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Formalin: Its Toxicity to Nontarget Aquatic Organisms, Persistence, and Counteraction

by

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Abstract

The acute toxicity of formalin to selected fishes and aquatic invertebrates was determined in standardized laboratory tests. Fish species exposed were chinook salmon *(Oncorhynchus tshawytscha)*, rainbow trout *(Salmo gairdneri)*, Atlantic salmon *(S. salar)*, lake trout *(Salvelinus namaycush)*, black bullhead *(Ictalurus melas)*, channel catfish *(I. punctatus)*, green sunfish *(Lepomis cyanellus)*, bluegill *(L. macrochirus)*, smallmouth bass *(Micropterus dolomieui)*, and largemouth bass *(M. salmoides)*. Invertebrates exposed were freshwater prawn *(Palaemonetes kadiakensis)*, seed shrimp (*Cypridopsis* sp.), Asiatic clam *(Corbicula leana)*, snail (*Helisoma* sp.), and backswimmer (*Notonecta* sp.). Black bullhead and channel catfish were the fish most sensitive to formalin (96-h LC_{50}'s, 62.1 and 65.8 μl/l), and Atlantic salmon and green sunfish were the most resistant (96-h LC_{50} for each, 173 μl/l). The $TILC_{50}$ (lethal concentration producing 50% mortality independent of time) for formalin against rainbow trout was 72.0 μl/l. Seed shrimp were the most sensitive invertebrates (96-h LC_{50}, 1.05 μl/l), and backswimmers were the most resistant (96-h LC_{50}, 835 μl/l). The toxicity of formalin was unchanged in solutions aged as long as 3 weeks; the biological half-life could not be determined. Formalin was not detoxified by oxidation or reduction, and filtration through activated carbon did not significantly reduce toxicity.

Formalin is one of the most effective and widely used compounds in fish culture for therapeutic and prophylactic treatment of fungal infections and external parasites of fish and fish eggs. Uses of formalin in fish culture were reviewed by Schnick (1974). Before about 1967, the registration of chemicals used to treat diseases of fish in hatcheries was not required. Since then the Food and Drug Administration and the Environmental Protection Agency have required specific information about each chemical and its use pattern before registration. Information required for the registration includes toxicity to target and nontarget organisms, efficacy, residues, metabolites, and means of counteraction (Lennon 1967). Standardized tests have been developed for generating toxicity information necessary for the registration of fishery chemicals (Marking 1975).

The purposes of this study were to determine (1) the toxicity of formalin to nontarget aquatic organisms, (2) the toxicity (safety) of maximum use-pattern exposures to formalin, (3) the toxicity of formalin to selected fishes in extended exposures, (4) the effects of certain water characteristics on the toxicity of formalin to fish, (5) the persistence of formalin in water, and (6) the feasibility of counteracting formalin by oxidation or reduction, or removal from water with activated carbon.

Materials and Methods

Stock solutions of commercial grade formalin (37% formaldehyde) obtained from North Central Chemical Co., La Crosse, Wisconsin, were prepared in water (the liquid formulation was measured volumetrically and diluted with water). All concentrations listed are based on the formulated product. To prepare test solutions of the desired concentrations, we pipetted portions of stock solutions into the test vessels, and stirred the resulting mixture to ensure homogeneity. In flow-through toxicity tests, required amounts of the stock solutions were delivered by a solenoid-activated pipetting pump (Micromedic Systems Automatic Pipette Model 2500).

Fish species exposed were chinook salmon *(Oncorhynchus tshawytscha)*, rainbow trout *(Salmo gairdneri)*, Atlantic salmon *(S. salar)*, lake trout *(Salvelinus namaycush)*, black bullhead *(Ictalurus melas)*, channel catfish *(I. punctatus)*, green sunfish *(Lepomis cyanellus)*, bluegill *(L. macrochirus)*, smallmouth bass *(Micropterus dolomieui)*, and largemouth bass *(M. salmoides)*. Invertebrates exposed were freshwater prawn *(Palaemonetes kadiakensis)*, seed shrimp (*Cypridopsis* sp.), clam *(Corbicula leana)*, snail (*Helisoma* sp.), and backswimmer (*Notonecta* sp.). The fish were obtained from State and Federal hatcheries and maintained in the laboratory; invertebrates were either cultured outdoors in partly shaded vinyl pools or collected in the field. Organisms collected in the field were held for 7 days in water identical with that used in the toxicity tests, before they were exposed to formalin. Fish and invertebrates were acclimated to test conditions for 24 h before the addition of formalin. Ten or more organisms were exposed at each concentration. Mortalities were recorded at 1, 3, and 6 h the first day and daily thereafter during the 96-h exposure period. Fish were regarded as dead when all opercular movements ceased and invertebrates when they became immobile or failed to respond to physical stimuli.

Laboratory toxicity tests were conducted according to standard procedures described by Lennon and Walker (1964) and the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Static tests were conducted in 2.5- or 15-liter glass jars, depending on the size of the test organism involved. Flow-through tests were conducted in 45 liters of test solution; the solution was replaced four times daily through a 1-liter dilution apparatus similar to that described by Mount and Brungs (1967).

Temperature was controlled by immersing test vessels in a water bath equipped with a chilling unit. Reconstituted water (Marking 1969) was used in tests with fish and clams and limed spring water (pH 6.5 ±0.1, total hardness 20 mg/l as $CaCO_3$) in tests with the other invertebrates. Chemical buffers were added to soft water in tests of the effect of pH (6.5–9.5), as recommended by Marking and Dawson (1973). The pH's of the test solutions were checked daily and adjusted to within ±0.2 pH units. For determination of the persistence of formalin, aqueous solutions were aged 1, 2, and 3 weeks, after which rainbow trout fingerlings were introduced and 96-h LC_{50}'s computed. Deactivation indices were computed from these data according to the method of Marking (1972).

In counteraction studies, potassium permanganate ($KMnO_4$) at a concentration of 1 mg/l and sodium thiosulfate ($Na_2S_2O_3$) at 10 mg/l were introduced into a series of formalin solutions of selected concentrations 6 h before the introduction of fish. In aeration tests, the solutions were aerated with air stones for 24 h before fish were added. We compared the 96-h LC_{50}'s with a reference standard to assess changes in toxicity. To determine if formalin could be removed from aqueous solutions, we filtered a concentrated solution (150 µl/l) at a flow rate of 100 ml/min through a 15-cm column of activated charcoal (Darco 20 × 40 mesh). Samples of effluent were taken at selected volumes (0–200 ml and 800–1,000 ml). These samples and a sample of the stock solution were bioassayed against rainbow trout and the 96-h LC_{50}'s compared.

We used the method of Litchfield and Wilcoxon (1949) to determine LC_{50}'s and 95% confidence intervals, and a modification of the method given by Green (1965) to compute $TILC_{50}$'s.

