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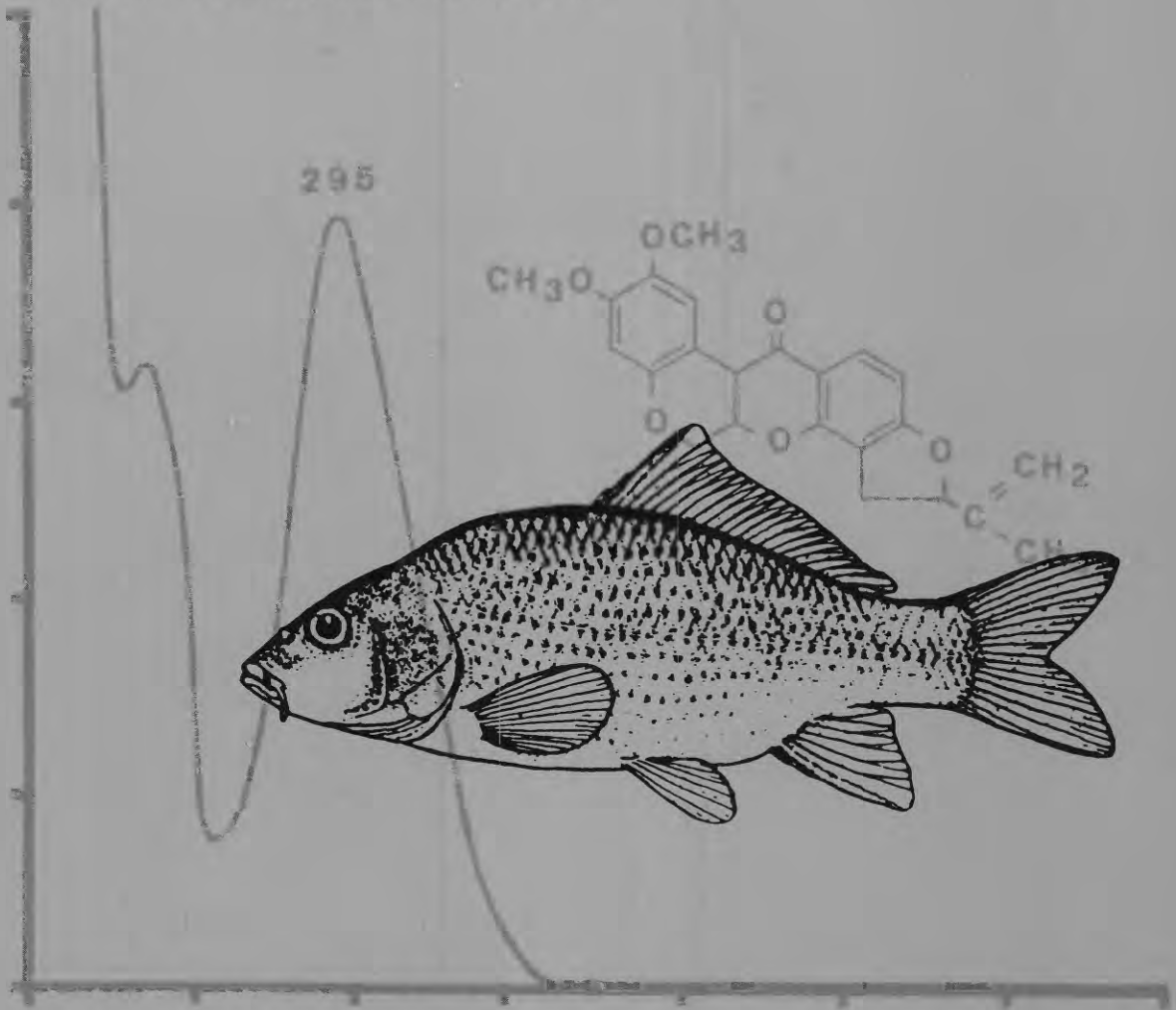

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

Investigations in Fish Control, published by the Fish and Wildlife Service, include reports on the results of work at the National Fisheries Research Center at La Crosse, Wisconsin, and reports of other studies related to fish control and acute toxicity of piscicides. Though each report is regarded as a separate publication, several may be issued under a single cover, for economy. See inside back cover for list of current issues.

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ISSN 0565-0704

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U.S. Fish and Wildlife Service
National Fisheries Research Center—La Crosse
P.O. Box 818
La Crosse, Wisconsin 54602

1990

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by

Philip A. Gilderhus

U.S. Fish and Wildlife Service
National Fisheries Research Center—La Crosse
P.O. Box 818
La Crosse, Wisconsin 54602

ABSTRACT.—Because concerns have been expressed about the effects of irrigating truck-garden crops with water from a stream treated with the lampricide 3-trifluoromethyl-4-nitrophenol (TFM), I conducted studies on the effects of TFM on young plants of common vegetables and fruits. Plants established in horticultural flats were irrigated for 12 h with water containing 10 mg/L of TFM and later compared with plants irrigated for a similar period with untreated water. Lettuce, radish, sweet corn, and potato plants were virtually unaffected. Green bean and tomato plants developed brown or dead spots on many leaves but growth rates and survival were not affected. Cucumber and cantaloupe plants were severely damaged; some were killed and about 40% of the leaves on surviving plants were dead or dying. Two weeks after treatment, the mean weight of surviving treated cantaloupe plants was significantly less than that of control plants.

The parasitic sea lamprey (*Petromyzon marinus*) is controlled in the Great Lakes by treating more than 400 nursery streams with the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) to kill the larvae. Some of these tributaries flow through agricultural land, where the stream water is used to irrigate truck-garden crops. Farmers have been reluctant to forego irrigation while a stream is being treated, without evidence that TFM would damage their crop. Lack of irrigation for even 1 day reportedly can affect crop growth, delay marketing, and prevent a farmer from getting full market price for a product. To date, sea lamprey control agencies have had no specific information to give to farmers about potential damage to their crops from using TFM-treated irrigation water.

