplating and photographic processes (WINDHOLZ et al. 1976). It is also a waste product from heavy metal mining and milling processes. Some silver may enter natural waters by weathering processes; however, most silver compounds are insoluble in water (MCKEE and WOLF 1963).

The present study was conducted to expand the data base on the toxicity of silver to aquatic organisms. The following organisms were exposed to silver nitrate in acute toxicity tests: flagfish (Jordanella floridae), fathead minnows (Pimephales promelas), scuds (Gammarus pseudolimnaeus) and parthenogenetic midges (Tanytarsus dissimilis Johannsen, 1937).

MATERIALS AND METHODS

Source and Culture of Test Organisms

All test organisms were reared in Lake Superior water. Flagfish and fathead minnows were obtained from the culture unit at the U.S. EPA Environmental Research Laboratory-Duluth (ERL-D), Duluth, Minnesota. They were fed live brine shrimp (Artemia sp.) and were raised in continuously renewed water at 25°C. Midges from ERL-D stock were fed a diet of Cerophyl® and trout pellets as described by ANDERSON et al. (1980). Scuds were collected from the Eau Claire River near Gordon, Wisconsin and were held in continuously renewed 20°C water for one month prior to testing. They were fed maple (Acer sp.), birch (Betula sp.) and oak (Quercus sp.) leaves which had been pre-soaked in Lake Superior water for approximately thirty days.

[1] Present address: Department of Geochemistry, University of Capetown, Rondebosch 7700, Capetown, South Africa.

0007-4861/82/0029-0184 $01.20

Test Procedures

Flagfish, fathead minnows, and scuds were exposed in a proportional diluter (MOUNT and BRUNGS, 1967) which was calibrated to provide a 0.5 dilution factor. Each test consisted of five concentrations of silver and one control with a duplicate of each. Tank dimensions were 26 x 17 x 15 cm. Each tank held 3.1 L of water and 13.3 volumes were added to each tank in 24 h. Scuds were placed into smaller glass chambers (6.3 x 9.3 x 6.3 cm) within the larger tanks. Two sides of the smaller chambers consisted of 202 Nitex mesh to allow an exchange of exposure water with the larger chambers. Light in the tanks ranged from 220-330 lux at 0.1 m above the water surface. The photoperiod was 16 h of light and 8 h of darkness with no transition period. Stock solutions of reagent grade silver nitrate ($AgNO_3$) were prepared in deionized water and were delivered from dark bottles to the diluter with a metering pump (FMI Corp.). Organisms were observed for deaths at 3, 6, and 12 h and twice daily thereafter. Flagfish, fathead minnows, and scuds were exposed to silver for 96 h. Midges were exposed for 48 h. Organisms were not fed during the test.

Flagfish and Fathead Minnow

Dividers made of stainless steel mesh were placed in the center of each tank. Fifteen 30 day old flagfish (0.044 ± 0.021 g) were randomly selected and placed in the upstream section of each tank and twenty 30 day old fathead minnows (0.079 ± 0.031 g) were randomly selected and placed in the downstream section of each tank. Fish death was defined as complete cessation of opercular movement. The average tank temperature was 24.7 ± 0.5°C. Mean silver concentrations were 3.4, 8.4, 13.7, 29.6, and 67.9 µg/L in the exposure chambers and <0.2 µg/L in the control chambers.

Scuds

Test organisms were selected based upon size uniformity (length = 0.67 ± 0.24 cm) with no attempt to determine age. Ten scuds were exposed in each duplicated concentration. Death was defined as lack of movement after gentle prodding. The average tank temperature was 19.9 ± 0.5°C. Mean silver concentrations were 0.80, 2.15, 4.9, 15.3, and 35.6 µg/L in the exposure chambers and <0.2 µg/L in the control chambers.

Midges

Ten 3rd instar midge larvae ranging in total length from 2.5 - 2.75 mm were transferred into each of twelve exposure chambers. Each 5.0 x 9.0 cm chamber with a 0.3 cm glass overflow tube held a fine layer of sand. Food and Lake Superior water (200 mL total) were prepared in a ratio of 2.5 mL:1 L. Chambers were then placed in a 20°C water bath. After the midges had acclimated for

48 h, five silver exposure concentrations (with a 0.5 dilution factor) and one control, in duplicate, were prepared. Water in the midge chambers was siphoned out to a depth of 2-3 mm and the silver solutions were slowly dripped in from a separatory funnel at a rate of 2 mL/min. After 500 mL of solution had flushed through each chamber, the remainder of the test was static. Death was defined as lack of movement after gentle prodding.

