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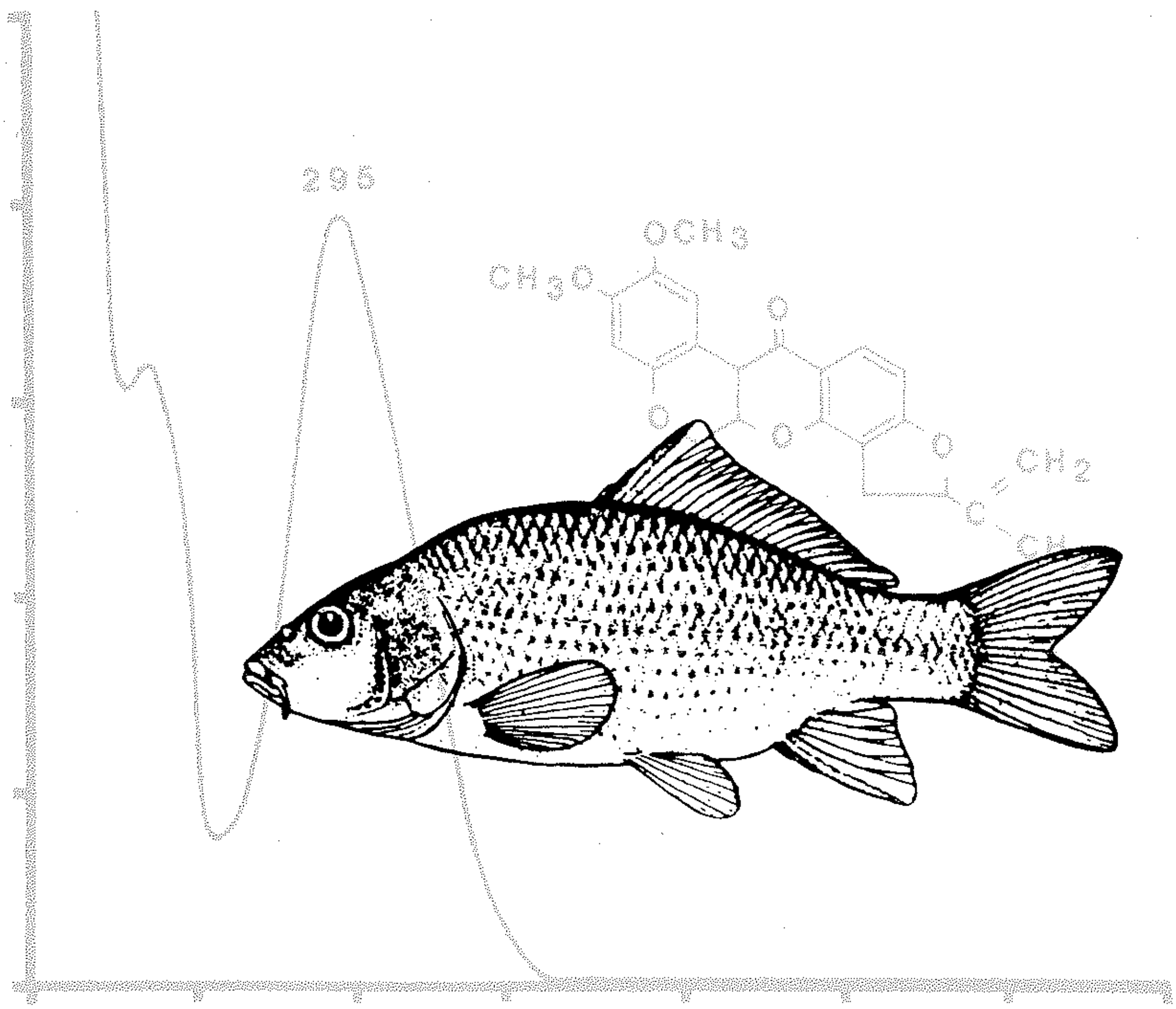
INVESTIGATIONS IN FISH CONTROL