Results

Toxicity of Formalin to Fish

The 96-h LC_{50}'s for formalin against nine species of fish ranged from 62.1 µl/l for black bullheads to 173 µl/l for green sunfish and Atlantic salmon (Table 1). Toxicity of formalin increased with time; for bluegills, for example, the 3- and 96-h LC_{50}'s were 2,290 µl/l and 100 µl/l, respectively. Ictalurids were twice as sensitive to formalin as the centrarchids or salmonids. Green sunfish were the most resistant centrarchid exposed, followed by largemouth bass, smallmouth bass, and bluegills. Atlantic salmon were the most resistant salmonid, followed by rainbow trout and lake trout (Table 1).

The effects of temperature, water hardness, and pH on toxicity were determined by exposing rainbow trout, channel catfish, and bluegills to formalin. In short exposures, formalin was significantly more toxic to these species at the higher temperatures; however, at 96 h the differences were insignificant except in rainbow trout (Tables 2, 3, and 4). Water hardness had no apparent effect on toxicity. For rainbow trout and channel catfish, formalin was more toxic in waters of pH 9.5 than in waters of pH 6.5, 7.5, or 8.5 (Tables 2 and 3).

In chronic toxicity tests the $TILC_{50}$ for formalin against rainbow trout was 72.0 µl/l as compared with LC_{50}'s of 157 µl/l at 24 h and 131 µl/l at 96 h.

Toxicity of Formalin to Invertebrates

Invertebrates differed widely in their responses to formalin. The 96-h LC_{50}'s ranged from 1.05 µl/l for seed shrimp to 835 µl/l for backswimmers; those for the bivalve and snail—126 and 93 µl/l—were similar

Table 1. *Toxicity of formalin to fingerling fish of nine species in standard toxicity tests at 12 C.*

Species	Average weight (g)	LC_{50} and 95% confidence interval (μl/l) at			
		3 h	6 h	24 h	96 h
Rainbow trout	0.63	1230 957-1581	655 580-740	300 237-380	118 99.7-140
Atlantic salmon	0.60	1410 1049-1896	840 751-939	389 333-455	173 149-201
Lake trout	0.50	—	603 444-819	141 114-174	100 78.2-128
Black bullhead	0.75	—	—	173 123-243	62.1 50.9-75.8
Channel catfish	0.40	495 430-570	232 178-303	122 102-145	65.8 58.1-74.5
Green sunfish	0.70	—	—	323 250-417	173 123-243
Bluegill	0.50	2290 1804-2907	1600 1165-2198	211 171-260	100 80.0-125
Smallmouth bass	0.68	—	—	222 171-288	136 90.2-205
Largemouth bass	1.00	—	1030 928-1140	283 229-350	143 129-159

Table 2. *Toxicity of formalin to fingerling rainbow trout at selected water temperatures, hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC_{50} and 95% confidence interval (μl/l) at				
			1 h	3 h	6 h	24 h	96 h
7	Soft	7.5	>3000	1810 1537-2131	940 762-1160	349 292-418	245 213-282
12	Soft	7.5	2310 1959-2724	1230 957-1581	655 580-740	300 237-380	118 99.7-140
17	Soft	7.5	1210 1015-1443	1000 788-1269	590 532-654	219 174-276	—
12	Very soft	6.6	>2500	1590 1070-2364	729 657-809	230 181-292	—
12	Hard	7.8	1740 1240-2441	1740 1240-2441	925 783-1093	388 332-454	172 108-274
12	Very hard	8.2	1690 1070-2670	1690 1070-2670	910 769-1077	334 272-411	171 122-240
12	Soft	6.5	1730 1233-2427	1730 1233-2427	835 749-931	321 231-446	171 122-240
12	Soft	8.5	1390 1043-1852	1150 917-1442	645 523-796	300 220-408	172 123-241
12	Soft	9.5	1740 1240-2441	875 750-1020	500 355-704	135 105-174	100 78.9-127

Table 3. *Toxicity of formalin to channel catfish at selected water temperatures, hardnesses, and pH's.*

Temp. (°C)	Water hardness	pH	LC_{50} and 95% confidence interval (μl/l) at 1 h	3 h	6 h	24 h	96 h
12	Soft	7.5	779 666-911	495 430-570	232 178-303	122 102-145	65.8 58.1-74.5
17	Soft	7.5	600 523-689	350 299-409	282 228-349	119 102-138	65.5 58.2-73.7
22	Soft	7.5	559 475-658	284 229-351	234 185-297	100 83.7-119	64.0 58.6-69.9
12	Very soft	6.6	771 660-901	490 425-565	490 425-565	99.0 86.0-114	69.9 63.7-76.7
12	Hard	7.8	1050 798-1382	450 360-563	355 298-422	117 100-137	49.0 43.3-55.5
12	Very hard	8.2	872 703-1081	439 346-557	285 229-355	111 94.5-130	61.9 53.9-71.1
12	Soft	6.5	779 666-912	424 356-505	282 228-349	118 103-135	62.0 54.4-70.7
12	Soft	8.5	630 554-717	455 365-567	285 230-353	94.0 84.0-105	56.5 51.4-62.1
12	Soft	9.5	559 474-659	282 228-349	235 185-298	63.9 54.7-74.7	42.9 36.2-50.9

Table 4. *Toxicity of formalin to fingerling bluegills at selected water temperatures, hardnesses, and pH's.*

Temp. (°C)	Water hardness	pH	LC_{50} and 95% confidence interval (μl/l) at 3 h	6 h	24 h	96 h
12	Soft	7.5	2290 1804-2907	1600 1165-2198	211 171-260	100 80.0-125
17	Soft	7.5	2300 1822-2904	780 670-908	189 153-234	73.5 63.5-85.0
22	Soft	7.5	1750 925-3312	469 403-545	142 115-176	91.0 81.2-102
12	Very soft	6.6	1800 1532-2115	1230 1071-1412	369 315-433	88.4 75.1-104
12	Hard	7.8	1720 1458-2029	1190 1027-1379	249 166-373	106 84.0-134
12	Very hard	8.2	1740 1499-2019	1310 1038-1654	233 181-300	117 101-136
12	Soft	6.5	2310 1961-2721	2290 1944-2697	335 284-395	125 89.1-175
12	Soft	8.5	2300 1822-2904	1650 1401-1943	230 182-290	86.2 72.6-102
12	Soft	9.5	2300 1950-2712	1055 883-1260	232 174-309	100 72.6-138

to those for fish. The freshwater prawn was intermediate in resistance to formalin, having a 96-h LC_{50} of 465 μl/l (Table 5).