During the 48 h acclimation period and first 24 h of the test, the photoperiod consisted of 16 h with a light intensity of 20 lux 0.1 m above the water surface and 8 h of darkness. Lights were turned off during the final 24 h because the exposure solutions turned gray as a result of the photoreduction of complexed silver. Mean silver concentrations were 371, 842, 1870, 3350, and 7190 µg/L in the exposure chambers and <0.2 µg/L in the control chambers.

Water Chemistry

Hardness, alkalinity, acidity, and pH were measured in the control, low, middle, and high concentrations following the methods described by the AMERICAN PUBLIC HEALTH ASSOCIATION (1975). Measurements were made once during each test except for the midge test in which water chemistries were analyzed from a pooled sample of all chambers. Mean values for 13 determinations of EDTA hardness, alkalinity, and acidity, expressed as mg/L of $CaCO_3$, were 44.3 ± 4.0, 43 ± 2, and 2.0 ± 0.7, respectively. Test water pH was 7.4 ± 0.2 (n=24). Dissolved oxygen was measured with an oxygen meter every 48 h in the control, low, middle, and high concentration chambers during each test. The mean, standard deviation, and range of percent dissolved oxygen were 94.0 ± 3.7 and 82.7 - 98.8 (n=34).

Silver Analytical Procedure

For the fish and scud tests, water samples were collected from each tank at 0 and 96 h. Half of the tanks were sampled at 24, 48, and 72 h. Samples were collected from all of the midge chambers after the solutions had completely dripped in and at the conclusion of the test. Water sample volumes for all tests ranged from 1 to 100 mL with subsequent dilution to 100 mL if required, depending on concentration. Samples were prepared and analyzed according to EPA Method #272.2 (U.S. EPA, 1979). Samples were not filtered or chemically treated to dissolve any precipitates prior to analysis. Silver concentrations were expressed as µg Ag/L, with a detection limit of 0.2 µg/L. The mean recovery of 24 spiked samples was 96.7 ± 9.0%. Percentage agreement for 12 duplicate samples was 95.9 ± 2.8.

Statistical Analysis

LC50 values for all tests were determined using mean measured concentrations on pooled replicate data by the Trimmed Spearman-Karber Method (HAMILTON et al. 1977).

RESULTS AND DISCUSSION

LC50 values extended over approximately three orders of magnitude with the four species tested (Table 1). There were no control mortalities in any of the tests. The 96 h LC50's were 10.7 µg/L for fathead minnows and 9.2 µg/L for flagfish. Most fish died shortly after they exhibited signs of stress, such as loss of equilibrium. All fathead minnows had bright red gills at death. LEMKE (1981) reported 96 h LC50's for fathead minnows to range from 3.9 - 12 µg/L in flow-through tests in water with hardness ranging from 40 - 49 mg/L as $CaCO_3$.

TABLE 1. Silver LC50 Concentrations and 95% Confidence Intervals for Pooled Duplicate Toxicity Tests with Fathead Minnows, Flagfish, Scuds, and Midges (µg/L)

Organism	24 h	48 h	72 h	96 h
Fathead minnow	15.2 (13.7-16.8)	11.6 (10.8-12.4)	10.7 (10.7-10.7)	10.7 (10.6-10.8)
Flagfish	43.7 (40.1-47.7)	25.9 (22.2-30.1)	15.8 (13.9-17.8)	9.2 (8.0-10.7)
Scud	4.7 (3.8-5.8)	4.7 (3.8-5.8)	4.5 (3.7-5.5)	4.5 (3.7-5.5)
Midge	5030 (4470-5650)	3160 (2490-4010)	n.d.[1/]	n.d.[1/]

[1/] No determinations were made beyond 48 h.

The 96 h LC50 for scuds was 4.5 µg/L. After 22 h of exposure to silver, all organisms in the two highest concentrations were dead. No more deaths occurred after 22 h. The scuds were much more sensitive than midges, which had a 48 h LC50 of 3160 µg/L. In water of similar hardness, *Daphnia magna* 48 h EC50's ranged from 0.39 to 2.9 µg/L (LEMKE 1981).