96. Effects of Environmental Factors on the Toxicity of Chloramine-T to Fish

97. Effects of Organic Matter and Loading Rates of Fish on the Toxicity of Chloramine-T



UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

Effects of Environmental Factors on the Toxicity of Chloramine-T to Fish

by

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Abstract

The toxicity of chloramine-T (*n*-sodium-*n*-chloro-para-toluenesulfonamide), a therapeutant used to treat bacterial gill disease in fish, was evaluated under a variety of physical and chemical conditions. The toxicity (96-h LC50) was 2.80 mg/L for rainbow trout, *Salmo gairdneri*; 3.75 mg/L for channel catfish, *Ictalurus punctatus*; and 7.30 mg/L for fathead minnows, *Pimephales promelas*. Chloramine-T was more toxic in warm water than in cold water at exposures of 24 h or less, but temperature had no significant effect on toxicity in 96-h exposures. The chemical was slightly less toxic to rainbow trout in hard than in soft water, but water hardness had little influence on its toxicity to channel catfish. The pH was the most important factor affecting chloramine-T toxicity; the chemical was about 6 times more toxic at pH 6.5 than at 9.5. At levels ×1, ×3, and ×5 the recommended use concentration of 12 mg/L for 1 h, there was no significant mortality in rainbow trout and no abnormal responses were observed in treated fish. In rainbow trout, the toxicity of solutions of chloramine-T aged for 4 weeks was about half that of fresh solutions (deactivation index = 2.10).

Introduction

Chloramine-T (*n*-sodium-*n*-chloro-para-toluenesulfonamide) was shown by From (1980) to be effective for controlling bacterial gill disease (BGD), which is one of the most common diseases of hatchery-reared salmonids and causes more fish losses than any other bacterial disease. The disease is strongly mediated by the stressful environmental conditions and marginal nutrition commonly associated with intensive culture. Flavobacteria and flexibacteria are associated agents (Snieszko 1981). Gills damaged by BGD pathogens are also prone to secondary invasion by fungi (Warren 1981). Because BGD is a limiting factor in fish production, a control agent, such as chloramine-T, is needed for use on food fish. Approval by the U.S. Food and Drug Administration will be required.

Researchers have reported that the activity of chloramine-T is affected by pH, water hardness, and temperature. Tooby et al. (1975) found that the toxicity of chloramine-T to fish and eggs increased as pH and water hardness decreased. Cross and Hursey (1973) observed that chloramine-T was more toxic to fish in soft, acid waters than in hard, alkaline waters, and less toxic at 10° C than at high temperatures.

The purpose of the present study was to delineate the five factors that affect the toxicity of chloramine-T to selected species of fish: (1) sensitivity differences between selected coldwater and warmwater fish species; (2) effects of pH, water hardness, and temperature; (3) persistence of toxicity in solutions over a 4-week period; (4) toxicity and observed effects at recommended use pattern concentrations for rainbow trout (*Salmo gairdneri*); and (5) the time-independent toxicity.

Materials and Methods

Static and flow-through test procedures used in this study followed those prescribed by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975), ASTM Committee E-35 on Pesticides (1980), and U.S. Department of Agriculture (1986). We exposed 20 fish to each concentration of chloramine-T in glass jars containing 15 L of oxygen-saturated test water. Reconstituted test waters were prepared according to standardized procedures to produce the desired water quality. The pH of test waters was controlled with chemical buffers (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975). The solutions were adjusted to the desired pH before each test began and readjusted with chemical buffers at 24-h intervals, as needed, to maintain the selected pH (± 0.2 unit). Temperatures were regulated by immersing the test jars in constant temperature water baths. To assess the effects of water hardness, we buffered solutions to a constant pH with sodium bicarbonate using the procedure of Marking (1975).

Rainbow trout, fathead minnows (*Pimephales promelas*), and channel catfish (*Ictalurus punctatus*), obtained from a Federal fish hatchery or produced at the National Fisheries Research Center, LaCrosse, Wisconsin, were maintained according to the standard procedures for handling bioassay fish described by Hunn et al. (1968). The fish were acclimated to the desired water chemistries and temperatures for 24 h before each test. Mortalities were recorded at 1, 3, 6, 12, 24, 48, 72, and 96 h.