Toxicity of Formalin at Use-Pattern Concentrations

Recommended use-pattern concentrations of formalin range as high as 250 μl/l for 1 h in tanks or raceways and are 15 to 25 μl/l for indefinite periods in earthen ponds. Exposure to use-pattern concentrations caused no mortality in chinook salmon, rainbow trout, Atlantic salmon, lake trout, black bullhead, channel catfish, green sunfish, bluegill, smallmouth bass, or largemouth bass. The seed shrimp was the only invertebrate affected; 99% mortality could be expected at a 25-μl/l indefinite treatment level.

Persistence of Formalin in Aqueous Solutions

The toxicity to rainbow trout fingerlings of formalin solutions that had been aged for 1, 2, and 3 weeks was not substantially different from that of fresh solutions (Table 6).

Formalin solutions were not detoxified by either oxidation or reduction. The 96-h LC_{50}'s for the formalin reference solution, an aerated solution, and a solution to which thiosulfate had been added were not significantly different. However, the 96-h LC_{50} for the formalin:potassium permanganate solution was 60.0 μl/l as compared with 107 μl/l for the formalin reference (Table 7).

When the first and last 200-ml portions of the filtrate of a 150-μl/l formalin stock solution filtered through a 15-cm column of activated carbon were bioassayed against rainbow trout along with a sample of the stock solution, the 96-h LC_{50}'s were 210 μl/l for the first 200-ml sample, 132 μl/l for the 800- to 1,000-ml sample, and 121 μl/l for the reference solution. Although this difference indicates some removal of formalin, the removal was insignificant when the relative amounts of formalin and carbon involved (1 mg formalin/1 g carbon) are considered (Table 8).

Table 6. *Effect of aging on the toxicity to rainbow trout of formalin in soft water at 12 C.*

Aging period (weeks)	96-h LC_{50} (μl/l) and 95% confidence interval	Deactivation index
0	119 91.3-155	1.00
1	111 94.5-130	0.933
2	141 114-174	1.18
3	122 87.5-170	1.03

Table 5.—*Toxicity of formalin to selected aquatic invertebrates in soft water at 16 C.*

Species	LC_{50} and 95% confidence interval (μl/l) at				
	1 h	3 h	6 h	24 h	96 h
Seed shrimp (ostracods)[a] *Cypridopsis* sp.	9.00 6.83-11.9	6.40 4.91-8.34	1.20 0.664-2.17	1.15 0.690-1.97	1.05 0.590-1.87
Freshwater prawn[a] *Palaemonetes kadiakensis*	—	2150 1948-2373	1900 1588-2273	1105 896-1362	465 368-588
Bivalves[b] *Corbicula* sp.	--	—	—	800 638-1003	126 80.9-196
Snail[c] *Helisoma* sp.	3525 3201-3881	1340 953-1883	780 629-967	710 544-925	93.0 69.5-124
Backswimmer[c] *Notonecta* sp.	—	—	—	4500 3006-6735	835 652-1069

[a] Toxicity based on immobility.

[b] Toxicity based on ability to resist attempts to open valves and respond to tactile stimulus.

[c] Toxicity based on ability to respond to tactile stimulus.

Table 7. *Toxicity of formalin solutions containing selected oxidizing and reducing agents to fingerling rainbow trout*[a].

Chemical	Concentration (mg/l)	96-h LC_{50} (μl/l) and 95% confidence interval
Formalin (reference)	—	107 89.9-127
Formalin:aeration[b]		117 90.0-152
Formalin:thiosulfate	10	99.0 81.4-120
Formalin:$KMnO_4$	1	60.0 53.8-66.9

[a] Fish were added to the reference, thiosulfate, and $KMnO_4$ solutions 6 h after the chemicals were added.

[b] Solutions were aerated vigorously for 24 h before addition of fish.

Discussion

Information regarding the toxicity of formalin to various aquatic organisms is abundant. However, the varied test conditions under which the data were developed make comparisons difficult, and some of the data are unacceptable for use in the evaluation of formalin for registration (Schnick 1974). Usually no reference has been made to temperature, pH, hardness, or other characteristics of water that directly affect toxicity and efficacy of other chemicals used in fisheries (Marking and Olson 1975; McKee and Wolf 1963).

Schnick (1974) pointed out the wide range of sensitivity for different species of fish, salmonids and centrarchids being the most resistant and ictalurids the most sensitive. Data from our study follow this pattern. Schnick also stated that, although various chemical characteristics of the water and physical condition of the fish appear to influence the toxicity of formalin, variations in sensitivity within a species may be due to genetic composition.

Table 8. *Toxicity of selected eluates of a 150-μl/l formalin stock solution filtered through a 15-cm column of activated carbon to rainbow trout in soft water at 12 C.*

Eluate	96-h LC_{50} (μl/l) and 95% confidence interval
Reference[a]	121 105-140
0 to 200 ml	210 189-233
800 to 1,000 ml	132 111-157

[a] Toxicity of stock solution before filtration.

The effect of water chemistry on the toxicity of formalin to fish is somewhat controversial. Birdsong and Avault (1971) reported that the toxicity of formalin to pompano *(Trachinotus carolinus)* was not affected by different salinity levels. Piper and Smith (1973) reported that water chemistry has no effect on the toxicity of formalin to fish; however, their data were based on questionnaires received from various hatcheries rather than on experimental data. Marking et al. (1972) also reported that the toxicity of formalin was not affected by water hardness or pH. Bills (1974) first demonstrated that formalin was more toxic to fish and fish eggs in alkaline than in acid water. This conclusion is further supported by data from the present study, which show that in soft water formalin was more toxic to channel catfish and rainbow trout at pH 9.5 than at lower pH's.

Formalin is frequently used at concentrations of 15 to 25 μl/l for control of parasites on fish in earthen ponds. Much information is available on the efficacy of formalin as a parasiticide; however, the effects of formalin on pond flora and fauna, particularly on aquatic invertebrates, have not been determined. Schnick (1974) reported few data on the toxicity of formalin to invertebrates. Our data show a wide range of sensitivities for invertebrates; the 96-h LC_{50}'s ranged from 1.05 μl/l for seed shrimp to 835 μl/l for backswimmers. Our data also show formalin to be persistent under laboratory conditions, and at use-pattern concentrations some invertebrates could be affected. Present governmental controls on the use of chemicals in the environment necessitate

counteraction of persistent compounds after they have accomplished their purpose (Dawson 1976); however, the two most commonly used techniques for removal of such compounds (chemical oxidation/reduction or adsorption on activated carbon) failed to neutralize the toxicity of formalin. In fact, under oxidative conditions the solutions became more toxic.

Although some formalin may be removed by activated carbon, the amount is insignificant and the technique probably would not be applicable to hatchery operations.