It is known that TFM has adverse effects on aquatic plants ranging from reduced production after exposure to 10 mg/L for 1 h to plants becoming limp and cyanotic after exposure to 20 mg/L for 3 h (National Research Council of Canada 1985). No studies of the effects of TFM on terrestrial plants have been published. In response to requests from control agents for information on how TFM affects terrestrial plants, I conducted studies at the National Fisheries Research Center, La Crosse, Wisconsin, to determine if irrigation with water containing TFM is deleterious to selected truck-garden plants.

Methods and Materials

Plants of eight common vegetables and fruits were raised in horticultural flats filled with sandy loam soil. Lettuce, radish, green bean, cucumber, and cantaloupe plants were started from seed in flats 53 × 27 cm and 6 cm deep. Corn and potatoes, which need deeper soil, were planted in flats 36 × 33 cm and 15 cm deep. Seedling tomato plants were transplanted from a garden plot into flats that were divided into sections 8 cm square and 6 cm deep. The plants were allowed to grow until they were well established (25 to 35 days after planting). Their sizes and the numbers used are listed in the Table.

The studies were conducted in a series of vertical-walled 0.005-ha concrete ponds (Figure). Two ponds held treatment water and the plants were kept in two unfilled ponds. For treatment, the plants were placed on racks with an oscillating sprinkler between the racks at the same height. A submersible pump transferred fresh water or

Table. *Size and number of plants used and effects of irrigating them with water containing TFM.*

Plant	Height of plants (cm)[a]	Plants per pan	Pans per treatment	Damage from TFM exposure[b]
Lettuce	8–10	15	3	None
Radish	8–10	15	3	None
Green bean	8–10	15	2	Leaf spotting
Sweet corn	25–30	16	3	None
Potato	25–30	4	3	None
Tomato	15–18	6	3	Leaf spotting
Cucumber	13–18	9	3	Severe
Cantaloupe	13–18	15	2	Severe

[a]At time of treatment.
[b]See text for details.

water containing TFM (field grade; 39.9% active ingredient) from the sump of the reservoir pond to the sprinkler (Figure). The spray crossed each rack twice per minute. Treatments were designed to simulate the maximum exposure likely to occur; the plants were sprayed with water containing 10 mg/L of TFM (calculated as the sodium salt) for 12 h. Controls were sprayed with clear water during the same period. Except during treatment, the plant flats were kept on the floor of the pond and covered with perforated aluminum screen to partly shield them from direct sunlight. Plants were watered daily before and after treatment, except when there was adequate rain. The water was applied from above the plants with a fine-spray watering can.

Mean weights of treated and control plants of green beans and cantaloupe were compared by use of the Student's *t* test.

Results

Lettuce, radish, sweet corn, and potato plants showed no adverse effects from the application of TFM. Green bean and tomato plants showed some damage.

At the time of treatment, the green bean plants were 8 to 10 cm high, each with two large unifoliate leaves. By 6 days after the TFM treatment, 70% of the leaves had brown spots of dead tissue that covered as much as 50% of individual leaves (average, about 15%). After 12 days, the dead spots on treated leaves were about the same as after 6 days, and all treated plants were growing and adding new trifoliate leaves at the same rate as the controls. Fifteen days after the first treatment, the bean plants were subjected to a second treatment to provide data on more advanced plants. The results were about the same as in the first treatment; about 90% of the new leaves sustained some damage (brown, dead spots) but all plants continued to grow. Two weeks after the second treatment, the plants were cut off at the ground and weighed to the nearest 0.1 g. Mean weights of treated and control plants ($N = 16$) did not differ significantly.

Two days after treatment, 50% of the leaves on treated tomato plants had numerous small brown spots; 4 to 5 days after treatment, the tomato plants began to look healthier and began developing new leaves; and 8 days after treatment, the treated plants were growing and blooming at about the same rate as the controls.

Cucumber and cantaloupe plants sustained severe damage from irrigation with water containing TFM. Some damage to cucumber plants was apparent 20 h after treatment, as evidenced by brown spots on some of the leaves. Two days after treatment, 60–70% of the leaves had large brown spots of dead tissue. At 4 days after treatment, five plants were dead and the brown areas on live plants were drying out. By 8 days after treatment, 40% of the leaves on surviving treated plants were dead or dying, but the plants continued to grow at their tips and produced blossoms. Overall, cucumbers sustained major damage; 20% of the plants were killed and 40% of the leaves were severely damaged on surviving plants.

Cantaloupe plants were vigorous and healthy at the time of treatment. At 6 days after treatment, 75% of the leaves on treated plants showed damage (brown spots), two plants were dead, and two were nearly dead. After 2 weeks all of the treated plants looked unhealthy; 40% of the leaves showed damage and new leaves were small and pale. The control plants were growing well, appeared healthier, and had added large, dark green leaves. At 2 weeks after treatment, the mean weight ($N = 16$) of treated plants (10.8 g) was 33% less than that of controls (16.2 g). The difference was highly significant ($P < 0.01$).