Two species (rainbow trout and channel catfish) were used in tests to determine the effects of water temperature, hardness, and pH on the toxicity of chloramine-T. In tests on the effects of use pattern levels, we exposed rainbow trout to chloramine-T (12 mg/L for 1 h) and to $\times 3$ and $\times 5$ levels, as prescribed in the IR-4 Guidelines for Investigation of Minor Use Drugs (U.S. Department of Agriculture 1986). We observed responses of fish after exposure for 14 days, using the criteria of Lennon and Walker (1964).

Commercial grade chloramine-T (lot 12605), obtained from Badger Pharmacal Inc. (Jackson, Wisconsin), was accompanied by a certificate of analysis that listed the assay as 100.03% available chlorine. When we tested the material, using the method provided by the manufacturer (U.S. Pharmacopeial Convention, Inc. 1979), the material yielded 98.4% available chlorine.

Concentrations of chloramine-T in test waters were determined by high performance liquid chromatography (HPLC) at 0, 6, 24, 48, 72, and 96 h. Samples were filtered through 0.45 μm Acrodiscs, and then injected directly onto the column with an automatic Waters Intelligent Sample Processor (WISP). Retention time was about 3.2 min. Quantification of the peaks was performed by a 730 Data Module having external standard calibration.

The Waters Associates, Inc., high performance liquid chromatography unit that we used consisted of a Model 481 Lambda-Max LC spectrophotometer, Model 510 pump, Model 710B WISP auto sampler, and 730 Data Module. The operating conditions were as follows: stationary phase, 30 cm $\times$ 4 mm Varian MicroPak MCH-10; mobile phase, acetonitrile: phosphate buffer (50:50, v/v); flow rate, 2.0 mL/min; chart speed, 2.0 cm/min; wavelength, 229 nm; and attenuation, 0.10 absorption units. A 0.45-μm disposal filter assembly was used to filter the sample.

Reagents were acetonitrile, HPLC grade; water, HPLC grade; phosphoric acid, American Chemical Society reagent grade 0.2 M, 13.6 mL diluted to 1 L with HPLC water; monobasic potassium phosphate, American Chemical Society reagent grade 0.2 M, consisting of 27.2 g of KH_2PO_4 diluted to 1 L with HPLC water; and buffer reagent, 0.1 M, consisting of 14.3 mL of 0.2 M H_3PO_4 + 10.7 mL of 0.2 M KH_2PO_4 diluted to 500 mL (pH = 2.6).

In tests to determine the persistence of chloramine-T in water, we aged solutions in glass containers under routine laboratory conditions with 12-h photoperiods for as long as 4 weeks. Residual concentrations of chloramine-T in the aged solutions were determined analytically each week and the toxicity was compared with that of fresh solutions. Deactivation indices were calculated by dividing the 96-h LC50 of the aged solutions by the 96-h LC50 of the fresh solutions (Marking 1972).

The methods of Litchfield and Wilcoxon (1949) were used to compute the LC50's and 95% confidence intervals. Time-independent LC50's (TILC50) were calculated according to the method of Green (1965).

Results and Discussion

Toxicity to Selected Species of Fish

Chloramine-T was toxic to all species exposed in soft water; the 96-h LC50's (mg/L) were 2.80 for rainbow trout, 3.75 for channel catfish, and 7.30 for fathead minnows (Table 1). Rainbow trout (coldwater) and channel catfish (warmwater) were thus twice as sensitive as fathead minnows (warmwater).

Table 1. *Toxicity of chloramine-T to three species of fish in soft water at 12° C.*

Species	Lot	Source[a]	Mean weight (g)	96-h LC50 and 95% confidence interval (mg/L)
Rainbow trout	8640	Erwin NFH	0.73	2.80 2.41–3.26
Fathead minnow	8631	LaCrosse NFRC	1.11	7.30 6.71–7.94
Channel catfish	8632	LaCrosse NFRC	1.50	3.75 3.30–4.26

[a]NFH = National Fish Hatchery; NFRC = National Fisheries Research Center.

