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UNITED STATES DEPARTMENT OF THE INTERIOR
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By R. H. Hudson

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Ethyl-*p*-aminobenzoate (Benzocaine): Efficacy as an Anesthetic for Five Species of Freshwater Fish

by

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Abstract

Ethyl-*p*-aminobenzoate (benzocaine) was tested for its efficacy as an anesthetic for rainbow trout (*Salmo gairdneri*), brown trout (*Salmo trutta*), northern pike (*Esox lucius*), carp (*Cyprinus carpio*), and largemouth bass (*Micropterus salmoides*). Since benzocaine is not water soluble, it was applied with acetone as a carrier. Concentrations of 100 to 200 mg/l were required for large adult northern pike, compared with 50 to 100 mg/l for small fish. Rates of sedation and recovery were slower in cold water than in warm water. Water hardness had little influence on the activity of benzocaine. Fish were anesthetized faster and recovered more slowly in acid than in alkaline water. Benzocaine produced deep anesthesia, but concentrations that rendered the fish handleable within 5 min were generally not safe for exposures longer than 15 min. Concentrations of benzocaine efficacious for fish were not acutely toxic to eggs of coho salmon (*Oncorhynchus kisutch*), chinook salmon (*Oncorhynchus tshawytscha*), rainbow trout, brown trout, or lake trout (*Salvelinus namaycush*). Benzocaine is not registered for fishery use and is neither more effective nor safer than the registered anesthetic, tricaine methanesulfonate (MS-222).

Many substances have been tested for their ability to anesthetize fish (McFarland 1959; Bell 1967; McErlean 1967; Schoettger and Julin 1967; Schoettger and Julin 1969; Howland and Schoettger 1969; Gilderhus et al. 1973). Few of these have been used extensively as anesthetics, however, and only one—tricaine methanesulfonate (MS-222)—is registered for fishery use by the U.S. Food and Drug Administration (Office of the Federal Register 1976).

McErlean (1967) suggested the use of ethyl-*p*-aminobenzoate (benzocaine) as a possible alternative to MS-222, and McErlean and Kennedy (1968) compared some anesthetic properties of the two compounds. Wedemeyer (1970) and Soivio et al. (1977) evaluated physiological effects of anesthesia with MS-222 and benzocaine on rainbow trout (*Salmo gairdneri*). Both studies revealed similarities between the two compounds at pH 7.0.

Benzocaine and MS-222 are similar in structure but differ in the addition of a methanesulfonate group to MS-222, to make it water soluble; the amine group is *meta*-substituted in MS-222 and *para*-substituted in benzocaine.

Although benzocaine is not registered for fishery use, it has been used extensively in veterinary (Windholz 1976) and human medicine (Baker 1976).

The present study was conducted to evaluate the potential of benzocaine as a fish anesthetic, under selected water conditions, for several species and sizes of fish. The investigation involved a laboratory study with subadult fish and a field study with adult northern pike (*Esox lucius*). The toxicity of benzocaine to fish eggs which might be exposed during hatchery spawning operations was also evaluated.

Materials and Methods

Benzocaine (90%) used in the tests was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin. Stock solutions were made up in acetone and added to the test vessels in the quantities needed to produce the desired concentrations. Fish eggs were exposed in glass jars containing 2.5 liters of soft reconstituted water. Subadult fish were exposed in polyethylene tanks containing 45 liters of water and adults in tanks containing 100 liters.

Hardness of water used in the tests was produced by adding selected amounts of salts to aerated, deionized water as described by Marking and Dawson (1973). Selected pH values (from 6.5 to 9.5) were produced and maintained with chemical buffers according to the method of Dawson et al. (1975). Temperatures of 7, 12,

Table 1. *Efficacy of benzocaine as an anesthetic against five species of fish in standard reconstituted water.*

Species and average weight (g)	Temp (°C)	Concn (mg/l)	Time (min) to loss of:		Exposure time (min)	Recovery time (min)	Survival (%)
			Equilibrium[a]	Reflex			
Rainbow trout							
65	12	50	2.8	5.0	10	12.0	60
Brown trout							
1	12	50	2.1	2.5	10	3.4	5
90	7	50[b]	3.9	8.8	10	6.7	100
90	12	50	2.4	4.5	10	7.4	100
90	17	50	2.5	4.3	10	7.5	10
Carp							
50	12	100	2.6	3.7	15	18.0	100
Largemouth bass							
50	12	50[b]	9.3	9.7	15	11.5	100
Northern pike							
6	7	60[b]	7.0	13.3	15	23.5	100
6	12	60[b]	4.0	8.0	15	20.0	100
6	17	60[b]	2.8	7.5	15	8.5	100
6	12	100	2.5	3.5	15	15.0	100
1,543	3	200	5.0	5.0	15	35.0	100
1,543	12	200	3.0	3.0	15	35.0	100

[a]Loss of equilibrium, stage 2 of Schoettger and Julin (1967).
[b]Loss of reflex did not occur within the desired period of 5 min.

and 17 C were maintained with water baths.

We tested the anesthetic against rainbow trout, brown trout (*Salmo trutta*), northern pike, carp (*Cyprinus carpio*), and largemouth bass (*Micropterus salmoides*), and evaluated its toxicity to green eggs of coho salmon (*Oncorhynchus kisutch*), chinook salmon (*Oncorhynchus tshawytscha*), rainbow trout, brown trout, and lake trout (*Salvelinus namaycush*). Eggs were exposed to the anesthetic within 24 h after fertilization.

Fish used in the laboratory tests were obtained from Federal or State fish hatcheries, maintained under a fish culturist's care, and acclimated to test waters 2 days before the benzocaine was added. Ten fish or 25 fish eggs were exposed to each concentration of anesthetic.

Field tests were conducted with adult northern pike collected from the Mississippi River with fyke nets in April. Most were females (average length, 61 cm, and weight, 1,543 g) that had been artificially spawned 3 days previously. Most tests were conducted at the State Fish Hatchery, Lansing, Iowa, in water from the Mississippi River (temperature, 3 C; pH, 7.8; total alkalinity, 135 mg/l as $CaCO_3$; and total hardness, 192 mg/l as $CaCO_3$). Tests at the National Fishery Research Laboratory were conducted in well water (temperature, 12 C; pH, 7.8; total alkalinity, 206 mg/l as $CaCO_3$; and total hardness, 224 mg/l as $CaCO_3$). Three fish were exposed to each concentration.