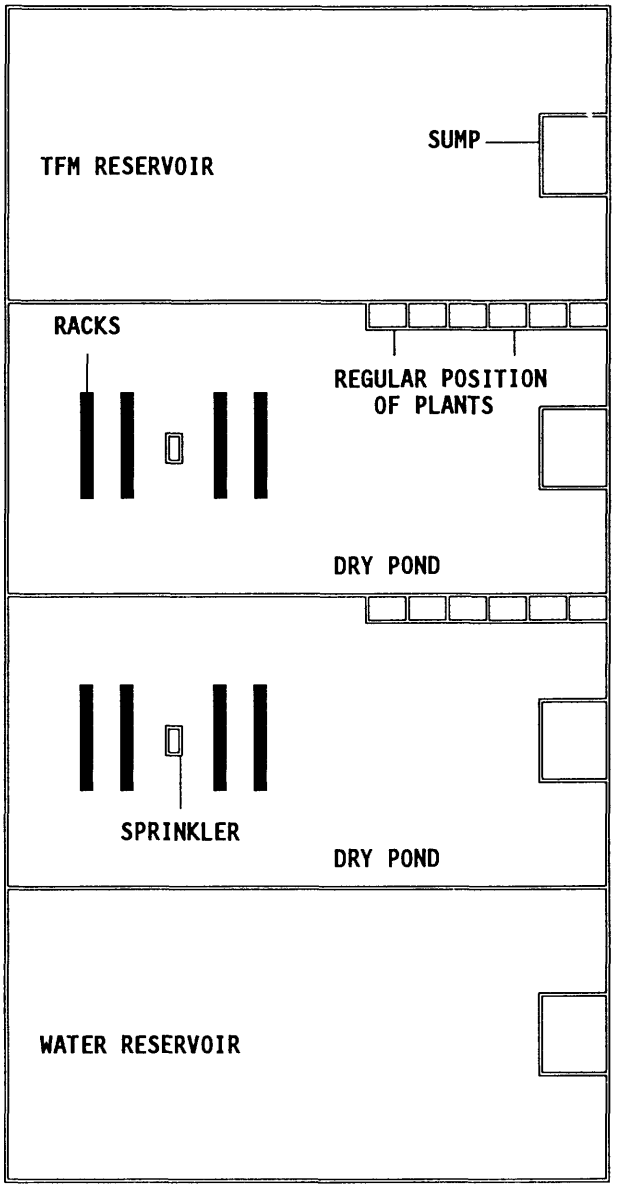

Figure. Schematic diagram of 0.005-ha ponds used for irrigating plants with water containing TFM.

Discussion

The treatment regimen used in these studies represented the maximum exposure that might occur when plants are irrigated with water from streams treated with TFM, and the length of treatment probably exceeded normal irrigation periods. Irrigation for a shorter period or at a lower concentration of TFM would probably show proportionately less effect on plants.

Although green beans and tomatoes showed no apparent growth inhibition, there was sufficient damage to individual leaves to indicate that exposure of these plants to TFM should be avoided. The severe damage sustained by cucumber and cantaloupe plants indicated that these plants and perhaps all plants of the family Cucurbitaceae should not be irrigated with water containing TFM. Because there is a wide variation in effects between types of plants, the best policy for types that have not been tested is to avoid irrigating them with water containing TFM.

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ABSTRACT.—Residues of malachite green in muscle, eggs, and fry of Atlantic salmon (*Salmo salar*) and chinook salmon (*Oncorhynchus tshawytscha*) were determined by colorimetric analysis after the fish had been routinely treated with the chemical at fish hatcheries (10–47 times, 1 ppm for 1 h). The concentration of residues in fish muscle generally depended on the elapsed time since the last treatment; concentrations were usually highest (about 1.0 to 2.5 µg/g) in fish sampled 1 or 2 days after the last treatment and had declined somewhat to values as low as 0.33 µg/g after 18–41 days. Residues in eggs taken from adults that had been treated with malachite green were about 0.1 to 4.2 µg/g in Atlantic salmon and 0.1 to 1.0 µg/g in chinook salmon; there was little relation between the residue concentrations in the eggs and the elapsed time since the last treatment. Residues of malachite green in fry newly hatched from eggs of treated chinook salmon ranged from 0.14 to 1.16 µg/g.

Malachite green has been used extensively in fish culture (usually in the zinc-free oxalate form) for the control of fungal infections and external parasites (Nelson 1974; Alderman 1985). It is the most effective antifungal treatment used in aquaculture (Meyer and Hoffman 1976; Bailey 1983; Alderman 1985). It has never been registered for use on food fish (such use was banned in 1978) because of potential health risks; use is currently limited to the treatment of nonfood fish (e.g., salmon brood stock) under an Investigational New Animal Drug Application issued by the U.S. Food and Drug Administration.

The potential threat of malachite green to human health was first pointed out by Werth and Boiteux (1958), who described marked increases in the incidence of internal tumors in the progeny of laboratory rats fed malachite green. Meyer and Jorgenson (1983) reported a significant increase in gross abnormalities in rainbow trout (*Oncorhynchus mykiss*) hatched from eggs treated with malachite green at concentrations of 1 mg/L for 1 h daily (30 applications); 3 mg/L for 1 h every other day (15 applications), and 5 mg/L for 1 h weekly (5 applications). They also reported that malachite green produced significant teratological effects at all levels of treatment when it was administered orally to New Zealand white rabbits at doses as low as 5 mg/kg body weight. Although a different delivery system and a higher dose rate were used in the rabbits than are normally used in fish culture, the existence of a serious potential hazard is obvious.

Information on residues of malachite green that might occur in fish flesh must be determined as part of the evaluation of its use. Poe and Wilson (1983), who reported the presence of malachite green in channel catfish (*Ictalurus punctatus*) after treatment, wrote that a greenish color appeared on the surface of the catfish muscle tissue after frozen storage for 13 to 60 days; and T. D. Bills (personal communication) indicated that a solution of 85% ethyl alcohol, 10% formalin, and 5% acetic acid (AFA) that was used to preserve fish exposed to malachite green developed a bluish color. Thus, the presence of malachite green can be determined by colorimetric analysis.

Allen and Hunn (1986) reported preliminary results of malachite green analysis after residues were extracted with AFA from muscle tissue of rainbow trout and muscle tissue and eggs of Pacific salmon (*Oncorhynchus* sp.). Colorimetric analysis showed that muscle of rainbow trout exposed to 0.1 mg/L of malachite green for 24 h at 14° C contained 1.87 µg/g of malachite green; the residue

declined to 0.22 µg/g in muscle after 6 days of withdrawal in fresh water. Residues in eggs from these fish ranged from 0.1 to 4.1 ppm. In the present study, I used AFA extraction and colorimetric analysis to examine adult salmonids treated with malachite green to obtain further data on residues of malachite green in the muscle tissue and eggs and, especially, newly hatched fry. The analyses were done on fish from national fish hatcheries treated and sampled under hatchery conditions.

Materials and Methods

Adult Atlantic salmon (*Salmo salar*) at the Berkshire (Massachusetts) National Fish Hatchery (NFH) were treated on 5 consecutive days each week 20 to 47 times with 1 ppm of malachite green for 1 h. Samples of muscle tissue and eggs were collected from 1 to 41 days after the last treatment of these fish, as well as from untreated adults (to determine background readings of malachite green).