Conclusions

1. Black bullheads were the species most sensitive to formalin (96-h LC_{50}'s = 62.1 μl/l).
2. Atlantic salmon and green sunfish were the most resistant species (96-h LC_{50}'s = 173 μl/l).
3. Lake trout were the most sensitive salmonids and bluegills were the most sensitive centrarchids.
4. The toxicity of formalin was not influenced by water hardness, but in soft water the chemical was more toxic to rainbow trout and channel catfish at pH 9.5 than at pH 6.5 or 8.5.
5. Formalin was more toxic to rainbow trout, channel catfish, and bluegills in warm than in cold water in 3-h exposures, but after 96 h the difference continued to be statistically significant only in rainbow trout.
6. Formalin was about twice as toxic in chronic exposures as in acute exposures.
7. Seed shrimp were the only organisms exposed that were affected by formalin at use-pattern concentrations.
8. Seed shrimp were the most sensitive invertebrates and backswimmers the most resistant; the 96-h LC_{50}'s were 1.05 μl/l and 835 μl/l.
9. The toxicity of formalin solutions persisted after 3 weeks of aging.
10. Formalin solutions were not detoxified by oxidation or reduction; in fact, they became more toxic under oxidative conditions.
11. Vigorous aeration for 24 h did not significantly change the toxicity of formalin solutions.
12. Only a small proportion of formalin was removed by filtration through activated carbon.

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Chlorine: Its Toxicity to Fish and Detoxification of Antimycin

by

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Abstract

The 96-h LC_{50}'s for chlorine ranged from 0.156 mg/l for channel catfish *(Ictalurus punctatus)* to 1.41 mg/l for black bullheads *(I. melas)* in standardized laboratory tests. The toxicity of chlorine was influenced little by pH, temperature, or water hardness. Chlorine at 0.25 and 0.5 mg/l effectively detoxified antimycin. The half-life of biological activity for antimycin with chlorine ranged from 1.1 h at pH 6.5 to 1.5 h at pH 9.5. Chlorine readily detoxified antimycin at pH 6.5, 7.5, and 8.5, but not at pH 9.5.

Chlorine is used extensively for disinfection of municipal water supplies and effluents (Fair et al. 1948; Chambers 1971) and occasionally for sterilization of fish hatchery water supplies (Hagen 1940; Bedell 1971) and culture ponds. Due to the widespread use of chlorine, precautions must be considered for protecting nontarget organisms such as fish, aquatic invertebrates, and plants. Brungs (1973), who summarized acute and chronic effects of residual chlorine on some aquatic organisms, recommended that total residual chlorine not exceed 0.2 mg/l for a period of 2 h/day for the more resistant species of fish. The evaluation of toxicity data in the literature is complicated by lack of information on the water chemistry, temperature, chlorine demand, solubility conditions, formation of chlorine complexes, and exposure time. Merkens (1958) stated that toxicity of chlorine depended on the amount of chlorine that remained in solution rather than on the amount added. Therefore the exact conditions of toxicity tests with aquatic organisms should be reported, and standardized procedures followed.

Because chlorine is a strong oxidizing agent, it can be used to detoxify chemicals that are subject to oxidation reactions. Preliminary studies on the fish toxicant antimycin indicated that it is effectively detoxified with 0.5 mg/l of chlorine in soft water at pH 7.5 (Dawson and Marking 1974); a concentration of 10 μg/l of antimycin was decreased to less than 0.2 μg/l after 2 h. The preliminary results were based on sensitivity bioassays with green sunfish *(Lepomis cyanellus)*. Although antimycin is nonpersistent in the aquatic environment (Marking and Dawson 1972), additional measures to accelerate its detoxification are needed when the possibility exists that treated water will enter municipal water supplies.

This study was designed to determine the toxicity of chlorine to fish and to establish the efficiency of chlorine for detoxifying the fish toxicant antimycin. Factors influencing toxicity of chemicals in water were included in the evaluation.

Materials and Methods

Technical grade antimycin was obtained from Ayerst Laboratories, Rouses Point, New York. Stock solutions were prepared by dissolving weighed portions in acetone and further diluting them in aqueous stock solutions just before use. Portions of the aqueous stock solutions delivered to the static test chambers yielded selected concentrations over a range which produced mortality at high concentrations but permitted survival at low concentrations. Aqueous stock solutions of chlorine were prepared from calcium hypochlorite (commercial grade HTH) containing 70% available chlorine. Test concentrations were based on active chlorine.

Procedures for the static toxicity tests followed those described by Lennon and Walker (1964), with some modifications. Test waters were prepared by adding mineral salts to deionized water in prescribed proportions to yield total hardness (as mg/l of $CaCO_3$) of 12 for very soft water, 44 for soft, 160 for hard, and 300 for very hard water (Marking 1969). The pH of reconstituted water was altered and stabilized by adding chemical buffers to soft water to yield pH's of 6.5, 7.5, 8.5, and 9.5 (Marking and Dawson 1973). The pH of water of different hardnesses was stabilized with sodium bicarbonate. The pH's of test solutions were checked daily and adjusted when necessary. Soft water was used in

standardized tests for the determination of the toxicity of chlorine to various species of fish.

Fish were obtained from Federal fish hatcheries and maintained by a trained fish culturist (Hunn et al. 1968). Ten fish, 2 to 5 cm in total length, were exposed to each concentration. Fish loading rates did not exceed 1 g per liter of water. Common and scientific names for fish used are listed in Table 1.

Mortalities were observed and recorded at 1, 3, and 6 h during the first day and at least daily thereafter. We analyzed the data by the methods of Litchfield and Wilcoxon (1949) to obtain LC_{50}'s (concentrations calculated to produce 50% mortality) and 95% confidence intervals. Chi-square tests indicated acceptability of all data reported. The half-life of antimycin:chlorine solutions was estimated by plotting deactivation indices against time on semilogarithmic coordinates (Marking and Dawson 1972).

Results

Toxicity of Chlorine to Fish

The toxicity of chlorine to fish varied with the species; 96-h LC_{50}'s ranged from 0.156 mg/l for channel catfish to 1.41 mg/l for black bullheads (Table 1). The three coldwater species (coho salmon rainbow trout, and lake trout) were more sensitive than the warmwater species (except for channel catfish). At 7 or 8 mg/l of chlorine, mortality occurred within 1 h of exposure for all species except goldfish, carp, fathead minnows, and black bullheads. The LC_{50}'s changed little after 24 h of exposure.