The tests were designed to establish the effective concentration that would result in loss of reflex and thus render the fish handleable within 5 min and still permit survival of all fish after at least 10 min of exposure. We determined the various stages of anesthesia by using characteristics defined by Schoettger and Julin (1967): sedation (decreased reactivity to stimuli); partial loss of equilibrium (swimming ability disrupted); total loss of equilibrium, stage 1 (usually the fish turn over but swimming ability persists); total loss of equilibrium, stage 2 (locomotion ceases but fish respond to pressure on the caudal peduncle); loss of reflex (failure to respond to external stimuli); and medullary collapse (opercular activity ceases).

We analyzed mortality data according to the method of Litchfield and Wilcoxon (1949) to determine LC_{50}'s (concentration causing 50% mortality) and 95% confidence intervals. A safe exposure index was obtained by dividing the time required for the first fish to reach medullary collapse by the time required for fish to reach loss of reflex (Schoettger and Julin 1967). An index value of less than 1.0 indicates that the anesthetic causes mortality before it produces the desired level of anesthesia.

Results

Efficacy to Subadult Fish

At 12 C, rainbow trout and brown trout exposed to 50 mg/l of benzocaine showed a total loss of equilibrium (stage 2) within less than 3 min and loss of

reflex within 5 min (Table 1). A 10-min exposure at 50 mg/l caused a mortality of 40% in rainbow trout and 95% in brown trout fry. Brown trout exposed to the 50-mg/l concentration at 7 C did not experience loss of reflex within the desired period of 5 min, and at 17 C, 90% died.

Warmwater fishes consistently withstood longer exposures and higher concentrations than did cold-water species. For example, largemouth bass and northern pike fingerlings exposed to concentrations of 50 and 60 mg/l, respectively, at 12 C did not reach loss of reflex within 5 min; at 100 mg/l, however, the northern pike—as well as carp—were completely anesthetized. However, exposure to the 100-mg/l concentration for 30 min killed subadults of all five species (data not shown). The safe exposure indices for the different species ranged from 1.5 to 2.5.

Efficacy to Adult Northern Pike

Adult northern pike did not progress through the stages of anesthesia shown by small fish. Most remained active until they turned on their sides. They had then lost reflex, having bypassed total loss of equilibrium, stages 1 and 2. Several fish were hyperactive for 20 to 30 s immediately before they turned over. Northern pike exposed to 200 and 300 mg/l in 12 C well water at the Laboratory exhibited some headshaking immediately after being placed in the solution, suggesting that the solution might be mildly irritating.

Anesthetization of adult northern pike at 3 C required exposure for 7 to 8 min to concentrations of 100 to 160 mg/l of benzocaine. A concentration of 200 mg/l rendered them motionless in 4.0 to 6.5 min at 3 C and in 2.5 to 3.5 min at 12 C. Anesthesia was induced in 3.5 to 4.8 min at 3 C and in 2.0 to 3.5 min at 12 C at a concentration of 300 mg/l of benzocaine; however, some mortality occurred at the higher concentration. The length of time required for fish to recover in fresh water varied from 35 to 100 min after 15 min of exposure to 200 mg/l at 3 C.

In the 3 C water at the Lansing (Iowa) tests, there were no mortalities among fish exposed to 200 or 300 mg/l for 30 min. In the 12 C water at the Laboratory, northern pike suffered 67% mortality (two of three fish) after 60 min of exposure to 200 mg/l and after 30 min of exposure to 300 mg/l.

Effect of Temperature

There was almost no difference in the rates of anesthesia at 12 and 17 C for brown trout, and the times to loss of equilibrium and reflex were only slightly longer for subadult northern pike at 12 C than at 17 C. At 7 C, however, the times to loss of equilibrium and reflex were significantly longer for both species. Little difference was noted in recovery time for brown trout at any of the test temperatures, but northern pike recovered considerably faster at 17 C than at 7 or 12 C (Table 1).

Effect of Water Hardness and pH

Water hardness had little influence on the average time to loss of reflex in subadult brown trout; the rate of anesthesia, however, appeared to be somewhat slower at higher pH's (Table 2). The rate of recovery after anesthesia (10-min exposure to 50 mg/l) was not influenced by water hardness or pH except that recovery tended to be slower at pH 6.5 than at pH 8.0 or 9.5 (Table 3).

Table 2. *Average time (min) for 10 subadult brown trout to reach loss of reflex in 50 mg/l of benzocaine at 12 C in waters of different total hardness and pH.*

	Water hardness		
pH	Soft	Hard	Very hard
6.5	3.3	3.1	4.2
8.0	4.9	3.7	5.0
9.5	5.3	4.3	5.2

Table 3. *Average recovery time (min) for 10 subadult brown trout after a 10-min exposure to 50 mg/l of benzocaine at 12 C in waters of different total hardness and pH.*

	Water hardness		
pH	Soft	Hard	Very hard
6.5	11.2	6.3	9.9
8.0	4.6	5.7	5.7
9.5	4.8	6.4	8.2

Safety to Fish Eggs

The toxicity of benzocaine to green eggs ranged from an LC_{50} of 88.0 mg/l for rainbow trout to 43.0 mg/l for coho salmon after 1 day of exposure (Table 4). The 5-day LC_{50}'s for eggs of these species were 47.0 and 38.4 mg/l, respectively. The most sensitive eggs after 5 days of exposure were those of brown trout (LC_{50} = 17.8 mg/l). Toxicity to eggs of all species tested was not substantially increased by exposures as long as 10 days.