Adult chinook salmon (*Oncorhynchus tshawytscha*) from the Warm Springs (Oregon) NFH and Winthrop (Washington) NFH were also treated on 5 consecutive days each week with 1 ppm of malachite green for 1 h. The fish at Warm Springs NFH were treated 27 to 30 times and samples of muscle tissue were collected 1, 6, 12, or 18 days after the last treatment; those at Winthrop NFH received 10 treatments at various intervals and were sampled 8 days after the last treatment. At both hatcheries, eggs were collected at fertilization (0 h) and 24 h after fertilization, and fry were sampled 24 h after hatching. Muscle tissue, eggs, and fry from untreated adults were collected at Warm Springs NFH to provide background values.

Samples of fish tissue, eggs, and fry from the several hatcheries were frozen and shipped to the La Crosse (Wisconsin) National Fisheries Research Center for analysis. I extracted 10-g samples (wet weight) with 25 mL of AFA at room temperature in the dark for 24 h, centrifuged samples at about 2,000 rpm, and filtered them through a glass fiber filter. The absorbance of sample extracts at 615 nm was determined on a Beckman DU6 spectrophotometer and compared to the absorbance of malachite green oxalate standards in AFA. Residue concentrations are reported as µg/g malachite green oxalate.

Results and Discussion

The presence of malachite green residues in the fish eggs and fry was readily apparent from the blue-green color of the tissue extracts. Because fish and eggs have a naturally occurring yellowish color, malachite green residues are seen as green by the human eye. The presence of naturally occurring colors also produced a low background reading in the untreated fish, eggs, and fry, but too few untreated samples were tested to establish a useful mean background reading for subtraction from the sample residue concentrations. Background color readings in the muscle from two untreated adult Atlantic salmon were 0.09 to 0.15 µg/g (malachite green equivalents) and 0.10 µg/g in the eggs of one (Table 1). The residue concentrations reported here include the background values.

Residues reported as malachite green in Atlantic salmon showed no relation between the number of treatments received and the concentration of residue in the muscle (Table 1). Residues ranged from 0.33 to 2.54 µg/g. The residue concentrations were lowest (0.33, 0.35 µg/g) in muscle from two adults that received the fewest treatments (20) and were sampled 24 and 33 days after the last treatment; however, the residue concentration in the muscle of one other adult was 2.54 µg/g after 20 treatments. Eggs from treated fish contained 0.21 to 4.16 µg/g of residual malachite green.

Chinook salmon from Warm Springs NFH tested for malachite green residues had received 27 to 30 treatments of 1.0 ppm for 1 h and were sampled at 1, 6, 12, and 18 days after the last treatment. Background color readings were 0.32 and 0.35 µg/g in muscle tissue, 0.03 to 0.08 µg/g in eggs, and 0.22 µg/g in fry (Table 2). The mean concentration of malachite green in muscle of the treated fish ranged from 0.473 to 1.08 µg/g. Residues in the muscle were slightly higher in fish sampled at 1 day after the last treatment than in those sampled after 6–18 days.

The mean concentration of malachite green in eggs taken from adults after different frequencies of treatment ranged from 1.16 to 1.86 µg/g. In eggs sampled 24 h after fertilization, the average levels ranged from 0.84 to 1.54 µg/g. In fry from treated adults, average residues ranged from 0.97 to 1.16 µg/g.

The mean concentration of malachite green in muscle of adult salmon from Winthrop NFH that had been treated 10 times at 1.0 ppm for 1 h was 0.43 µg/g (Table 2). The mean concentration in eggs was 0.173 µg/g at collection and 0.113 µg/g in 24 h; the average concentration in the fry was 0.14 µg/g.

In general, malachite green seemed to be readily taken up by adult salmon treated with it and was incorporated into their eggs before spawning. Residues in the fry hatched from eggs of treated fish indicated that little of the chemical was lost during incubation.

Table 1. *Residues of malachite green oxalate (μg/g) in muscle and eggs of Atlantic salmon subjected to 20 to 47 1-h treatments with 1 ppm of malachite green at the Berkshire National Fish Hatchery. Each row of data represents one fish.*

Time after last treatment (days)	Number of treatments at 1 ppm	Sample	
		Muscle	Eggs
No treatment	0	0.15	0.10
No treatment	0	0.09	—
1	42	1.66	3.15
1	45	2.13	3.26
1	47	—	4.16
2	20	2.54	2.51
3	45	—	3.60
5	20	1.11	0.72
6	47	0.64	2.67
7	47	2.22	3.28
24	20	0.33	2.29
33	20	0.35	—
41	45	0.91	0.21

Table 2. *Mean residues (SD in parentheses) of malachite green (μg/g) in muscle, eggs, and fry from treated (1 ppm for 1 h) and untreated chinook salmon at two national fish hatcheries (NFH).*

Time from last treatment (days)	Number of fish	Number of treatments at 1 ppm	Sample			
			Muscle	Eggs (0 h)	Eggs (24 h)	Fry (24 h)
Warm Springs NFH						
1	3	27	0.997 (0.197)	1.34 (0.835)	0.843 (0.401)	0.970 (0.423)
1	3	30	1.08 (0.159)	1.82 (0.300)	1.40 (0.332)	1.04 (0.091)
6	3	29	0.75 (0.14)	1.86 (0.681)	1.45 (0.561)	1.00 (0.246)
6	3	30	0.473 (0.186)	1.47 (0.941)	0.973 (0.601)	1.16[a]
12	3	30	0.577 (0.228)	1.16[a]	1.24 (1.06)	1.01 (0.946)
18	3	30	0.743 (0.104)	1.69 (0.662)	1.54 (0.611)	1.03 (0.375)
No treatment	2	0	0.335[a]	0.08[a]	0.03[a]	0.22[b]
Winthrop NFH						
8	3	10	0.43 (0.044)	0.173 (0.055)	0.113 (0.04)	0.14 (0.01)

[a]Average for two fish.
[b]Represents one fish.