In rainbow trout exposed at different temperatures, chlorine was more toxic at 17 and 12 C than at 7 C, after 1 h of exposure (Table 2). This trend was

Table 1. *Toxicity of chlorine (from commercial grade HTH) to 12 species of fish in soft, reconstituted water at 12 C.*

Species	LC_{50} and 95% confidence interval (mg/l) at				
	1 h	3 h	6 h	24 h	96 h
Coho salmon *Oncorhynchus kisutch*	4.25 3.43-5.26	0.599 0.541-0.664	0.434 0.383-0.492	0.310 0.247-0.389	0.289 0.226-0.370
Rainbow trout *Salmo gairdneri*	0.969 0.886-1.06	0.640 0.533-0.769	0.550 —	0.236 0.199-0.280	0.172 0.148-0.200
Lake trout *Salvelinus namaycush*	1.19 0.974-1.45	0.615 0.554-0.682	0.450 0.371-0.545	0.246 0.177-0.343	0.200 0.147-0.272
Goldfish *Carassius auratus*	>7.00	— —	2.39 1.87-3.06	1.42 1.14-1.76	1.18 0.902-1.54
Carp *Cyprinus carpio*	>8.00	3.65 2.91-4.57	2.83 2.56-3.13	0.825 —	0.800 —
Fathead minnow *Pimephales promelas*	>7.00	2.43 1.57-3.77	1.38 0.964-1.98	1.00 0.843-1.19	0.998 0.841-1.18
White sucker *Catostomus commersoni*	2.00 1.65-2.42	0.880 0.830-0.933	0.631 0.538-0.740	0.379 0.318-0.452	0.379 0.318-0.452
Black bullhead *Ictalurus melas*	>8.00	>8.00	2.21 —	1.41 1.14-1.74	1.41 1.14-1.74
Channel catfish *I. punctatus*	1.38 1.06-1.79	0.346 0.273-0.438	0.286 0.231-0.354	0.156 0.106-0.229	0.156 0.106-0.229
Green sunfish *Lepomis cyanellus*	9.42 6.84-13.0	3.00 2.21-4.08	1.72 1.23-2.41	1.28 1.01-1.62	1.28 1.01-1.62
Bluegill *L. macrochirus*	10.8 6.41-18.2	1.32 0.996-1.75	1.11 0.797-1.55	0.569 0.484-0.669	0.555 0.468-0.658
Yellow perch *Perca flavescens*	1.32 0.994-1.75	1.16 0.842-1.19	0.735 0.658-0.821	0.570 0.489-0.665	0.558 0.474-0.657

Table 2. *Toxicity of chlorine (from commercial grade HTH) to rainbow trout at selected temperatures, water hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC_{50} and 95% confidence interval (mg/l) at 1 h	3 h	6 h	24 h	96 h
7	Soft	7.5	3.00 2.34-3.85	1.11 0.912-1.35	0.695 0.594-0.814	0.310 0.224-0.428	0.141 0.114-0.174
12	Soft	7.5	0.969 0.886-1.06	0.640 0.533-0.769	0.550 —	0.236 0.199-0.280	0.172 0.148-0.200
17	Soft	7.5	0.800 0.721-0.888	0.619 0.561-0.683	0.434 0.348-0.541	0.320 0.245-0.419	0.192 0.145-0.254
12	Very soft	8.0	3.00 2.20-4.10	0.900 0.763-1.06	0.830 0.757-0.910	0.362 0.270-0.485	0.200 0.144-0.278
12	Soft	8.0	2.87 2.12-3.89	1.00 0.859-1.16	0.815 0.768-0.865	0.590 —	0.211 0.168-0.265
12	Hard	8.0	2.81 2.28-3.46	1.24 1.03-1.49	0.879 0.806-0.958	0.510 0.414-0.628	0.350 —
12	Very hard	8.0	2.75 2.24-3.37	0.900 0.839-0.965	0.851 0.806-0.898	0.439 0.327-0.590	0.228 —
12	Soft	6.5	0.999 0.871-1.15	0.476 0.436-0.520	0.405 0.372-0.441	0.250 0.218-0.287	0.143 0.115-0.178
12	Soft	8.5	3.30 2.36-4.62	1.23 0.957-1.58	0.900 0.833-0.972	0.350 0.321-0.382	0.189 0.158-0.226
12	Soft	9.5	>3.00	1.65 1.39-1.96	1.00 0.901-1.11	0.308 0.274-0.346	0.200 0.164-0.244

reversed after 24 h, however, and at 96 h chlorine was most toxic in the coldest water—probably because chlorine is more residual at low than at high temperatures.

Water hardness at a constant pH of 8.0 (maintained by adding equal amounts of bicarbonate to water at each level of hardness) did not affect the toxicity of chlorine to rainbow trout (Table 2). Although chlorine toxicity was influenced little by pH, the general trend was toward decreasing toxicity with increasing pH.

Detoxification of Antimycin

Green sunfish were exposed to antimycin, chlorine, and a mixture of antimycin and chlorine to determine the detoxification efficiency of chlorine. Detoxification was represented by a deactivation index, which is the quotient of the LC_{50} for antimycin and chlorine in aged and in fresh solutions. For example, the deactivation index, at pH 7.5 after 1 h of interaction time, was 11.5/6.90, or 1.67 (Table 3). The time required for the deactivation index to reach 2.0—indicating that the toxicity has been decreased by one-half—coincides with the half-life of the toxicant. The half-life of antimycin in combination with chlorine at pH 7.5 was estimated at 1.3 h by plotting the deactivation indices against time on semilogarithmic coordinates (Fig. 1). Similar curves prepared for the data at other pH's yielded estimated half-lives of 1.1 h at pH 6.5 and 1.5 h at pH's 8.5 and 9.5.

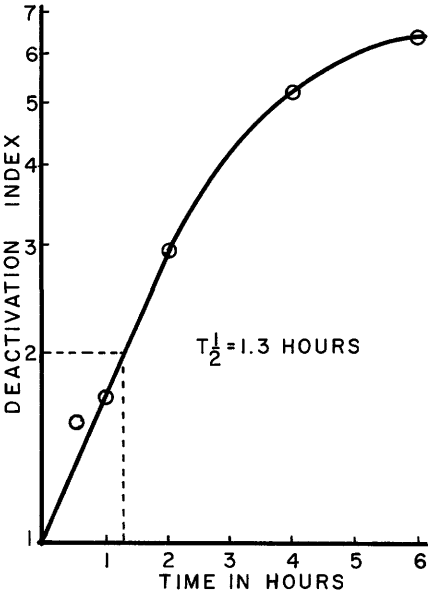

Fig. 1. Half-life (T½) curve for antimycin and 0.5 mg/l of chlorine in soft water at pH 7.5 and 12 C.

