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Do we have the
One on White sucker?

ACUTE TOXICITY OF AMMONIA TO CHANNEL CATFISH

by

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INTRODUCTION

Ammonia is an important pollutant in natural waters both as a toxicant and as an oxygen demanding material. It commonly occurs in municipal and industrial waste discharges and in runoff from agricultural feed lots. Aqueous solutions of ammonia are very toxic to fishes under certain environmental conditions, and the toxicity is primarily attributed to the un-ionized form. The percent of total ammonia occurring in the un-ionized form depends upon pH, temperature and, to a lesser degree, ionic strength.

Frequency of occurrence and the high cost of treatment for adequate removal have made the issue of water quality criteria for ammonia very difficult. It is essential to develop a broad data base for ammonia toxicity to fish. Information on toxicity during all life stages is needed to insure protection throughout the entire life-cycle.

Early life stage tests are less costly and time consuming than entire life-cycle tests and may allow a realistic estimate of the maximum allowable concentration of a chemical to a fish species. However, acute toxicity data from tests conducted under conditions similar to early life stage tests are needed in order to determine realistic application factors. Information on the effects of exposure to ammonia on the egg and larval stages are available for channel catfish (Reinbold and Pescitelli 1982). Acute toxicity data exist for channel catfish but not under conditions similar to an early life stage test.

The purpose of this study was to determine 96-h LC50 values for juvenile channel catfish using the same dilution water and under similar conditions as tests conducted on the early life stages of this species (Reinbold and Pescitelli 1982).

Test Organisms

Two 96-h flow-through toxicity tests were conducted on juvenile (Age 1) channel catfish. The fish were obtained from Opel's Fish Farm, Worden, Illinois, where they were reared in outdoor ponds. Fish were transported to the INHS laboratory in 500 liters of pond water. Transport time was less than 4 hours. The transport water was gradually replaced with laboratory dilution water which had been adjusted to the proper temperature, and then the fish were placed into flow-through holding tanks. Ninety percent replacement time (Sprague 1973) for the holding tanks was never greater than 20 hours during the acclimation period. Catfish were held at the test temperature in the laboratory where the tests were conducted for at least 2 weeks prior to testing. During acclimation, the fish were fed a trout production diet (Rangen, Inc., Buhl, ID), two times a day, equalling approximately 3-4 percent of body weight. Two days after arrival, fish were treated with 150 mg/liter formalin for approximately 1 hour (American Society for Testing and Materials 1980) to prevent disease and eliminate external parasites. During acclimation, the photoperiod was increased by 15-minute increments every 2-3 days from 15 to 16 h. Mean size (and range) of channel catfish used in the test was 123 (99-145) mm and 12.8 (6.1-20.0) g.

Dilution Water

Dilution water was taken from municipal well at depths of 230 to 370 feet in the Mahomet-Teays aquafer near Champaign-Urbana, Illinois. The water was passed through two in-line charcoal filters to remove chlorine and through an ultraviolet sterilizer to eliminate microorganisms. It was then delivered through PVC pipes to a 670-liter stainless steel

holding tank. Sodium thiosulfate was metered into the tank to remove any trace of chlorine which might remain after charcoal filtration. Characteristics of the dilution water are listed in Table 1.

Dilution water was aerated in the holding tank and maintained above 90 percent of saturation. Water temperature in the tank was controlled by a thermister in conjunction with two solenoid valves which allowed hot or cold water to pass through a water jacket surrounding the tank.

Exposure Systems

For each test the dilution water was pumped through PVC pipes from the holding tank to a 0.5 liter proportional diluter, modified from Mount and Brungs (1967) and Lemke, Brungs and Halligan (1978), which was used to deliver a logarithmic series of five ammonia concentrations and a control through mixing chambers to two replicate test aquaria. The flow rate to each test chamber was 0.25 liter of water every 3 minutes. The test chambers used for the fish were constructed of glass and silicone sealant. Each aquarium measured 30 x 40 x 20 cm with an overflow outlet at a height of 25 cm and contained a volume of 20 liters. Test aquaria were placed in a stratified random arrangement. The tests were conducted in the laboratory in which fish were acclimated, and the test chambers were maintained in a water bath to control temperature fluctuations.

All apparatus, including holding tank and diluter, were cleaned prior to each test. Test chambers and other equipment coming in contact with test organisms were sterilized with hypochlorite before each test.

Reagent grade ammonium chloride was used as the toxicant. Stock solutions were prepared in glass distilled water and delivered to diluters from a Mariotte bottle. The pH of the stock solution was adjusted to that of the control chambers with sodium hydroxide solution.