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P.O. Box 818
La Crosse, Wisconsin 54602

ABSTRACT.—The toxicity of benzocaine (ethyl 4-aminobenzoate; 98%) to eleven freshwater fishes was evaluated under various physical and chemical conditions. Under standard test conditions (pH 7.8, 12° C, soft water), the 24-h LC50 (mg/L) was 17.2 for lake sturgeon (*Acipenser fulvescens*), 22.5 for rainbow trout (*Oncorhynchus mykiss*), 34.0 for muskellunge (*Esox masquinongy*), 24.0 for northern pike (*Esox lucius*), 19.0 for common carp (*Cyprinus carpio*), 25.9 for fathead minnow (*Pimephales promelas*), 28.0 for channel catfish (*Ictalurus punctatus*), 28.1 for striped bass (*Morone saxatilis*), 21.9 for green sunfish (*Lepomis cyanellus*), 21.9 for bluegill (*L. macrochirus*), and 22.0 for walleye (*Stizostedion vitreum*). Tests conducted at three water temperatures, four hardnesses, and three pH's showed that none of these variables influenced toxicity. The higher temperatures tested (to 22° C) did not increase the rate of toxicosis. Rainbow trout survived exposure to the recommended use concentration of 25 mg/L for 15 min, but did not survive exposure to 75 or 125 mg/L. Users of benzocaine should be cautioned that overdoses may lead to undue stress or mortality in exposed fish. Comparison of benzocaine concentrations by high performance liquid chromatography at 0 and 96 h showed no degradation of the compound during the exposure period.

Anesthetics are used for a number of fishery applications, ranging from mild sedation for transport to total anesthetization for marking, tagging, spawn-taking, and surgical procedures. Although many chemicals have been used to anesthetize fish (McFarland 1959; Bell 1967), tricaine methanesulfonate (MS-222) is the only chemical registered for use on fish in the United States. The label for MS-222 forbids anesthetized fish to be released to the wild or used as food until after a 21-day withdrawal. Salmon anesthetized with MS-222 and killed during spawning must be discarded rather than used as human or animal food (Gilderhus 1989). Because of the withdrawal period for MS-222, there is a need for an alternate anesthetic.

Many substances have been tested as potential anesthetics for fish (McFarland 1959; Bell 1967; Gilderhus et al. 1973; Dawson and Gilderhus 1979). McErlean (1967) first suggested that ethyl-ρ-aminobenzoate be used as an anesthetic for cold-blooded vertebrates. The 183 fishery workers who responded to a survey conducted by Marking and Meyer (1985) used a total of 11 different chemicals to anesthetize fish. Gilderhus and Marking (1987) identified benzocaine, from a group of 16 anesthetics, as a possible candidate for use in fisheries. Chemically, it is similar to MS-222, differing only by the position of a single substituent: the amino group is in the meta position in MS-222 and in the para position on benzocaine. Also, because benzocaine is widely used in human over-the-counter drug preparations, its registration for fishery use may be easier and less costly than for other candidate anesthetics (Gilderhus 1989).

Previous studies of benzocaine (Dawson and Gilderhus 1979; Gilderhus 1989) determined the compound's effectiveness as a fish anesthetic but provided little information on its toxicity to fish. Standardized toxicity information is required to satisfy requirements of the U.S. Food and Drug Administration for minor use of animal drugs. The purpose of our study was to determine the toxicity of benzocaine to representative coldwater and warmwater fish in laboratory tests; to evaluate the effects of water temperature, hardness, and pH on toxicity; and to determine the safety to fish of treatment concentrations that were 3 and 5 times the effective rate for trout.

Materials and Methods

Static test procedures used in this study followed those prescribed by the Committee on Methods for Acute Toxicity Tests with Aquatic Organisms (1975), ASTM Committee E-35 on Pesticides (1980), and the U.S. Department of Agriculture (1986). We exposed 20 fish to each concentration of benzocaine 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 solutions were adjusted to a selected pH (± 0.2 unit) with chemical buffers (Committee on Methods for Acute Toxicity Tests with Aquatic Organisms 1975), before each test and at 24-h intervals, as needed. Temperatures were regulated by immersing the test jars in constant-temperature water baths. To assess the effects of water hardness, we buffered the test solutions to a constant pH with sodium bicarbonate, using the procedure of Marking (1975).

The test species were lake sturgeon (*Acipenser fulvescens*), rainbow trout (*Oncorhynchus mykiss*), muskellunge (*Esox masquinongy*), northern pike (*Esox lucius*), common carp (*Cyprinus carpio*), fathead minnow (*Pimephales promelas*), channel catfish (*Ictalurus punctatus*), striped bass (*Morone saxatilis*), green sunfish (*Lepomis cyanellus*), bluegill (*L. macrochirus*), and walleye (*Stizostedion vitreum*). They were obtained from a State or Federal fish hatchery or produced at the National Fisheries Research Center, La Crosse, Wisconsin, and were maintained according to the standard procedures for handling experimental fish. The fish were acclimated to the desired water chemistries and temperatures for 24 h before each test. Mortalities were recorded at 1, 3, 6, and 12 h on the first day of exposure and daily thereafter for 96 h. The methods of Litchfield and Wilcoxon (1949) were used to compute the LC50's (concentration causing 50% mortality) and 95% confidence intervals.

Three species (rainbow trout, channel catfish, and bluegill) were used in tests to determine the effects of water temperature, hardness, and pH on the toxicity of benzocaine. In tests to determine safe use pattern levels, we exposed groups of 300 rainbow trout to benzocaine at the prescribed effective treatment concentration of 25 mg/L for 15 min (Gilderhus 1989) and to 3 and 5 times this effective level (U.S. Department of Agriculture 1986). We observed the fish for a 14-day postexposure period for unusual behavior or delayed mortality, using the criteria set forth by Lennon and Walker (1964).

Benzocaine (ethyl 4-aminobenzoate, 98%; Aldrich Chemical Company, Milwaukee, Wisconsin) was dissolved in ethanol to make stock solutions, and aliquots of these solutions were pipetted into test vessels to reach the desired test concentrations.