Table 3. *Toxicity and deactivation of antimycin in static tests at 12 C with green sunfish in water containing 0.25 mg/l of chlorine at pH 6.5 and 0.50 mg/l of chlorine at pH's 7.5, 8.5, and 9.5.*

Compound, and interaction time[a] of chlorine and antimycin (h)	96-h LC_{50}, 95% confidence interval, and (in parentheses) deactivation index at			
	pH 6.5	pH 7.5	pH 8.5	pH 9.5
Chlorine (mg/l)	0.745 0.623-0.891	0.949 0.837-1.08	0.840 0.688-1.03	0.715 0.578-0.885
Antimycin (μg/l)	0.107 0.084-0.136	0.164 0.138-0.194	0.640 0.539-0.761	16.2 14.1-18.6
Antimycin (μg/l) plus chlorine				
0	0.779 0.644-0.942 (1.00)	6.90 4.69-10.1 (1.00)	5.19 4.44-6.07 (1.00)	4.45 2.93-6.76 (1.00)
0.5	1.72 1.23-2.41 (2.21)	10.7 8.73-13.1 (1.55)	6.00 4.99-7.22 (1.16)	7.09 5.47-9.18 (1.59)
1.0	1.39 1.03-1.87 (1.78)	11.5 8.64-15.1 (1.67)	9.00 7.40-10.9 (1.73)	8.69 6.86-11.0 (1.95)
2.0	2.87 2.15-3.84 (3.68)	20.0 16.9-23.7 (2.90)	13.3 — (2.56)	7.05 5.29-9.39 (1.58)
4.0	4.28 3.38-5.41 (5.49)	36.0 30.0-43.2 (5.22)	15.2 11.6-19.9 (2.93)	15.0 11.3-19.9 (3.37)
6.0	8.51 7.36-9.83 (10.9)	44.8 — (6.49)	25.5 20.6-31.5 (4.91)	23.4 18.5-29.5 (5.26)
8.0	7.60 7.05-8.19 (9.76)	— — —	34.5 29.9-40.0 (6.65)	28.0 25.4-30.8 (6.29)

[a] Time antimycin and chlorine were in solution before fish were introduced.

Toxicity of antimycin decreased significantly at successively higher pH's; the 96-h LC_{50}'s ranged from 0.107 μg/l at pH 6.5 to 16.2 μg/l at pH 9.5 (Table 3). The drop was particularly sharp between pH's 8.5 and 9.5—as previously observed by Marking and Dawson (1972). As in the earlier tests, the toxicity of chlorine was affected little by pH.

The toxicity of antimycin decreased significantly immediately after chlorine was added to the solution at pH 6.5, 7.5, and 8.5—with no interaction time (Table 3). For example, at pH 7.5 the 96-h LC_{50} for antimycin was 0.164 μg/l and that for antimycin plus 0.5 mg/l of chlorine was 6.90 μg/l. The difference was perhaps due to the time required for antimycin to produce a lethal effect, commonly called the effective exposure time (Gilderhus 1972). Therefore the biological measure of concentrations remaining after aging was delayed by the latent response of fish.

Toxicity to fish increased when chlorine was added to the antimycin solution at pH 9.5 (Table 3). The 96-h LC_{50} was 16.2 μg/l for antimycin alone, and 4.45 μg/l after the addition of 0.5 mg/l of chlorine. Most likely the 0.5 mg/l of chlorine contributed toxicity, rather than detoxifying the antimycin, and its effect was additive rather than antagonistic (Marking and Dawson 1975). As the interaction time increased, however, antimycin was detoxified; its half-life was estimated to be 1.5 h.

Marking and Dawson (1972) demonstrated that antimycin detoxifies in water without the addition of chemical detoxifiers (half-lives for antimycin alone ranged from 310 h at pH 6.5 to 1.5 h at pH 10.0); however, the chemicals greatly increase the rate of

detoxification. Although chlorine detoxifies antimycin much less rapidly than does potassium permanganate (Marking and Bills 1975), it destroys the biological activity of antimycin within a suitably short time.

The detoxification of antimycin without detoxifiers followed a first order decay curve; with detoxifiers, however, the curve was nonlinear. The nonlinearity results from loss of chlorine during the interaction time due to reactions such as reduction, volatilization, and adsorption. The initial detoxification rate is most important, however, because antimycin is generally nontoxic after a time equal to one or two of the initial half-life periods.

When chlorine concentrations were monitored for 96 h to ascertain the dissipation rate during a typical toxicity test, the concentrations measured (Taras et al. 1971) in solutions containing fish were significantly lower than those measured in solutions without fish (Table 4). Little, if any, chlorine remained after 24 h in the fish assays.

Table 4. *Dissipation of chlorine (calcium hypochlorite, 70% active chlorine) in water without fish and in water with green sunfish (0.75 g/l) in soft water at 12 C.*

Time after addition (h)	Chlorine (mg/l)			
	Without fish		With fish	
	0.5	1.0	0.5	1.0
0	0.525	1.075	0.275	0.725
24	0.350	0.825	0.050	0.075
48	0.350	0.925	<0.01	0.025
72	0.325	0.975	<0.01	<0.01
96	0.300	0.925	<0.01	<0.01

Conclusions

1. The 96-h LC_{50}'s for chlorine at pH 7.5 ranged from 0.156 mg/l for channel catfish to 1.41 mg/l for black bullheads.
2. Toxicity of chlorine to fish was influenced little by pH.
3. Toxicity of antimycin to green sunfish decreased as pH increased; the 96-h LC_{50}'s ranged from 0.107 μg/l at pH 6.5 to 16.2 μg/l at pH 9.5.
4. Chlorine effectively detoxified antimycin. Half-lives of antimycin with chlorine ranged from 1.1 h at pH 6.5 to 1.5 h at pH 9.5.

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Malachite Green: Its Toxicity to Aquatic Organisms, Persistence and Removal with Activated Carbon

by

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Abstract

The acute toxicity of malachite green was determined in standardized laboratory tests for chinook salmon *(Oncorhynchus tshawytscha)*, coho salmon *(O. kisutch)*, Atlantic salmon *(Salmo salar)*, brown trout *(S. trutta)*, rainbow trout *(S. gairdneri)*, brook trout *(Salvelinus fontinalis)*, channel catfish *(Ictalurus punctatus)*, largemouth bass *(Micropterus salmoides)*, smallmouth bass *(M. dolomieui)*, bluegill *(Lepomis macrochirus)*, snails (*Pleurocera* sp.), Asiatic clams *(Corbicula leana)*, ostracods (*Cypridopsis* sp.), freshwater prawns *(Palaemonetes kadiakensis)*, larval midges *(Tanytarsus dissimilis)*, naiads of mayflies (*Callibaetis* sp.), adult newts *(Notophthalmus viridescens)*, larval leopard frogs *(Rana pipiens)*, and larval toads (*Bufo* sp.). Bluegills were the most sensitive (96-h LC_{50}, 0.0305 mg/l), and coho salmon the most resistant (0.383 mg/l). The $TILC_{50}$ (lethal concentration producing 50% mortality independent of time) for rainbow trout was 0.0998 mg/l. The responses of frog and toad larvae (96-h LC_{50}, 0.173 and 0.0680 mg/l) were similar to those of fish, whereas adult newts were more resistant (1.03 mg/l). The invertebrates exposed were generally more resistant than the fish and amphibians; the 96-h LC_{50}'s ranged from 0.510 to 3.45 mg/l, except for the Asiatic clam, which was extremely resistant (122 mg/l), and the mayfly naiad, which was very sensitive (0.0790 mg/l). The toxicity of malachite green to fish was not affected by water hardness or pH, except bluegills, in which toxicity was about half as great at pH 6.5 as at pH 7.5 to 9.5, and was increased only slightly by increases in water temperatures. Malachite green was very persistent in aqueous solutions; it did not detoxify after 3 weeks of aging in glass containers. The chemical is readily absorbed from aqueous solutions (pH 7.5, total hardness 44 mg/l, temperature 12 C) by filtration through activated carbon; the capacity was 23.4 mg of malachite green per gram of carbon.