Eggs of rainbow trout were resistant to benzocaine exposures that might be encountered during hatchery spawning operations. No mortalities occurred after a

Table 4. *Toxicity (LC_{50} and 95% confidence interval, mg/l) of benzocaine to fish eggs in standard reconstituted water at 12 C.*

Species	Exposure period (days) 1	5	10
Rainbow trout	88.0	47.0	42.5
	75.3-103	36.2-61.0	33.0-54.3
Brown trout	62.0	17.8	14.5
	53.9-71.4	14.1-22.4	11.9-17.7
Lake trout	65.0	36.5	29.2
	56.9-74.3	30.1-44.2	23.1-37.0
Coho salmon	43.0	38.4	32.0
	34.0-54.4	32.2-45.8	28.1-36.5
Chinook salmon	64.1	46.0	44.0
	54.6-75.3	37.7-56.1	33.2-58.4

1-h exposure to 500 mg/l of benzocaine or to a 3-h exposure to 100 mg/l (Table 5). Exposure for 3 h to 200 and 500 mg/l of benzocaine resulted in mortalities of 24 and 100%, respectively.

Table 5. *Mortalities (%) of green eggs of rainbow trout after exposures to different concentrations of benzocaine for 1 to 3 h and transfer to fresh water for 96 h.*

Concentration (mg/l)	Exposure period (h) 0.5	1	3
100	0	0	0
200	0	0	24
500	0	0	100

Discussion

The observed progression of adult northern pike exposed to the anesthetic, from partial loss of equilibrum directly to loss of reflex, would present no use problem, since fish in the loss of reflex stage are easier to handle.

The activity of many chemicals is influenced by the pH of the test solution, often as a result of an ionization equilibrium where only the un-ionized form of the molecule is able to penetrate the gill membrane (Sills and Allen 1971; Dawson and Marking 1973; Hunn and Allen 1974; Dawson et al. 1977). However, the ionization constant (pKa) for benzocaine (2.38; Fasman 1976) is so low that little ionization occurs at the pH's of 6.5 to 9.5 used in our tests. Therefore most benzocaine molecules should have been in the lipid-soluble, un-ionized form. The apparent increase in activity at lower pH's may be related to increased stress on the fish in acidic water.

The safe exposure indices for benzocaine (1.5-2.5) are closely similar to those of its structural homologue, MS-222, as reported by Schoettger and Julin (1967).

Benzocaine does not depress the pH of poorly buffered solutions as do the fish anesthetics MS-222 and quinaldine sulfate (Dawson and Marking 1973). Benzocaine, however, is not water soluble and is not registered for fishery use. Furthermore, by comparison with the reported efficacy (Schoettger and Julin 1967) and toxicity (Marking 1967) of the registered anesthetic, MS-222, benzocaine is neither more effective nor safer.

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Influences of Selected Environmental Factors on the Activity of a Prospective Fish Toxicant, 2-(Digeranylamino)-ethanol, in Laboratory Tests

by

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Abstract

Fathead minnows (*Pimephales promelas*), brown trout (*Salmo trutta*), and rainbow trout (*S. gairdneri*) were used to assess the influences of temperature, pH, turbidity, ultraviolet light, and aquatic vegetation on the toxicity of an experimental fish toxicant, 2-(digeranylamino)-ethanol (GD-174). Also examined was the feasibility of chemical counteraction by oxidation or reduction. The activity of the compound was reduced in cold water, in acid water, and in turbid water where the turbidities were produced by bentonite or Crowley silt loam, and in water containing waterweed (*Elodea* sp.). The activity was not affected by exposure to ultraviolet light, by the presence of duckweed (*Lemna* sp.), or by turbidities produced by kaolin or barium sulfate. GD-174 can be counteracted by either oxidation or reduction; oxidation by potassium permanganate was the most effective procedure tested.

Rotenone and antimycin are currently the only two piscicides registered for use in the United States. Both are general toxicants. Through an intensive screening program seeking selective chemicals for the control of undesirable fishes, Marking (1974) determined that a terpine derivative, 2-(digeranylamino)-ethanol (GD-174), was more toxic to carp than to other warmwater species under laboratory conditions. Although the chemical has shown great promise in laboratory studies as a general fish toxicant and potential for the selective control of carp, results in small-scale field applications have been inconsistent. We conducted several laboratory studies to assess the effects of water temperature, pH, turbidity, aquatic vegetation, and ultraviolet radiation on the activity of GD-174. We also examined the effectiveness of selected oxidizing and reducing agents for counteracting the chemical.

Materials and Methods

Stock solutions of technical grade GD-174 obtained from Glidden Durkee, Division of SCM Corporation, Jacksonville, Florida, were prepared by solubilizing the chemical in an equal volume of 20% glacial acetic acid and then diluting to the desired volume with water. To prepare test solutions of desired concentrations, we pipetted portions of the stock solutions into test vessels and then stirred the mixture to ensure homogeneity.

Fish species tested were brown trout, *Salmo trutta* (average weight 7.5 g); rainbow trout, *S. gairdneri* (2.5 g); and fathead minnows, *Pimephales promelas* (1.1 g). Fish were either obtained from Federal fish hatcheries or cultured at the National Fishery Research Laboratory, La Crosse, Wisconsin.

Acute toxicity tests for the effects of water temperature and pH on the activity of GD-174 were conducted according to methods described by the Committee on Methods for Acute Toxicity Tests with Aquatic Organisms (1975).

In photodegradation studies, stock solutions of GD-174 were exposed to UV light (21 $\mu W/cm^2 \times 100$) from a GE-275W sunlamp at a distance of 25 cm from the surface for periods of 6, 12, or 24 h. These solutions were maintained in a temperature-controlled water bath and continuously stirred to ensure homogeneity. Portions of these solutions were then added to test vessels and their toxicities compared with those of reference solutions aged in darkness for the same period.

In studies on turbidity, GD-174 was added to a series of solutions containing either barium sulfate, kaolin, bentonite, or Crowley silt loam (classified as a Typic Albaqualf; consists of 6% sand, 83% silt, 11% clay) and having turbidities of 25, 50, 75, and 100 nephelometric turbidity units (NTU). Turbidity was measured with an HF DRT-100 model turbidimeter. The solutions were compared to a reference series of clear water (0.53 NTU), and the toxicities compared.