Benzocaine concentrations in water samples were quantified at 0 and 96 h by high performance liquid chromatography (HPLC). Quantification equipment and conditions were as follows: Waters HPLC system, consisting of a 710B WISP autosampler, a 510 variable speed pump, a 481 spectrophotometer detector, and a 730 data module integrator. A Micro Pak, C_{18}, reverse-phase column (30 cm × 4 mm) was used with a mobile phase consisting of 70% HPLC methanol, 26% HPLC water, and 4% acetic acid. Flow rate for the mobile phase was 2.0 mL/min and the detector was set at a wavelength of 286 nm. This method resulted in a retention time of about 3.6 min. To quantify peak areas, we used the Waters External Standard Quantification program.

Results

Toxicity to Eleven Species of Fish

Benzocaine was toxic to all species exposed in soft water at 12° C; the 24-h LC50's ranged from 17.2 mg/L for lake sturgeon to 34.0 mg/L for muskellunge (Table 1). Coldwater and warmwater species responded similarly; the 24-h LC50 was 22.5 mg/L for rainbow trout and 21.9 mg/L for bluegills. The toxicity of benzocaine did not increase significantly with longer exposures; the LC50's changed little between 1 and 24 h for the species exposed.

Influence of Temperature, Water Hardness, and pH

The toxicity of benzocaine was not significantly affected by any of the water characteristics tested. Increased water temperature, which increases the metabolic rate of poikilotherms, usually increases the rate of uptake of toxic chemicals and results in greater mortality. However, the toxicity of benzocaine was not affected by changes in water temperature in these exposures. For example, with rainbow trout, the 24-h LC50 was 17.0 mg/L in water at 7° C and 20.5 mg/L in water at 17° C (Tables 2, 3, and 4). The 24-h LC50's for channel catfish were 28.0 mg/L at 12° C and 27.9 mg/L at 22° C.

Likewise, neither water hardness nor pH affected toxicity. For example, in channel catfish, the 24-h LC50 was 30.2 mg/L in very soft water (10–12 mg/L, total hardness as milligrams per liter $CaCO_3$) and 30.0 mg/L in very hard water (300–320 mg/L total hardness). The 24-h LC50's for channel catfish were 30.1 mg/L in acidic water (pH 6.5) and 29.0 mg/L in alkaline water (pH 9.5).

The HPLC analysis of benzocaine concentrations in water from randomly selected test vessels agreed closely

Table 1. *Toxicity (LC50 in mg/L, and 95% confidence interval) of benzocaine to eleven species of fish in soft water at 12° C.*

	Duration of exposure (hours)					
Species	1	3	6	12	24	96
Lake sturgeon[a]	28.0	24.2	20.5	—	17.2	17.2
	25.3–31.0	20.0–29.2	18.4–22.8	— —	15.5–19.1	15.5–19.1
Rainbow trout	27.0	24.2	23.0	22.5	22.5	11.0
	26.7–28.4	23.3–25.1	21.8–24.2	21.3–23.8	21.3–23.8	10.2–11.8
Northern pike	35.0	35.0	35.0	27.5	24.0	20.0
	28.0–43.7	28.0–43.7	28.0–43.7	26.1–29.0	21.6–26.6	18.1–22.0
Muskellunge[a]	34.0	34.0	34.0	34.0	34.0	30.0
	29.5–39.1	29.5–39.5	29.5–39.1	29.5–39.1	29.5–39.1	26.7–33.7
Fathead minnow	35.0	29.0	26.0	25.9	25.9	25.9
	33.0–37.2	27.2–30.9	24.8–27.4	24.9–26.9	24.9–26.9	24.9–26.9
Common carp	22.6	21.9	21.9	19.3	19.0	19.0
	21.2–24.1	20.7–23.2	20.7–23.2	18.6–20.0	17.9–20.1	17.9–20.1
Channel catfish	36.0	35.0	29.5	29.0	28.0	18.5
	33.1–39.1	32.5–37.7	27.8–31.3	27.2–30.9	26.7–29.3	12.3–14.8
Striped bass[a]	32.9	28.1	28.1	28.1	28.1	28.1
	27.6–39.2	22.7–34.8	22.7–34.8	22.7–34.8	22.7–34.8	22.7–24.8
Bluegill	26.5	22.8	22.8	21.0	21.9	17.0
	22.6–31.0	20.4–25.4	20.4–25.4	19.5–22.6	20.3–23.6	15.8–18.3
Green sunfish	26.5	25.0	23.0	22.0	21.9	20.2
	25.0–28.1	22.8–27.4	21.8–24.3	20.8–23.3	20.6–23.2	19.0–21.4
Walleye[a]	37.7	25.9	22.0	22.0	22.0	—
	32.4–43.9	22.1–30.4	19.7–24.6	19.7–24.6	19.7–24.6	— —

[a]Ten fish per test concentration; 20 for other species.

Table 2. *Toxicity (LC50 in mg/L, and 95% confidence interval) of benzocaine to rainbow trout in water of different temperatures, hardnesses, and pH's.*