Influence of Temperature, Water Hardness, and pH

The toxicity of chloramine-T was affected by water characteristics, and alterations in toxicity were consistent between the two species. The chemical was more toxic in warm water than in cold water in exposures of 24 h or less. For example, the 3-h LC50 for rainbow trout was 28.0 mg/L at 17° C and 43 mg/L at 12° C; at 7° C there was no mortality at 60 mg/L (Table 2). Channel catfish did not respond as quickly to the chemical; there was no mortality in 3 h at 60 mg/L at any temperature (Table 3). After 6 h at temperatures of 22, 17, and 12° C, the LC50's were 14.0, 25.3, and >60.0 mg/L, respectively. Temperature apparently affected the time of response rather than the ultimate toxicity of chloramine-T; the 96-h LC50's did not differ significantly for either species.

Water hardness had only a slight effect on the toxicity of chloramine-T to rainbow trout and no effect on channel catfish (Tables 2 and 3). For rainbow trout, the 96-h LC50 was 7.35 mg/L in very soft water (10–13 mg/L as $CaCO_3$) and 14.2 mg/L in very hard

Table 2. *Toxicity (LC50, mg/L and 95% confidence interval) of chloramine-T to rainbow trout in water of different temperatures, water hardnesses, and pH levels.*

Temperature (°C)	Hardness	pH	Duration of test (h): 1	3	6	12	24	96
7	Soft	7.5	>60.0	>60.0	28.0 22.8–34.4	18.0 15.3–21.1	11.7 10.5–13.0	4.30 3.69–5.01
12	Soft	7.5	>60.0	43.0 36.9–50.1	17.5 14.8–20.7	14.0 11.3–17.3	6.90 6.31–7.54	2.80 2.41–3.26
17	Soft	7.5	>60.0	28.0 22.7–34.5	10.0 9.07–11.0	8.00 7.57–8.46	4.90 4.48–5.35	2.80 2.27–3.46
12	Very soft	8.1	>60.0	>60.0	>60.0	38.7 33.9–44.2	20.2 17.7–23.0	7.35 6.92–7.80
12	Soft	8.1	>60.0	>60.0	>60.0	47.5 — —	25.4 21.0–30.6	9.00 9.33–9.72
12	Hard	8.1	>60.0	>60.0	>60.0	45.0 38.5–52.6	25.0 20.7–30.1	10.0 9.04–11.1
12	Very hard	8.1	>60.0	>60.0	>60.0	49.0 — —	25.5 21.2–30.7	14.2 12.2–16.5
12	Soft	6.5	55.8 48.4–64.3	13.5 11.2–16.2	8.22 7.71–8.76	6.05 5.17–7.08	2.81 2.41–3.27	1.89 1.63–2.19
12	Soft	8.5	>60.0	>60.0	>60.0	55.7 49.1–63.1	46.0 39.5–53.6	11.0 9.78–12.4
12	Soft	9.5	>60.0	>60.0	>60.0	>60.0	>60.0	10.8 9.96–12.2

Table 3. *Toxicity (LC50, mg/L and 95% confidence interval) of chloramine-T to channel catfish in water of selected temperatures, water hardnesses, and pH levels.*

Temperature (°C)	Hardness	pH	Duration of test (h)					
			1	3	6	12	24	96
12	Soft	7.5	>60.0	>60.0	>60.0	14.2 12.2–16.5	10.0 9.07–11.0	3.75 3.30–4.26
17	Soft	7.5	>60.0	>60.0	25.3 21.0–30.4	14.2 12.2–16.5	7.20 6.78–7.64	3.73 3.29–4.22
22	Soft	7.5	>60.0	>60.0	14.0 12.0–16.3	9.00 8.51–9.51	5.63 5.08–6.24	3.80 3.33–4.34
12	Very soft	8.1	>60.0	>60.0	>60.0	>60.0	28.0 24.1–32.6	7.70 6.98–8.49
12	Soft	8.1	>60.0	>60.0	>60.0	>60.0	30.5 26.3–35.4	11.0 9.13–13.2
12	Hard	8.1	>60.0	>60.0	>60.0	>60.0	33.0 29.2–37.3	7.80 6.53–9.31
12	Very hard	8.1	>60.0	>60.0	>60.0	>60.0	37.0 32.7–41.8	9.80 8.64–11.1
12	Soft	6.5	>60.0	27.0 21.0–35.0	10.0 9.07–11.0	5.60 5.21–6.01	2.85 2.52–3.22	1.75 1.48–2.06
12	Soft	8.5	>60.0	>60.0	>60.0	>60.0	51.5 46.8–56.7	10.5 9.14–12.1
12	Soft	9.5	>60.0	>60.0	>60.0	>60.0	>60.0	12.3 9.75–15.5