In studies to determine the effects of aquatic vege-

tation on the toxicity of GD-174, waterweed (*Elodea* sp.) and duckweed (*Lemna* sp.) collected from the wild were used. In outdoor tests, GD-174 was added to polyethylene tanks containing reconstituted water and either 0, 5, 10, or 15 g/l of waterweed or 0 or 5 g/l of duckweed. In outdoor tests, 0, 2, 4, 8, or 12 g/l of waterweed were used. After 24 h, the vegetation was removed, fish were introduced, and the toxicities were compared with those of reference solutions which had contained no vegetation.

In counteraction studies, potassium permanganate ($KMnO_4$) at 2 mg/l, chlorine at 0.05 mg/l (active Cl_2 from commercial grade calcium hypochlorite), or sodium thiosulfate ($Na_2S_2O_3$) at 10 mg/l was introduced into a series of GD-174 solutions 6 h before the introduction of fish. The toxicities of these solutions were then compared with that of reference solutions.

The method of Litchfield and Wilcoxon (1949) was used to calculate LC_{50}'s and 95% confidence intervals. Significant differences in all data were based on $P = 0.05$. All reported data met the chi-square test for acceptability.

Table 1. *Toxicity of 2-(digeranylamino)-ethanol to fathead minnows in water of selected temperatures and pH's.*

Temp (°C)	pH	LC_{50} and 95% confidence interval (µl/l) 24 h	96 h
7	7.5	>1.0	0.547
			0.461-0.649
12	7.5	0.467	0.200
		0.377-0.578	0.164-0.244
17	7.5	0.196	0.150
		0.169-0.228	0.126-0.179
22	7.5	0.100	0.0750
		0.0803-0.125	0.0573-0.0982
12	6.5	0.820	0.324
		0.712-0.943	0.274-0.383
12	7.5	0.386	0.137
		0.329-0.452	0.113-0.166
12	8.5	0.243	0.100
		0.225-0.263	0.0788-0.126
12	9.5	0.178	0.0730
		0.146-0.216	0.0653-0.0815

Results

Water Temperature

Toxicity of GD-174 was greater at higher than at lower water temperatures (Table 1). At 96 h, the LC_{50}'s for fathead minnows were significantly higher at 12 C than at 7 C and at 22 C than at 17 C. Furthermore, the toxic action was more rapid at the higher temperatures: there were no significant differences between the 24- and 96-h LC_{50}'s at either 17 or 22 C, whereas at 7 and 12 C the LC_{50}'s for 96 h equaled only about half those for 24 h (Table 1).

pH

The GD-174 was more toxic to fathead minnows in alkaline than in acid waters (Table 1). At 24 h, there were significant differences between the LC_{50}'s at each pH tested (6.5, 7.5, 8.5, and 9.5), and a fourfold difference between pH 6.5 and pH 9.5. At 96 h, there were no significant differences between pH's 7.5 and 8.5 or between pH's 8.5 and 9.5.

Ultraviolet Light

In tests in which stock solutions of GD-174 exposed to UV light were compared with unexposed reference solutions, no significant differences were found in the toxicities to fathead minnows, based on 96-h LC_{50}'s (Table 2).

Turbidity

The activity of GD-174 was severely reduced in turbid water where the primary source of turbidity was produced by either bentonite or Crowley silt loam. In fact, no mortality of fathead minnows was observed in solutions where bentonite was the source of turbidity. A slight reduction in toxicity was observed in kaolin turbidities, and barium sulfate turbidities had no significant effect on toxicity (Table 3).

Table 2. *Toxicity of 2-(digeranylamino)-ethanol solutions exposed to ultraviolet light (21 µW/cm² X 100) to fathead minnows in soft water at 12 C.*

Exposure[a] or aging time (h)	LC_{50} and 95% confidence intervals (µl/l) Unexposed solutions[b]	Exposed solutions
6	0.268	0.250
	0.241-0.298	0.219-0.285
12	0.200	0.250
	0.172-0.232	0.220-0.283
24	0.172	0.150
	0.127-0.232	0.121-0.185

[a]Time the solutions were irradiated with ultraviolet light.
[b]Unexposed solutions were shielded with aluminum foil.

Table 3. *Effects of selected turbidities on the toxicity of 2-(digeranylamino)-ethanol to fathead minnows.*

Turbidity (NTU)	Source of turbidity[a], exposure time, and LC_{50}'s and 95% confidence intervals (µl/l)							
	Bentonite		Crowley silt loam		Kaolin		Barium sulfate	
	24 h	96 h	24 h	96 h	24 h	96 h	24 h	96 h
0.53[b]	0.410	0.142	0.353	0.128	0.500	0.128	0.560	0.141
	0.345-0.487	0.119-0.169	0.285-0.438	0.110-0.149	0.413-0.605	0.108-0.152	0.475-0.660	0.118-0.168
25	> 2.0	> 2.0	0.353	0.247	0.542	0.194	0.448	0.123
			0.285-0.438	0.234-0.357	0.435-0.675	0.165-0.227	0.320-0.627	0.108-0.139
50	> 2.0	> 2.0	0.690	0.289	0.560	0.209	0.531	0.140
			0.552-0.862	0.234-0.357	0.477-0.658	0.187-0.234	0.400-0.704	0.109-0.179
75	> 2.0	> 2.0	0.790	0.290	0.557	0.196	0.474	0.184
			0.681-0.916	0.249-0.338	0.446-0.696	0.165-0.232	0.394-0.570	0.155-0.218
100	> 2.0	> 2.0	0.750	0.292	0.640	0.221	0.532	0.143
			0.582-0.967	0.250-0.341	0.549-0.745	0.181-0.270	0.430-0.658	0.120-0.171

[a]Grams of substance per 15 liters of solution used to produce turbidities of 25, 50, 75, and 100 NTU's were: bentonite—1.5, 3.75, 7.5, and 15.0; Crowley silt loam—1.8, 3.45, 5.1, and 6.75; kaolin—0.375, 0.75, 1.125, and 1.5; and barium sulfate—0.27, 0.615, 0.99, and 1.35.