Malachite green has been used in fish culture as a fungicide and parasiticide for about 40 years. It was first used as a dip treatment by Foster and Woodbury (1936) to treat fungal infections of four species of trout and largemouth bass *(Micropterus salmoides)*. More recently it has been used in combination with formalin to treat *Ichthyophthirius*, a serious parasite of catfishes (Leteux and Meyer 1972).

Although the use of malachite green as a therapeutant in fish culture has many advantages, it also poses various potential problems (Nelson 1974): toxicity to fishes (Willford 1967); possible teratogenic and mutagenic effects (Lieder 1961; Nelson 1974; T.D. Bills and L.L. Marking, in preparation); and stress induced during and after the treatment of fry of certain fishes (Glagoleva and Malikova 1968; Bills and Hunn 1976).

Malachite green is not registered for aquatic use by either the Food and Drug Administration or Environmental Protection Agency, because information required for registration—toxicity, efficacy, residues, metabolites, and counteraction—is incomplete. The purpose of the present study was to contribute laboratory data on (1) the toxicity of malachite green to nontarget aquatic organisms; (2) its toxicity to rainbow trout *(Salmo gairdneri)* and bluegills *(Lepomis macrochirus)* in extended exposures; (3) the effects of certain water characteristics on its toxicity to fish; (4) its persistence in water; and (5) its possible removal from water with activated carbon.

Materials and Methods

Concentrated stock solutions of commercial grade zinc-free malachite green (4-[P-(dimethylamino)-α-phenylbenzylidene]-2,5-cyclohexadien-1-ylidene dimethyl-ammonium chloride) manufactured by MCB Manufacturing Chemists, Norwood, Ohio, were prepared by mixing weighed portions with water. To prepare test solutions of the desired concentrations, we pipetted portions of stock solutions into test vessels and stirred the resulting mixture to ensure homogeneity. In flow-through toxicity tests, the required amounts of the stock solution were delivered by a solenoid-activated pipette pump (Micromedic Systems Automatic Pipette Model 2500).

Tests were conducted according to the methods outlined by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) and the protocol described by Marking (1975). Glass jars of 3.78 or 18.9 liters were used, depending on the size of the test organism. Reconstituted water was used in tests with fish (Marking 1969), and limed spring water (pH, 7.5 ± 0.1; total hardness, 20 mg/l as $CaCO_3$) in the tests with amphibians and invertebrates. Chemical buffers were added to soft water to adjust the pH (6.5–9.5), as described by Marking and Dawson (1973).

Flow-through tests were conducted in a proportional diluter similar to that of Mount and Brungs (1967). Test vessels were 45-liter glass aquariums supplied with a flow sufficient to replace the entire volume at least four times daily. Carbon-filtered, municipal well water (total hardness 300 mg/l, pH 7.5) was used in the flow-through system. Temperature was maintained by immersing test vessels in a water bath equipped with a chilling unit.

Fish species exposed were chinook salmon *(Oncorhynchus tshawytscha)*, coho salmon *(O. kisutch)*, Atlantic salmon *(Salmo salar)*, brown trout *(S. trutta)*, rainbow trout, brook trout *(Salvelinus fontinalis)*, channel catfish *(Ictalurus punctatus)*, largemouth bass, smallmouth bass *(Micropterus dolomieui)*, and bluegills. Test fishes weighed 0.5 to 1.5 g. Other aquatic organisms exposed were snails (*Pleurocera* sp.), Asiatic clams *(Corbicula leana)*, ostracods (*Cypridopsis* sp.), freshwater prawns *(Palaemonetes kadiakensis)*, larval midges *(Tanytarsus dissimilis)*, naiads of mayflies (*Callibaetis* sp.), adult newts *(Notophthalmus viridescens)*, larval leopard frogs *(Rana pipiens)*, and larval toads (*Bufo* sp.).

In tests for the determination of persistence of malachite green, aqueous solutions were aged for 1, 2, and 3 weeks in glass containers. Rainbow trout were introduced concurrently to these and a freshly prepared reference solution for comparison of mortality. Deactivation indices were computed from these data according to the method of Marking (1972).

We used the method of Litchfield and Wilcoxon (1949) to determine LC_{50}'s and 95% confidence intervals, and a modification of the method published by Green (1965) for computing $TILC_{50}$'s (lethal concentration producing 50% mortality independent of time).

To determine if malachite green could be removed from aqueous solutions (pH 7.5, total hardness 44 mg/l, temperature 12 C), we filtered a concentrated solution (2 mg/l) at a flow rate of 100 ml/min through a glass 2.7 cm ID column containing 15 cm (35.5 g dry weight) of activated carbon (Darco 20 × 40 mesh). Samples were taken periodically and concentrations in the effluent determined colorimetrically (620 nm). The carbon bed was considered saturated when the concentration in the effluent reached 10% of that in the original stock solution (0.2 mg/l). The capacity of activated carbon for the chemical was determined by the following formula:

$$\text{Milligrams of malachite green adsorbed per gram of carbon} = \frac{\text{Concentration (mg/l)} \times \text{liters passed through filter}}{\text{Grams of carbon (dry weight)}}$$

Results

Toxicity to Fish

Malachite green was toxic to all species of fish exposed; LC_{50}'s ranged from 0.0305 to 0.383 mg/l in 96-h exposures in soft water at 12 C (Table 1). Centrarchids were 1.5 to 3.5 times more sensitive to the chemical than the ictalurids and 3 to 7 times more sensitive than the salmonids. The bluegill was the most sensitive species (96-h LC_{50}, 0.0305 mg/l) and the coho salmon the most resistant (0.383 mg/l). The toxicity of the chemical increased as exposures lengthened in all species; for bluegills the LC_{50} was 6.00 mg/l at 3 h and 0.0305 mg/l at 96 h.