Photoperiod was automatically controlled using a combination of

Table 1. Chemical characteristics of the dilution water.[a]

Al	<0.051
As	0.057
Ba	0.064
Ca	12.3
Cd	<0.002
Co	<0.004
Cr	<0.010
Cu	<0.008
Fe	<0.014
Hg	<0.00005
Mg	10.4
NI	<0.014
P	<0.097
Pb	<0.026
Se	<0.045
Si	2.64
Zn	0.011
Soluble orthophosphate	0.01
Total dissolved ionizable solids	137
Total organic carbon	5.6
Specific conductance (mhos/cm at 25°C)	180
Total residue	141
Chloride	8.1
Sulfate	1.87
Fluoride	1.0

[a]All values are in mg/liter unless otherwise stated.

incandescent and fluorescent (including wide spectrum Durotest "Vita Lite") light bulbs. For both tests a 16-h photoperiod was maintained, including a 30-minute gradual brightening and dimming to simulate dawn and dusk.

Analytical Procedures

Water quality parameters were measured by using standard methods (American Public Health Association et al. 1976, U.S. Environmental Protection Agency 1979). Water samples were taken from the center of each test chamber.

During each test, simultaneous measurements of total ammonia nitrogen, pH, and temperature were made in each test chamber at least once each day during the first 4 days of the test. Total ammonia nitrogen concentrations were determined by the phenate method (American Public Health Association et al. 1976) using a standard curve prepared by linear regression. Colorimetric measurements were made with a Coleman 124D double beam spectrophotometer. Un-ionized ammonia concentrations were determined from total ammonia nitrogen, pH, and temperature using the tables of Thurston et al. (1979). The pH in each test chamber was determined at least twice daily with an Orion 701A digital pH meter. Dissolved oxygen was measured with an oxygen specific electrode calibrated to titration accuracy (Altex 0260 oxygen analyzer by Beckman). Measurements were made at least three times per week.

Hardness, nitrate nitrogen, nitrite nitrogen and soluble orthophosphate were determined using a Technicon Autoanalyzer (U.S. Environmental Protection Agency 1979). Other water quality parameters such as alkalinity, conductivity, and TOC were determined according to analytical procedures described in American Public Health Association et al. (1976). Analyses of metals in the dilution water were performed by

induction-coupled argon plasma spectrometry (American Society for Testing and Materials 1980).

Test Procedures

The methodology for the tests generally followed that recommended in Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates and Amphibians (American Society for Testing and Materials 1980). The test organims were distributed into the test chambers one-at-a-time and were acclimated to the test chambers for 2 days while the diluter operated without toxicant addition. The test was initiated when toxicant addition was started.

Mortality was recorded after 1, 3, 6, 12 and 24 hours and at least daily thereafter to the end of the test. No mortality occurred in control tanks. Death was determined by absence of gill movement and the lack of response to gentle prodding. Total length and weight were measured individually as mortality occurred or at test termination. Fish were not fed during the tests.

The 96-h median lethal concentrations (LC50) and their 95 percent confidence intervals were calculated from the un-ionized ammonia-nitrogen values in the test chambers by using the Trimmed Spearman-Karber method (Hamilton, Russo, and Thurston 1977).

RESULTS AND DISCUSSION

The 96-h LC50 values and their 95 percent confidence intervals are reported in Table 11. Mean temperature and the range of pH values in all test chambers for each test are listed because of the importance of these variables in the equilibrium of ammonia in water, and mean values of other selected characteristics of the test water are also reported.

Table 2. Acute toxicity of ammonia to channel catfish. Conditions in test aquaria and 96-h LC50.

	Test I	Test II
Temperature[a](C°)	23.8 (22.8-24.3)	23.8 (22.6-24.9)
pH	7.75 - 8.20	7.77 - 8.12
Dissolved oxygen[a] (% saturation)	89 (72-97)	88 (73-95)
Total alkalinity (mg/l as $CaCO_3$)	114 (109-118)	104 (86-115)
Hardness (as $CaCO_3$)	59 (46-71)	58 (51-64)
Nitrate nitrogen (mg/l)	<0.02	<0.02
Nitrite nitrogen (mg/l)	<0.01	<0.01
96-h LC50 with 95% CL[b] (mg/l un-ionized ammonia-N)	1.45 (1.29-1.64)	1.44 (1.44-1.44)

[a]Values reported are mean values of all measurements in all tanks with ranges in parentheses.
[b]Confidence level.