[b]Turbidity of standard reconstituted water.

Table 4. *Toxicity of 2-(digeranylamino)-ethanol to three species of fish after 24-h exposure of test solutions to duckweed or waterweed.*

Species of fish, and type and amount (g/l) of vegetation	LC_{50} and 95% confidence interval (µl/l)	
	24 h	96 h
Brown trout		
Duckweed		
0	0.347	0.323
	0.278-0.433	0.270-0.386
5	0.323	0.263
	0.275-0.378	0.198-0.347
Rainbow trout		
Waterweed		
0	0.298	0.264
	0.248-0.358	0.222-0.314
5	0.562	0.562
	0.495-0.638	0.495-0.638
10	0.800	0.800
	0.694-0.921	0.694-0.921
15	1.07	1.07
	0.931-1.23	0.931-1.23
Fathead minnows		
Waterweed		
0	0.728	0.218
	0.688-0.771	0.183-0.259
2	0.800	0.390
	0.736-0.869	0.344-0.440
4	1.06	0.363
	0.889-1.26	0.300-0.439
8	1.00	0.618
	0.871-1.15	0.560-0.681
12	1.40	0.720
	1.05-1.88	0.657-0.789

Aquatic Vegetation

Duckweed appeared to have no effect on the toxicity of GD-174, whereas waterweed reduced the toxicity significantly (Table 4). The reduction was directly proportional to the amount of waterweed in the solutions. In rainbow trout, toxicity decreased 20.1% for each 1 g increase of waterweed per liter of solution, and results with fathead minnows were similar.

Counteraction

Our study indicated that GD-174 was subject to detoxification by both oxidation and reduction. However, sodium thiosulfate and chlorine were considerably less effective than potassium permanganate (Table 5).

Table 5. *Toxicity of 2-(digeranylamino)-ethanol solutions containing selected oxidizing or reducing agents to fathead minnows in soft water at 12 C.*

Agent and concentration	LC_{50} and 95% confidence interval (µl/l)	
	24 h	96 h
Reference solution	0.250	0.0895
	0.192-0.324	0.0688-0.116
$KMnO_4$ (2 mg/l)	2.45	1.42
	2.04-2.97	1.14-1.77
Cl_2[a] (0.05 mg/l)	0.970	0.450
	0.844-1.12	0.382-0.529
$Na_2S_2O_3$ (10 mg/l)	0.635	0.263
	0.560-0.720	0.197-0.351

[a]Commercial grade calcium hypochlorite (65% available Cl_2).

Discussion and Conclusions

The compound GD-174, like rotenone (Gersdorff 1943; Spitler 1970) and antimycin (Berger et al. 1969), was more toxic at high than at low temperatures. However, unlike rotenone (Brooks 1961; Spitler 1970) and antimycin (Berger et al. 1969), GD-174 was more toxic in alkaline than in acid waters. Toxic action of GD-174 did not appear to be altered by ultraviolet light, whereas that of both antimycin and rotenone is decreased (Dawson 1973; Cheng et al. 1972).

In tests with plants, the toxicity was reduced by the submerged waterweed, whereas the floating duckweed had no effect. Although it is difficult to assess the cause of the differences, it could be related to differences in metabolism, or to differential sorption of the toxicant onto the surface of the plants. Because submerged vegetation has much more area in contact with the solution than the floating variety, it would reduce activity by a greater degree.

In field situations, the inhibiting effects of aquatic vegetation and turbidity on the activity of GD-174 are likely to be additive. Furthermore, interactions between those factors and pH and temperature, which may themselves either antagonize or enhance the activity, will make difficult the choice of the proper concentration for selective carp control. Therefore, a precise on-site toxicity test will be necessary to prescribe the proper concentration for field application.

The counteraction studies showed that the toxicant could be deactivated rapidly with strong oxidizing or reducing agents. Potassium permanganate was the most effective and would be the chemical of choice for field application. However, no chemicals are currently registered for aquatic use of this type.

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Toxicities of the Lampricides 3-Trifluoromethyl-4-nitrophenol (TFM) and the 2-Aminoethanol Salt of 2',5-Dichloro-4'-nitrosalicylanilide (Bayer 73) to Four Bird Species[1]

by

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Abstract

The acute oral toxicities of the lampricides 3-trifluoromethyl-4-nitrophenol (TFM) and the 2-aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide (Bayer 73, Bayluscide) were determined in mallards (*Anas platyrhynchos*), ring-billed gulls (*Larus delawarensis*), bobwhites (*Colinus virginianus*), and California quail (*Lophortyx californicus*). Ring-billed gulls were the most sensitive to both chemicals, alone and in combination. Field grade TFM (35% TFM in N,N-dimethylformamide) was toxic; median lethal dosages (LD_{50}'s) ranged from 250 mg/kg in gulls to 546 mg/kg in California quail. Toxicity of Bayer 73 (70%) was lower; LD_{50} values ranged from 500 mg/kg in gulls to more than 2,000 mg/kg in mallards and bobwhites. Field grade TFM was more toxic than purified TFM (> 96% pure) to mallards. Toxicity of a mixture of Bayer 73 with field grade TFM was additive in gulls and possibly additive in mallards. Mallards exposed for 48 h to drinking and swimming water containing the lampricides showed mild signs of intoxication but no birds became severely ill. On the basis of these studies, use of TFM or Bayer 73, alone or in combination, presents little toxic hazard to birds when applied in the amounts routinely used for lamprey control.

The toxicant 3-trifluoromethyl-4-nitrophenol (TFM) has been used extensively in the Great Lakes region for controlling the sea lamprey (*Petromyzon marinus*). Since Howell et al. (1964) reported that the 2-aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide (Bayer 73, Bayluscide) acts synergistically to increase the toxicity of TFM to sea lamprey larvae, Bayer 73 has sometimes been used in combination with TFM at concentrations corresponding to 2% or less of the TFM concentrations, based on active ingredients of each.