water (280–320 mg/L as $CaCO_3$). Tooby et al. (1975) and Cross and Hursey (1973) reported that chloramine-T was more toxic in soft, acid waters than in hard, alkaline waters, but they did not isolate the effects of hardness from those of pH.

The toxicity of chloramine-T increased significantly as the pH of test waters decreased for both species (Tables 2 and 3). At pH 9.5, no rainbow trout or channel catfish died after 24 h of exposure to 60 mg/L, but the chemical was about 6 times more toxic to both species at pH 6.5. The 96-h LC50's for rainbow trout and channel catfish were 1.89 and 1.75 mg/L in acid water (pH 6.5) compared with 10.8 and 12.3 mg/L in alkaline water (pH 9.5), respectively (Tables 2 and 3).

Use Pattern Exposure

Responses of rainbow trout exposed to chloramine-T for 1 h to ×1, ×3, and ×5 the recommended use pattern concentration (12 mg/L) did not differ from those of control fish during the 14-day posttreatment recovery period. These fish were exposed in soft water at 12° C and pH 7.5. Therefore, there was little chance of causing mortality with overtreatment.

Persistence

The toxicity of aged solutions of chloramine-T decreased with aging (Table 4). Toxicity to rainbow trout was decreased by a factor of 1/2 after 4 weeks; the 96-h

Table 4. *Toxicity and deactivation index for chloramine-T for rainbow trout in soft water at 12° C.*

Aging period (weeks)	96-h LC50 (mg/L) and 95% confidence interval	Deactivation index[a]
0	2.85 2.57–3.16	1.0
1	4.10 3.54–4.72	1.44
2	3.80 3.34–4.32	1.33
3	8.00 6.15–10.4	2.81
4	6.00 5.27–6.82	2.10

[a] $\frac{\text{LC50 of aged solution}}{\text{LC50 of fresh solution}}$

Table 5. *HPLC analysis of chloramine-T concentrations of 6, 10, and 40 mg/L (calculated) remaining in soft water without fish at 12° C for periods as long as 4 weeks.*

Age of solution at sampling time (weeks)	Calculated concentration (mg/L)														
	6	10	40	6	10	40	6	10	40	6	10	40	6	10	40
0	6.12	10.26	40.59	5.84	9.88	39.33	5.78	9.58	37.94	5.88	9.87	39.60	5.94	10.02	39.65
1	5.82	9.82	39.60	5.44	9.37	37.46	5.76	9.73	39.06	5.83	9.85	39.75			
2	5.43	9.34	37.48	5.62	9.38	39.22	5.89	9.87	40.29						
3	5.41	9.33	38.98	5.58	9.66	41.11									
4	5.50	9.56	40.81												

LC50 was 6.00 mg/L, in comparison with 2.85 mg/L for freshly prepared solutions (deactivation index = 2.10).

In analytical checks of the concentrations of chloramine-T made at weekly intervals throughout the aging experiment, the measured concentrations (Table 5) did not decrease as rapidly as the toxicity of the aged solutions. For example, the 6 mg/L (calculated) concentration was measured at 6.12 mg/L at time 0 and 5.50 mg/L after 4 weeks of aging. For the same aging period, the toxicity decreased by a factor of 2. When calculated exposure concentrations of 6, 10, and 40 mg/L were analyzed by HPLC at the beginning of all tests to verify the accuracy of the chemical additions, the means and standard deviations (mg/L) for the three analyzed concentrations from 40 tests were 5.90±0.17, 9.89±0.27, and 38.9±1.39.