			Duration of exposure (hours)					
Temperature (° C)	Hardness	pH	1	3	6	12	24	96
7	Soft	7.8	23.0	19.8	19.0	17.0	17.0	13.3
			21.8–24.3	18.8–20.9	18.0–20.1	15.7–18.4	15.7–18.4	12.3–14.3
12	Soft	7.8	27.0	24.2	23.0	22.5	22.5	11.0
			25.7–28.4	23.3–25.1	21.8–24.2	21.3–23.8	21.3–25.8	10.2–11.8
17	Soft	7.8	26.0	23.8	22.0	22.0	20.5	7.20
			23.6–26.4	22.7–24.9	20.7–23.3	20.7–23.3	19.2–21.9	5.92–9.76
12	Very soft	8.2	31.0	26.8	24.0	24.0	22.0	8.60
			28.4–33.8	24.7–29.1	21.4–26.9	21.4–26.9	20.3–23.9	7.98–9.26
12	Soft	8.2	28.0	26.0	24.0	24.0	24.0	7.50
			26.2–29.9	23.7–28.5	21.4–26.9	21.4–26.9	21.4–26.9	6.53–8.62
12	Hard	8.2	24.0	24.0	24.0	24.0	24.0	9.70
			21.5–26.8	21.5–26.8	21.5–26.8	21.5–26.8	21.5–26.8	8.90–10.6
12	Very hard	8.2	30.0	27.0	27.0	27.0	23.2	23.2
			27.6–32.6	24.9–29.2	24.9–29.2	24.9–29.2	21.1–25.5	21.1–25.5
12	Soft	6.5	27.0	24.0	24.0	24.0	21.0	—
			24.9–29.2	21.4–26.9	21.4–26.9	21.4–26.9	19.5–22.6	— —
12	Soft	8.5	35.5	30.4	29.0	24.0	22.0	—
			32.5–38.8	27.9–33.1	26.8–31.4	21.4–26.9	20.4–23.8	— —
12	Soft	9.5	31.0	24.2	24.2	21.0	19.0	—
			28.5–33.7	21.5–27.2	21.5–27.2	19.7–22.4	17.3–20.8	— —

Table 3. *Toxicity (LC50 in mg/L, and 95% confidence interval) of benzocaine to bluegills in water of different temperatures, hardnesses, and pH's.*

Temperature (° C)	Hardness	pH	Duration of exposure (hours)					
			1	3	6	12	24	96
12	Soft	7.8	26.5	22.8	22.8	21.0	21.9	17.0
			22.6–31.0	20.4–25.4	20.4–25.4	19.5–22.6	20.3–23.6	15.8–18.3
17	Soft	7.8	27.0	25.0	25.0	25.0	23.0	18.0
			20.7–35.2	22.4–27.9	22.4–27.9	22.4–27.9	21.1–25.1	16.2–20.0
22	Soft	7.8	27.0	22.0	21.0	18.6	17.8	9.20
			24.8–29.4	19.5–24.8	19.5–22.6	16.9–20.4	16.5–19.2	7.48–11.3
12	Very soft	8.2	29.0	27.0	25.0	22.3	22.3	16.0
			27.4–30.7	25.4–28.7	22.7–27.6	21.1–23.6	21.1–23.6	14.6–17.5
12	Soft	8.2	28.5	25.0	23.0	22.9	22.0	17.0
			26.6–30.5	23.8–26.2	21.8–24.3	21.7–24.2	20.8–23.3	15.8–18.3
12	Hard	8.2	29.5	24.5	23.0	23.0	23.0	18.0
			26.7–32.5	22.6–26.5	21.8–24.3	21.6–24.5	21.6–24.5	16.9–19.2
12	Very hard	8.2	29.0	28.0	25.6	24.3	21.3	15.1
			27.2–30.9	26.7–29.4	24.3–27.0	23.4–25.3	19.8–22.9	13.6–16.7
12	Soft	6.5	25.5	22.0	21.2	21.2	21.2	19.0
			23.4–27.8	20.2–24.0	20.1–22.3	20.1–22.3	20.1–22.3	17.4–20.8
12	Soft	8.5	29.0	25.0	23.0	21.5	20.6	19.5
			26.1–32.2	23.0–27.1	21.3–24.8	19.9–23.2	19.3–22.0	18.6–20.4
12	Soft	9.5	25.0	23.5	23.0	22.5	22.5	22.0
			22.2–28.1	21.2–26.1	20.8–25.4	20.8–24.3	20.8–24.3	20.7–23.3

Table 4. *Toxicity (LC50 in mg/L, and 95% confidence interval) of benzocaine to channel catfish in water of different temperatures, hardnesses, and pH's.*

Temperature (° C)	Hardness	pH	Duration of exposure (hours)					
			1	3	6	12	24	96
12	Soft	7.8	36.0	35.0	29.5	29.0	28.0	13.5
			33.1–39.1	32.5–37.7	27.8–31.3	27.2–30.9	26.7–29.3	12.3–14.8
17	Soft	7.8	34.5	33.0	33.0	31.0	29.0	16.0
			32.4–36.7	31.2–34.9	31.2–34.9	29.4–32.7	26.9–31.2	14.1–18.2
22	Soft	7.8	34.0	33.0	30.0	28.0	27.9	13.8
			31.3–36.9	31.2–34.9	28.3–31.2	26.7–29.4	26.5–29.3	11.3–16.8
12	Very soft	8.2	49.0	35.0	34.0	31.0	30.0	20.0
			44.8–53.4	32.9–37.3	31.9–36.2	29.5–32.5	28.2–31.9	17.9–22.5
12	Soft	8.2	48.0	34.7	34.7	30.2	30.2	15.8
			43.9–52.3	32.6–37.0	32.6–37.0	28.7–31.8	28.7–31.8	13.7–18.2
12	Hard	8.2	49.0	33.0	33.0	30.0	30.0	28.0
			43.4–55.3	31.2–34.9	31.2–34.9	28.4–31.7	28.2–31.9	26.6–29.4
12	Very hard	8.2	49.0	34.7	34.7	30.0	29.4	15.0
			44.8–53.5	32.6–36.9	32.6–36.9	28.2–31.9	27.6–31.3	13.8–16.3
12	Soft	6.5	49.0	38.0	32.0	31.0	30.1	27.5
			44.8–53.5	34.8–41.5	30.5–33.6	29.5–32.6	28.5–31.8	26.1–29.0
12	Soft	8.5	48.0	35.5	35.0	31.5	31.0	28.0
			43.9–52.5	33.0–38.2	32.9–37.3	30.0–33.1	29.3–32.7	26.2–30.0
12	Soft	9.5	42.0	35.1	33.0	29.0	29.0	26.0
			39.2–45.0	32.6–37.8	31.2–34.9	27.2–30.9	27.2–30.9	23.7–28.5

with the calculated concentrations (Table 5). At the beginning of the exposures, concentrations in the water samples were within 5% of the calculated values. Analysis of the same vessels 96 h later showed little, if any, change in the benzocaine concentration. Also, water temperature, hardness, and pH did not affect the rate of degradation of benzocaine over the 96-h period.