Under ideal conditions TFM is 2 to 10 times more toxic to sea lamprey larvae than to most fish, but it is also toxic to many other organisms (Schnick 1972). Bayer 73, on the other hand, is extremely toxic to a wide variety of aquatic organisms, but appears to have little effect on mammals (Marking and Hogan 1967). Although the toxicity of TFM to sea lampreys is increased by the addition of Bayer 73, its selectivity is decreased; consequently fish kills occur more frequently when the combination of the two chemicals is used (Schnick 1972). The present study was conducted to gather basic data on the toxicity of TFM, Bayer 73, and a mixture of the two chemicals to four bird species—mallards (*Anas platyrhynchos*), ring-billed gulls (*Larus delawarensis*), bobwhites (*Colinus virginianus*), and California quail (*Lophortyx californicus*)—and to assess the toxic hazard of aqueous solutions of these compounds to mallards.

Materials and Methods

The lampricides used in these studies, obtained from the National Fishery Research Laboratory, La Crosse, Wisconsin, consisted of purified TFM (TFM[A]; > 96% pure, Aldrich Chemical Co., Lot No. 060217); field grade TFM (TFM[FG]; 35% TFM in N,N-dimethylformamide); and Bayer 73 (70% pure, Chemagro Corp., Lot No. 8059410). Reagent grade N,N-dimethylformamide (DMF), obtained locally, was tested alone and in combination with TFM(A) to assess its contribution to

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toxicity in the field grade formulation.

Mallards used in this study were raised at the Denver Wildlife Research Center, either from stock lines (for the studies with purified and field grade TFM, and DMF) or from 1-day-old ducklings obtained from the Max McGraw Wildlife Foundation, Dundee, Illinois (for the studies on Bayer 73 and the mixture of Bayer 73 with field grade TFM). Ring-billed gulls were obtained from a captive breeding colony maintained at the Denver Wildlife Research Center, and California quail were raised at the Center, from stock lines. Bobwhites came from Highland Game Bird Farms, Franktown, Colorado. Age and sex of test birds varied, depending on availability. Previous studies have indicated that, unlike mammals, nonbreeding birds show only minor sex-dependent differences in sensitivity to oral administration of pesticides in acute toxicity tests (Tucker and Crabtree 1970; Tucker and Haegele 1971), and differences between age groups are generally small (Hudson et al. 1972). However, these differences occasionally are significant and should not be ignored. The age and sex of the birds used in the present tests are shown in Table 1.

Table 1. *Acute oral toxicity (LD_{50})[a] of lampricides to four species of birds.*

	Lampricide[c]			
Species[b]	TFM(A)	TFM(FG)	Bayer 73	TFM(FG) + Bayer 73
Mallard	458 (324-649)	308 (237-400)	> 2,000	472 (408-546)
Ring-billed gull		250 (170-368)	500 (77.8-3,210)	154 (118-200)
Bobwhite			> 2,000	435 (335-565)
California quail		546 (313-950)		

[a] LD_{50} values are expressed as milligrams of toxicant per kilogram of body weight; 95% confidence limits are shown in parentheses.

[b] Age and sex of birds exposed to the different chemicals follow. Mallards: TFM(A) and TFM(FG), 1-year-old drakes; Bayer 73, 1-year-old hens; TFM(FG) + Bayer 73, 14- to 17-week-old drakes. Ring-billed gulls: TFM(FG), immature males and females; Bayer 73, adult females; TFM(FG) + Bayer 73, immature and adult males and females. Bobwhites: all tests, 18- to 24-week-old cocks. California quail: TFM(FG), 1-year-old hens.

[c] Lampricides: TFM(A) = purified, > 96% active 3-trifluoromethyl-4-nitrophenol; TFM(FG) = field grade, 35% active TFM in the carrier N,N-dimethylformamide (DMF); and Bayer 73 = 70% active 2-aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide. The LD_{50}'s of DMF alone (not shown) were > 2,000 mg/kg in mallards, > 185 in ring-billed gulls, and > 460 in bobwhites.

Mallards and ring-billed gulls were exposed in groups of 2 to 16 birds in unheated, concrete-floored pens (2.4 × 3.0 m, 2.4 m high), which were enclosed and covered, and contained a 37-liter swimming pond with about 2,450 cm^2 of water surface. Quail were held individually in indoor cages (30 × 56 cm, 23 cm high) provided with a water trough. All birds were held in the pens for at least 1 day before they were exposed to lampricides. Temperature in the indoor test room was maintained at 20 to 24 C. Mallards and quail were fed a commercially prepared game bird diet, and gulls a commercially prepared dry dog food. Feed was available ad libitum, except during a 20-h fasting period before oral administration of toxicants for the acute toxicity tests. Water was available ad libitum, and fresh water was constantly available in the mallard and gull cages, except during 48-h exposures of mallards to lampricides in the water under static conditions.

For determination of the acute oral median lethal dosage (LD_{50}), the test compounds were administered either by stomach tube or in gelatin capsules inserted through glass tubing. Each compound was delivered to the level of the proventriculus in mallards and gulls and to the crop in both species of quail. Gelatin capsules were used for the administration of TFM(A) to mallards; of Bayer 73 to mallards, gulls, and bobwhites; and of DMF to gulls and bobwhites. A stomach tube was used to deliver TFM(FG) to mallards, gulls, and California quail, and DMF to mallards.

In tests to assess the toxicity of a mixture of the lampricides, TFM(FG) and Bayer 73 were administered sequentially (because the chemicals could not be successfully administered simultaneously) to mallards, gulls, and bobwhites as follows: (1) Bayer 73 in gelatin capsules was administered through glass tubing; (2) a small amount of pure DMF was administered by stomach tube; and (3) within 5 to 18 min, TFM(FG) was administered by stomach tube. Dosages of the individual lampricides administered to each animal provided the active ingredients in the ratio of 98 parts TFM to 2 parts Bayer 73 (34.3% active TFM and 0.7% active Bayer 73 in the mixture).

Three to six birds were treated at each of two to four geometrically spaced dosage levels. Acute oral LD_{50} values were computed by the methods of Thompson (1947) and Weil (1952).