Time-independent Toxicity

Rainbow trout exposed to chloramine-T in flow-through tests were considerably more resistant than those exposed in static tests. In two separate exposures, the 96-h LC50's were 23.0 and 30.0 mg/L in flow-through tests and 7.0 and 4.6 mg/L in static tests (Table 6). We speculate that chlorine is released from chloramine-T more completely under static conditions and believe that this increased release causes the higher toxicity. Mortalities of fish exposed to chloramine-T did not continue beyond 96 h; the time-independent LC50's were similar to the 96-h LC50's in flow-through toxicity tests (Table 6).

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Table 6. *Acute and chronic (time-independent) toxicity of chloramine-T to rainbow trout in well water at 12° C.*

Fish source and type of test	LC50's (mg/L) and 95% confidence intervals: 96 h	TILC50[a]
Lot 8675		
Static	4.60	—
	4.14–5.11	— —
Flow-through	30.0	24.3
	27.6–32.6	21.5–27.5
Lot 8706		
Static	7.00	—
	6.44–7.61	— —
Flow-through	23.0	23.0
	20.2–26.2	20.1–26.2

[a]Time-independent LC50.

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Effects of Organic Matter and Loading Rates of Fish on the Toxicity of Chloramine-T

by

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Abstract

Chloramine-T (*n*-sodium-*n*-chloro-para-toluenesulfonamide) is effective for the control of bacterial gill disease in fishes but data on its toxicity and safety are lacking. We examined the effect of fish loading rates, feed levels, and fecal material on the availability and toxicity of the chemical. Its toxicity increased (24-h LC50's were 36 mg/L and 14 mg/L) as loading rates of fathead minnows (*Pimephales promelas*) increased from 0.52 to 2.07 g/L of water. The presence of fish food decreased the toxicity of the chemical. The 96-h LC50 was 7.30 mg/L without feed and 14.2 and 20.8 mg/L after the addition of 0.07 and 0.20 g/L of fish food. The presence of fecal material also

. Concentrations in water decreased more rapidly
l matter was present. The decreases were greatest
l material.

Bills, Terry D., Leif L. Marking, Verdel K. Dawson, and Jeffery J. Rach. 1988. **Effects of environmental factors on the toxicity of chloramine-T to fish.** U.S. Fish Wildl. Serv., *Invest. Fish Control* 96. 6 pp.

The toxicity of chloramine-T (*n*-sodium-*n*-chloro-para-toluenesulfonamide), a therapeutant used to treat bacterial gill disease in fish, was evaluated under a variety of environmental conditions. The 96-h LC50 (mg/L) was 2.80 for rainbow trout, *Salmo gairdneri*; 3.75 mg/L for channel catfish, *Ictalurus punctatus*; and 7.30 mg/L for fathead minnows, *Pimephales promelas*. Chloramine-T was more toxic in warm water than in cold water only at exposures of 24 h or less; it was about 6 times more toxic at pH 6.5 than at 9.5, but nontoxic at levels ×5 the use pattern level (12 mg/L; 1 h), and detoxified to half the original activity in about 4 weeks.

Key words: Toxicity, chloramine-T, pH, water hardness, temperature.

(1973) demonstrated that chloramine-T lost its activity in the presence of excessive organic matter and showed that its toxicity to fish was greater in soft, acid waters than in hard, alkaline waters. Bills et al. (1988) reported that the acute toxicity of chloramine-T was greater at a given concentration in warm than in cold water and that the compound was more toxic in acid than in alkaline water.

The purpose of the present study was to determine (1) the effects of fish loading rates, feed levels, and fecal material on the toxicity of chloramine-T to fish; and (2) the available concentration of chloramine-T in water containing fish, feed, and fecal material.

Materials and Methods

Static test procedures used in this study followed those prescribed by the Committee on Methods for Toxicity with Aquatic Organisms (1975), ASTM Committee E-35 on Pesticides (1980), and U.S. Department of Agriculture