Mortality data, including the approximate time death occurred, are given in Appendix A. The 96-h LC50 values for the two tests were 1.45 and 1.44 mg/liter of un-ionized ammonia nitrogen. The LC50 values found in this study at 23.8°C and a pH range of 7.75 to 8.20 compare with values reported by Roseboom and Richey (1977) of 1.5 and 3.0 mg/liter of un-ionized ammonia nitrogen for fish of a similar size at 22° and 28°C, respectively, at a pH range of 7.8 to 8.4 in a dilution water of greater hardness and alkalinity than that used in this study. Colt and Tchobanoglous (1978) reported a 96-h LC50 of 1.6 mg/liter of un-ionized ammonia nitrogen for smaller fingerlings (1 g) at 28°C and a pH range of 8.3 to 8.4.

In an earlier study of the effects of ammonia on early life stages of channel catfish, using the same dilution water as in the acute toxicity tests reported here, a delay in time to swim-up of catfish fry was observed at a concentration of un-ionized ammonia nitrogen as low as 0.05 mg/liter, and a significant reduction in growth was found at a concentration of 0.32 mg/liter, with no significant effect found at 0.17 mg/liter.

American Public Health Association, American Water Works Association, Water Pollution Control Federation. 1976. Standard Methods for the Examination of Water and Wastewater. 14th ed., American Public Health Association, Washington, D.C. 1193 pp.

American Society for Testing and Materials. 1980a. Annual Book of Standards, Part 31, Water. Philadelphia, Pennsylvania. 1401 pp.

American Society for Testing and Materials. 1980b. Standard Practice for Conducting Acute Toxicity Tests with Fishes, Macroinvertebrates, and Amphibians. American Society for Testing and Materials, Designation E 729-80.

Colt, J., and G. Tchobanoglous. 1978. Chronic exposure of channel catfish, Ictalarus punctatus, to ammonia: effects on growth and survival. Aquaculture 15:353-372.

Hamilton, M. A., R. C. Russo, and R. V. Thurston. 1977. Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays. Environ. Sci. Technol. 11:714-719; correction, ibid. 1978, 12:417.

Lemke, A. E., W. A. Brungs, and B. J. Halligan. 1978. Manual for construction and operation of toxicity-testing proportional diluters. EPA-600/3-78-072. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, Minnesota. 70 p.

Mount, D. I., and W. A. Brungs. 1967. A simplified dosing apparatus for fish toxicity studies. Water Res. 1:21-29.

Reinbold, K. A., and S. M. Pescitelli. 1982. Effects of exposure to ammonia on sensitive life stages of aquatic organisms. Report to U.S. Environmental Protection Agency, Region V, Chicago, Illinois (draft).

Rsoeboom, D. P., and D. L. Richey. 1977. Acute toxicity of residual

chlorine and ammonia to some native Illinois fishes. Report of Investigation 85, Illinois State Water Survey, Urbana, Illinois. 42 pp.

Sprague, J. B. 1973. The ABC's of pollutant bioassay using fish. In Biological Methods for the Assessment of Water Quality. ASTM STP 528, American Society for Testing and Materials. Baltimore, Mayland. pp. 6-30.

Thurston, R. V., R. C. Russo, and K. Emerson. 1979. Aqueous ammonia equilibrium-tabulation of percent un-ionized ammonia. EPA-600/3-79-091. U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, Minnesota. 428 pp.

Thurston, R. V., R. C. Russo, and G. A. Vinogradov. 1981. Ammonia toxicity to fishes. Effect of pH on the toxicity of the un-ionized ammonia species. Environ. Sci. Technol. 15:837-840.

U.S. Environmental Protection Agency. 1975. Methods for acute toxicity tests with fish, macroinvertebrates and amphibians. EPA-660/3-75-009. National Environmental Research Center, Corvallis, Oregon. 61 pp.

U.S. Environmental Protection Agency. 1979. Methods for chemical analysis of water. EPA-600/4-79-020. U.S. Environmental Protection Agency, Environmental Monitoring and Support Laboratory, Cincinnati, Ohio.

Appendix A. Acute toxicity of ammonia to channel catfish time/percent mortality observations.

Time (hours)	Tank numbers											
	4	12	3	7	6	10	5	9	2	8	I	II
						Test I						
NH_3-N,mg/l	4.85	4.50	2.60	2.66	1.43	1.46	0.93	0.87	0.60	0.56	controls	
24	100	100	0	0	0	0	0	0	0	0	0	0
48	100	100	50	30	0	0	0	0	0	0	0	0
72	100	100	100	90	0	0	0	0	0	0	0	0
96	100	100	100	100	80	30	0	0	0	0	0	0
						Test II						
NH_3-N,mg/l	3.48	3.60	2.92	3.34	1.75	1.89	1.09	1.19	0.60	0.51	controls	
24	100	100	70	90	0	0	0	0	0	0	0	0
48	100	100	100	100	0	20	0	0	0	0	0	0
72	100	100	100	100	80	90	0	0	0	0	0	0
96	100	100	100	100	100	100	0	0	0	0	0	0