The indices used to describe the toxicity of lampricides administered in combination were derived by Marking and Dawson (1975). An additive index greater than 0 indicates greater than additive toxicity (synergism), an index less than 0 indicates less than additive toxicity (antagonism), and an index of 0 indicates simple additive toxicity of the toxicants in a mixture. If the confidence interval for the additive

index overlaps 0, simple additive toxicity is indicated.

Mallards were exposed to lampricide-contaminated water in their swimming ponds for 48 h under static conditions. Experimental groups in these tests were composed of five males and five females. The ages of the ducks exposed to the different chemicals were as follows: TFM(A), 6-8 weeks; TFM(FG) and Bayer 73, 1 year; and the mixture of TFM(FG) with Bayer 73, 17 weeks. Appropriate quantities of TFM(A) were dissolved in 59.2 ml ethanol and then added to the tap water in the swimming ponds to make up treatment levels of 5, 15.8, 50, and 500 mg/l of active TFM. Only the solvent (59.2 ml of ethanol) was added to the swimming pond of the control group. In other swimming ponds TFM(FG) was at concentrations of 57.1 and 286 mg/l (20 and 100 mg/l active TFM); Bayer 73 at concentrations of 0.01, 0.10, and 1.0 mg/l (0.007, 0.07, and 0.70 mg/l of active Bayer 73); and the mixture of TFM(FG) and Bayer 73 (of the same composition as that administered orally) at concentrations of 11.7, 58.4, and 292 mg/l (4.01 and 0.082, 20.0 and 0.409, and 100 and 2.04 mg/l of active TFM and Bayer 73, respectively). At the end of the 48-h exposure, the lampricide-contaminated water was flushed out and replaced with tap water, augmented by a small continuous flow. The swimming ponds of the controls for all tests other than TFM(A) contained untreated tap water.

Mortality, signs of intoxication, and body weight changes were observed for 14 to 21 days after treatment. (In general, the weights of the survivors of all studies were normal by the end of the observation periods.)

Results

Acute Oral Toxicity Tests

Of the species exposed, ring-billed gulls were the most sensitive to all of the compounds (TFM[A] not tested), alone or in combination (Table 1). Median lethal dosages for TFM(FG) ranged from 250 mg/kg in gulls to 546 mg/kg in California quail. Bayer 73 was less toxic than TFM; LD_{50} values ranged from 500 mg/kg in gulls to $>2{,}000$ mg/kg in mallards and bobwhites.

The additive toxicity indices of combinations of lampricides and solvent (DMF) are presented in Table 2. TFM(A) and DMF produced an additive index of 1.98 (greater than additive toxicity) with mallards. TFM(FG) and Bayer 73 produced an additive index of -0.506, which suggests less than additive toxicity, although the range for that index could not be calculated because there was no 95% confidence interval for Bayer 73 tested individually. Since the index is close to zero, however, the toxicity possibly was additive. The combination of TFM(FG) and Bayer 73 was additive in toxicity to ring-billed gulls (the interval for the additive index, -0.179 to 2.17, overlapped zero).

Mallards treated with oral doses of TFM(FG) drank excessive amounts of water and showed a number of other responses: regurgitation, stumbling, excessive sitting, weakness, incoordination, imbalance, labored breathing, immobility, and terminal wing-beat convulsions accompanied by arching of the head and neck over the back. Mallards treated with TFM(A) also displayed these signs of intoxication (except for weakness), in addition to social withdrawal, imperturbability, convulsions, and temporary whole-body rigidity. Signs of intoxication were produced more rapidly by TFM(FG) than by TFM(A): signs of TFM(FG) intoxication appeared as soon as 1 min after treatment, and deaths from 4 to 30 min after treatment, whereas the effects from TFM(A) did not appear for 13 min and deaths occurred 20 to 50 min after treatment. Recovery (when it occurred) usually was complete within 4 h after treatment with TFM(FG), but was prolonged for up to 7 or 8 days after treatment with TFM(A).

The signs of intoxication observed in ring-billed gulls dosed with TFM(FG) were similar to those observed in mallards with the addition of rapid breathing, imperturbability, wing-droop, outspread wings, and temporary whole-body rigidity, but the gulls did not display stumbling, weakness, imbalance, immobility, or terminal wing-beat convulsions. The timing of the appearance of signs and deaths was usually similar to that in mallards; however, one gull died 11 days after treatment. There was no apparent sex-related difference in response among the gulls.

After treatment with TFM(FG), California quail displayed signs of intoxication similar to those observed in gulls and mallards, except that regurgitation did not occur. The timing of the appearance of signs and deaths was also similar.

Bayer 73 produced intoxication in all of the species tested at all of the dosage levels administered. Signs observed in mallards included tenseness of the masseter, regurgitation, high carriage, excessive drinking and sitting, imperturbability, incoordination, imbalance, rapid and labored breathing, tremors, and temporary whole-body rigidity followed by death. Signs observed in bobwhites and gulls were similar; however, regurgitation in gulls was more profuse, especially at the higher dosage levels, and probably accounts for the wide confidence interval (Table 1) calculated for this test (regurgitation did not occur in bobwhites). Deaths occurred at 500, 1,000, and 2,000 mg/kg in mallards (one of the six mallards treated at each of these levels died), and one of three bobwhites treated at 2,000 mg/kg died. Toxic signs in all three species appeared as soon as 15 min after treatment and persisted for up to 24 h; deaths occurred from

30 min to 3 h after treatment.

After treatment with the mixture of TFM(FG) and Bayer 73, signs observed in mallards included most of those observed after treatment with the individual chemicals. The timing of the appearance of effects, deaths, and recovery in mallards was similar to that after treatment with TFM(FG) alone. Toxic signs observed after treatment of ring-billed gulls with the mixture were similar to those observed in mallards, although the appearance of signs, deaths, and recovery was more rapid: deaths usually occurred in 2 to 12 min and recovery within 1 h. Signs of intoxication observed after treatment of bobwhites with the mixture were similar to those observed in mallards, except that regurgitation did not occur. Timing of the appearance of signs, deaths, and recovery was also similar to that observed in mallards.