In order to obtain mortality with midges some concentrations were prepared to exceed the solubility of silver in exposure water. The midges were lightly blanketed with white precipitate and their movements stirred up the precipitate in the three highest ($\geq$1870 µg/L) exposure concentrations. The precipitate formed was most likely silver chloride (AgCl). With a $[Cl^-]$ in Lake Superior water of 1,200 µg/L (BIESINGER and CHRISTENSEN 1972) AgCl would precipitate at concentrations $\geq$ 596 µg Ag^+/L (25°C).

It is unknown how photoreduction of silver or sustained contact of the organism with the precipitate affect toxicity.

Silver has been shown to adsorb onto manganese dioxide, ferric hydroxide, clay minerals, and organic materials (KHARKAR et al. 1968; ANDERSON et al. 1973; CHAO and ANDERSON 1974; FREEMAN 1977). In a series of acute toxicity tests with rainbow trout, DAVIES et al. (1978) observed that the toxicity of silver decreased with an increase in water hardness. They proposed that this occurred because silver was more likely to complex into less toxic forms in hard water. LEMKE (1981) also reported that silver toxicity to rainbow trout, fathead minnows, and *Daphnia magna* was reduced in hard (300 mg/L $CaCO_3$) water. Because Lake Superior test water used in the present study was relatively soft (44.3 mg/L $CaCO_3$) and the concentrations of organic materials and particulates were also presumably low, the test water likely had a limited capacity for reducing silver toxicity by adsorption or complexation. The LC50's of this study would then represent values obtained under conditions resulting in relatively sensitive estimates of toxicity.

ACKNOWLEDGEMENTS

This work was funded through a Cooperative Agreement (CR 8064020) between the U.S. Environmental Protection Agency and the University of Wisconsin-Superior. This paper has been approved for publication by the Director of the Center for Lake Superior Environmental Studies as publication no. 47 of the Center series.

REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION: Standard Methods for the Examination of Water and Wastewater. 14 ed. Washington, D.C.: APHA (1975).

ANDERSON, B.J., E.A. JENNE, and T.T. CHAO: Geochim. Cosmochim. Acta 37, 611 (1973).

ANDERSON, R.L., C.I. WALBRIDGE, and J.T. FIANDT: Arch. Environ. Contam. Toxicol. 1, 329 (1980).

BIESINGER, K.E. and G.M. CHRISTENSEN: J. Fish. Res. Bd. Can. 29, 1961 (1972).

BIRGE, W.J., J.E. HUDSON, J.A. BLACK, and A.G. WESTERMAN, p. 97. In: D.E. Samuel, C.H. Hocutt, and W.T. Mason (eds.): Surface Mining and Fish/Wildlife Needs in the Eastern United States. U.S. Dept. Int. Fish Wildlife Serv., FWS/OBS - 78/81 (1978).

CHAO, T.T. and B.J. ANDERSON: Chem. Geol. 14, 159 (1974).

DAVIES, P.H., J.P. GOETTL, and J.R. SINLEY: Water Res. 12, 113 (1978).

FREEMAN, R.A.: Aquatic Toxicol., ASTM STP 667, 342; L. L. Marking and R.A. Kimerle, eds.; Amer. Soc. for Testing and Materials (1979).

HAMILTON, M.A., R.C. RUSSO, and R.V. THURSTON: Environ. Sci. Technol. 11, 714 (1977).

JOHANNSEN, D.A.: Aquatic Diptera. Part IV. Chironomidae: Subfamily chironominae. Cornell Univ., Agr. Exp. Stat. Memoir 210, 3 (1937).

KHARKAR, D.P., K.K. TUREKIAN, and K.K. BERTINE: Geochim. Cosmochim. Acta 32, 285 (1968).

LEMKE, A.E.: Interlaboratory Comparison: Acute Testing Set. EPA-600/S3-81-005. (1981).

MCKEE, J.E. and H.W. WOLF, Ed.: Water Quality Criteria. 2 ed. Sacramento, California: State Water Quality Control Board. (1963).

MOUNT, D.I. and W.A. BRUNGS: Water Res. 1, 21 (1967).

U.S. ENVIRONMENTAL PROTECTION AGENCY: EPA 600/4-79-020. Environmental Monitoring and Support Laboratory, Cincinnati, Ohio. (1969).

WINDHOLZ, M., S. BUDAVI, L.Y. STROUMTSOS, and M.M. FERTOG (eds.): The Merck Index: An encyclopedia of chemicals and drugs. 9th Ed. Rahway, N.J.: Merck & Co., Inc. (1976).

Accepted June 7, 1982