Use Pattern Exposure

Rainbow trout exposed to benzocaine at the use pattern concentration of 25 mg/L for 15 min recovered from the anesthesia within 15 min or less and responded much like control fish during the 14-day postexposure period. However, all fish exposed to 3 or 5 times the effective concentration died within 30 min.

Discussion

The use pattern concentration of 25 mg/L was chosen to represent the level that was effective for anesthetizing small salmonids (Gilderhus 1989). At that level, fish were effectively anesthetized in 3 min or less, recovered from anesthesia in less than 5 min, and survived 15 min of exposure. In our use pattern exposures, the fish were also exposed for 15 min, which is about 5 times the duration necessary for effective anesthesia. Although the X3 and X5 concentrations were lethal in 15-min exposures, they would be much less toxic in 3-min exposures. However, the margin of safety to treated fish is not high, and users should be aware that overdosing could result in undue stress or mortality.

Benzocaine is uniformly toxic to different species of fish and at various water temperatures, pH's, and hardnesses. This consistency is an advantage because users need not be concerned about alterations in safety to the fish. The toxicity of many other fishery chemicals is influenced by water characteristics, especially pH (Hunn and Allen 1974). Fasman (1976) reported the ionization constant (pka) for benzocaine to be 2.38. At this pH, half the benzocaine would be in the ionized form and half in the un-ionized form (Dawson and Gilderhus 1979). At the pH's of 6.5–9.5, there would be little change in the concentration of the un-ionized, lipid soluble form of benzocaine available for uptake; thus, little, if any, change in toxicity at these pH's would be expected.

The 96-h exposures were of little relevance for evaluating safety, but the values generated demonstrate that exposure time beyond 1 h does not critically increase the toxicity. The concentrations of benzocaine in the water solutions remained nearly constant for 96 h, indicating that the chemical was not degraded and the fish did not remove a significant portion of the chemical at this loading rate. In some of the exposures for 96 h, dissolved oxygen fell below 50% saturation. As a result, toxicity increased in those tests. The ethanol solvent seemingly provided bacteria with a nutrient source that allowed them to proliferate in some test vessels. Variability in the 96-h results is probably due to these bacterial growths.

Toxicity of benzocaine to fish compares closely to that of MS-222. Marking (1967) reported that for MS-222, the

Table 5. *HPLC analysis of benzocaine concentrations of 7.5, 10, and 30 mg/L (calculated) in water at selected temperatures, hardness, and pH's at 0 and 96 h.*

Temperature (° C)	Hardness	pH	Benzocaine concentration (mg/L)			
				Measured		Measured
			Calculated	0 h[a], 96 h[a]	Calculated	0 h[a], 96 h[a]
7	Soft	7.8	10	9.5, 9.4	30	28.5, 29.4
12	Soft	7.8	10	9.4, 9.3	30	28.4, 29.4
17	Soft	7.8	10	9.6, 9.0[b]	30	29.2, 29.3
12	Very soft	8.2	7.5	7.1, 6.4	30	28.5, 28.1
12	Soft	8.2	7.5	7.3, 6.7	30	28.2, 29.1
12	Hard	8.2	7.5	7.3, 6.9	30	28.4, 29.4
12	Very hard	8.2	7.5	7.3[b], 6.8	30	27.3, 27.6
12	Soft	6.5	7.5	6.8, 6.6	30	27.0, 27.8
12	Soft	8.5	7.5	7.1[b], 6.8	30	28.3, 29.1
12	Soft	9.5	7.5	6.9, 6.7	30	26.8, 26.9

[a]Duplicate samples.
[b]Average of triplicate analysis.

24-h LC50's ranged from 34 to 64 mg/L. Although the values for MS-222 are slightly higher than those for benzocaine, MS-222 was formulated as a methanesulfonate salt. The higher molecular weight of the formulation would be expected to yield an anesthetic less active than the benzocaine formulation. The toxicity of MS-222 also was affected little by water hardness or temperatures, and safety for rainbow trout in 15-min exposures to MS-222 was similar to that for benzocaine.

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U.S. GOVERNMENT PRINTING OFFICE: 1990-773-216/20017

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The toxicity of benzocaine (ethyl 4-aminobenzoate; 98%) to eleven freshwater fishes was evaluated under various physical and chemical conditions. Under standard test conditions (pH 7.8, 12° C, soft water), the 24-h LC50 (mg/L) 17.2 for lake sturgeon (*Acipenser fulvescens*), was 22.5 for rainbow trout (*Oncorhynchus mykiss*), 34.0 for muskellunge (*Esox masquinongy*), 24.0 for northern pike (*Esox lucius*), 19.0 for common carp (*Cyprinus carpio*), 25.9 for fathead minnow (*Pimephales promelas*), 28.0 for channel catfish (*Ictalurus punctatus*), 28.1 for striped bass (*Morone saxatilis*), 21.9 for green sunfish (*Lepomis cyanellus*), 21.9 for bluegill (*L. macrochirus*), and 22.0 for walleye (*Stizostedion vitreum*). Tests conducted at three water temperatures, four hardnesses, and three pH's showed that none of these variables influenced toxicity. The higher temperatures tested (to 22° C) did not increase the rate of toxicosis. Rainbow trout survived exposure to the recommended use concentration of 25 mg/L for 15 min but did not survive exposure to 75 or 125 mg/L. Users of benzocaine should be cautioned that overdoses may lead to undue stress or mortality in exposed fish. Comparison of benzocaine concentrations by high performance liquid chromatography at 0 and 96 h showed no degradation of the compound during the exposure period.

Key words: Toxicity, benzocaine, anesthetic, environmental factors, fish.

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(Reports 87 through 89 are in one cover.)

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88. Influences of Selected Environmental Factors on the Activity of a Prospective Fish Toxicant, 2-(Digeranyl-amino)-ethanol, in Laboratory Tests, by C. A. Launer and T. D. Bills. 1979. 4 pp.
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