Signs of intoxication in mallards (1-year-old drakes) given DMF at dosages up to 2,000 mg/kg were limited to slowness, incoordination, and falling, which developed within 10 min after treatment. All ducks used in the tests had recovered within about 24 h. Bobwhites (19- to 20-week-old cocks) treated at dosages up to 505 mg/kg and gulls (immature and adult males and females) treated at dosages up to 249 mg/kg showed no signs of intoxication after DMF treatment.

Water Exposure Toxicity Tests

No mortalities occurred in mallards exposed to lampricides in the ponds in their enclosures, and body weights by the end of the observation periods were normal. However, various clinical signs of poisoning were observed.

All of the 6- to 8-week-old mallards exposed to 5 mg/l TFM(A) appeared normal throughout the 48-h exposure and subsequent 12-day observation period. In the group treated with 15.8 mg/l, several birds appeared wobbly and showed slight masseter tenseness about 7 h after exposure began. About 22 h later most of the mallards in this test group may have had slight degrees of incoordination, and 49 h after exposure the group was underactive and all birds tended to sit; one drake showed a moderate degree of incoordination and jerky movements. All birds in the 50-mg/l group were strongly affected after 3 h of exposure. They displayed excessive sitting, underactivity, and weakness of the leg muscles, and some ran and fell with moderate to extreme degrees of incoordination. Although one bird was extremely incoordinated 22 h after exposure began, most birds had begun to recover. All were normal 24 to 48 h after exposure ended. The group exposed to 500 mg/l apparently avoided consumption of the solution after initial exposure. This group sat huddled together more than controls and stumbled and showed slight to moderate degrees of incoordination; they appeared normal 24 h after exposure ended.

The 1-year-old mallards exposed to 57.1 mg/l TFM(FG) appeared normal throughout the study. Those exposed to 286 mg/l displayed incoordination, slowness, and stumbling; however, recovery occurred within 24 h after the end of treatment.

During the water exposure studies of Bayer 73 and the mixture of Bayer 73 with TFM(FG), only mild signs of intoxication were observed. One mallard exposed to 0.10 mg/l Bayer 73 showed slight incoordination 24 to 48 h after the beginning of treatment, and one exposed to 292 mg/l of the mixture showed slight incoordination on the day treatment was begun.

Discussion

The present tests showed that TFM is moderately toxic to birds exposed by acute oral administration, and that it has about the same magnitude of toxicity in birds as in mammals. Bayer 73 appears to have a low order of toxicity in birds, although birds may be more susceptible than mammals to acute oral administration. The analysis for additive toxicity revealed that the mixture of TFM(A) and DMF worked synergistically in mallards (Table 2). Maki et al. (1975), who studied the toxicity of purified and field grade TFM to several species of algae, noted that DMF in the field grade material may augment the toxicity of TFM; Sanders and Walsh (1975) found a synergistic effect of the field grade formulation in crayfish (*Orconectes nais*); and Marking and Olson (1975) noted that the field grade TFM was more toxic to several species of fish. Apparently the carrier (DMF) increases dispersion, reduces particle size, or enhances the ionic state of the TFM molecule (Marking and Olson 1975). The increased activity of TFM(FG) in mallards can probably be attributed to increased absorption of TFM in the gastointestinal tract as a result of the presence of DMF. Whether DMF acts by altering the epithelial lining, or merely by changing the state of the TFM (solution vs. solid) is not known, but DMF is known to increase the epidermal absorption of many substances (Wurster 1972).

In these studies it appears that the mixture of TFM(FG) with Bayer 73 was additive in ring-billed gulls and possibly additive in mallards. Kawatski et al. (1975) noted that the toxicity of TFM and Bayer 73 to larvae of a midge (*Chironomus tentans*) also was usually additive.

Concentrations of the lampricide TFM routinely used for sea lamprey control range from 1 to 15 mg/l (active ingredient), and exposures generally last 12 to 15 h. Concentrations of Bayer 73 routinely used are 2% or less of the TFM concentration. Exposures of mallards to drinking and swimming water containing

Table 2. *Additive toxicity indices of lampricides administered individually and in combination to birds in acute oral tests.*

Species and lampricide[a]	LD_{50}[b] and 95% confidence limits		Additive index[c]
	Individually	In combination	
Mallard	458	108	
TFM(A)	(324-649)	(83.0-140)	
and			1.98
DMF	> 2,000	200	
		(154-260)	
Mallard	308	463	
TFM(FG)	(237-400)	(400-535)	
and			-0.506
Bayer 73	> 2,000	4.72	
		(4.08-5.46)	
Ring-billed gull	250	151	
TFM(FG)	(170-368)	(116-196)	
and			0.647
Bayer 73	500	1.54	(-0.179-2.17)
	(77.8-3,210)	(1.18-2.00)	

[a]Lampricides: TFM(A) = purified, > 96% active 3-trifluoromethyl-4-nitrophenol; DMF = reagent grade N,N-dimethylformamide; TFM(FG) = field grade, 35% active TFM in DMF; and Bayer 73 = 70% active 2-aminoethanol salt of 2',5-dichloro-4'-nitrosalicylanilide.

[b]LD_{50} values are expressed as milligrams of toxicant per kilogram of body weight.

[c]For discussion of derivation, see Marking and Dawson (1975).

lampricides lasted longer than those used in field treatments; no mortalities occurred at any of the concentrations tested; and toxic responses were lacking or mild at the concentrations used in sea lamprey control.

Conclusions

- The lampricide TFM was toxic to birds in acute oral tests, and apparently has about the same magnitude of toxicity in birds as in mammals.
- Bayer 73 appeared to have a low order of toxicity in birds, but may be more toxic to birds than to mammals.
- Field grade TFM appeared to be more toxic than purified TFM to mallards.
- Mixtures of Bayer 73 and TFM (2:98) appeared to have additive toxic action in ring-billed gulls and possibly additive action in mallards.
- Among mallards exposed to lampricides in the drinking and swimming water, none died at the concentrations tested, and toxic responses to use-pattern concentrations were lacking or mild.
- The addition of TFM or Bayer 73, alone or in combination, to the environment in the amounts routinely used for lamprey control appears to present little toxic hazard to birds.

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