Toxicity to Other Aquatic Organisms

In 96-h exposures, the LC_{50}'s for malachite green to frog larvae (0.173 mg/l) and toad larvae (0.0680 mg/l) were similar to those for fish (Table 2). Adult newts were more resistant than frog or toad larvae (96-h LC_{50}, 1.03 mg/l), but about equally or less resistant than most of the invertebrates exposed. Mayfly naiads were the most sensitive invertebrate

Table 1. *Toxicity of malachite green to fingerling fish in soft water at 12 C.*

Species	LC_{50} and 95% confidence interval (mg/l) at			
	3 h	6 h	24 h	96 h
Chinook salmon	1.72	1.38	0.292	0.224
(Oncorhynchus tshawytscha)	1.22-2.42	1.04-1.82	0.245-0.348	0.209-0.240
Coho salmon	—	>3.00	0.569	0.383
(O. kisutch)	—		0.486-0.662	0.327-0.449
Atlantic salmon	3.56	1.09	0.497	0.283
(Salmo salar)	2.77-4.58	0.929-1.28	0.415-0.595	0.229-0.350
Brown trout	1.73	1.27	0.352	0.237
(S. trutta)	1.23-2.43	0.991-1.63	0.280-0.443	0.209-0.268
Rainbow trout	1.41	0.760	0.360	0.248
(S. gairdneri)	1.14-1.74	0.649-0.890	0.305-0.425	0.193-0.319
Brook trout	3.00	1.44	0.300	0.220
(Salvelinus fontinalis)	2.06-4.37	1.05-1.98	0.259-0.348	0.188-0.257
Channel catfish	>3.00	1.10	0.181	0.112
(Ictalurus punctatus)		0.904-1.34	0.123-0.266	0.0893-0.140
Largemouth bass	—	—	0.282	0.0728
(Micropterus salmoides)	—	—	0.211-0.376	0.0604-0.0877
Smallmouth bass	1.36	—	0.154	0.0453
(M. dolomieui)	1.09-1.70	—	0.117-0.202	0.0366-0.0561
Bluegill	6.00	2.19	0.231	0.0305
(Lepomis macrochirus)	4.41-8.17	1.66-2.89	0.184-0.290	0.0218-0.0427

Table 2. *Toxicity of malachite green to selected nontarget aquatic organisms in limed water at 16 C.*

Organism	LC_{50} and 95% confidence interval (mg/l) at		
	6 h	24 h	96 h
Snail	—	—	0.720
(Pleurocera sp.*)*	—	—	0.483-1.07
Asiatic clam	—	—	122
(Corbicula leana)	—	—	93.8-159
Ostracod	5.85	5.85	3.45
(Cypridopsis sp.*)*	4.00-8.57	4.29-7.97	2.49-4.80
Freshwater prawn	—	9.10	1.90
(Palaemonetes kadiakensis)	—	7.29-11.3	1.76-2.06
Midge (larvae)	5.00	1.00	0.510
(Tanytarsus dissimilis)	3.13-7.99	0.636-1.57	0.295-1.10
Mayfly naiads	5.75	2.75	0.0790
(Callibaetis sp.*)*	4.95-6.69	2.07-3.65	0.0442-0.141
Newts (adult)	—	3.90	1.03
(Notophthalmus viridiscens)	—	3.47-4.38	0.672-1.58
Leopard frog (larvae)	1.00	0.380	0.173
(Rana pipiens)	0.875-1.14	0.351-0.412	0.149-0.200
Toad (larvae)	1.70	0.355	0.0680
(Bufo sp.*)*	1.54-1.87	0.235-0.276	0.0530-0.0860

exposed (96-h LC_{50}, 0.0790 mg/l), and the Asiatic clam was by far the most resistant to the chemical; it tolerated concentrations in excess of 100 mg/l. The other invertebrates exposed were more resistant than fish or amphibians, but less resistant than the Asiatic clam. The 96-h LC_{50}'s for these organisms were between 0.510 and 3.45 mg/l.

Effects of Temperature, Water Hardness, and pH on Toxicity

In short exposures of 3 or 6 h, malachite green was more toxic to rainbow trout, channel catfish, and bluegills in warm water (17 and 22 C) than in cool water (7 and 12 C), but at 96 h the LC_{50}'s at different temperatures were not significantly different, except for channel catfish (Tables 3, 4, 5). Neither water hardness nor pH influenced the toxicity of the chemical to any species except bluegills, in which toxicity was about half as great as pH 6.5 as at pH 7.5 to 9.5.

Chronic Toxicity

Rainbow trout and bluegills were exposed simultaneously to the chemical in a flow-through toxicity test to determine the $TILC_{50}$. Mortality increased with time in both species. The 24-h LC_{50} for bluegills was 0.151 mg/l. A $TILC_{50}$ could not be calculated because mortality continued until all organisms succumbed at the lowest concentration (0.0316 mg/l) after 16 days of exposure. The 24-h LC_{50} was 0.230 mg/l for rainbow trout, and mortality continued through 30 days. A $TILC_{50}$ of 0.0998 mg/l was determined after 36 days of exposure.

Persistence of Malachite Green in Water

Bioassays with rainbow trout of aqueous solutions of malachite green aged for 1, 2, and 3 weeks in glass jars indicated no significant loss of activity. The LC_{50} was 0.173 mg/l for the freshly prepared reference solution and 0.179 mg/l for the solution aged for 3 weeks.

Table 3. *Toxicity of malachite green to fingerling rainbow trout at selected temperatures, water hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC_{50} and 95% confidence interval (mg/l) at			
			3 h	6 h	24 h	96 h
7	Soft	7.5	>2.00	2.30 1.71-3.10	0.400 0.330-0.486	0.168 0.137-0.206
12	Soft	7.5	1.41 1.14-1.74	0.760 0.649-0.890	0.360 0.305-0.425	0.248 0.193-0.319
17	Soft	7.5	1.42 1.15-1.76	0.567 0.517-0.621	0.569 0.516-0.627	0.284 0.229-0.353
12	Very soft	8.0	2.00 1.55-2.58	0.780 0.726-0.838	0.362 0.307-0.426	0.286 0.230-0.355
12	Soft	8.0	2.31 1.72-3.10	0.800 0.659-0.971	0.280 0.226-0.347	0.234 0.179-0.305
12	Hard	8.0	2.30 1.43-3.71	1.40 1.13-1.73	0.345 0.296-0.403	0.288 0.233-0.356
12	Very hard	8.0	2.35 1.74-3.17	0.820 0.701-0.959	0.280 0.226-0.347	0.249 0.195-0.318
12	Soft	6.5	>2.00	1.01 0.764-1.34	0.279 0.207-0.375	0.280 0.227-0.345
12	Soft	8.5	2.60 1.86-3.63	0.980 0.851-1.13	0.284 0.229-0.351	0.212 0.172-0.262
12	Soft	9.5	>2.00	1.26 0.978-1.62	0.367 0.311-0.434	0.173 0.136-0.220

Table 4. *Toxicity of malachite green to fingerling channel catfish at selected temperatures, water hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC_{50} and 95% confidence interval (mg/l at 6 h	24 h	96 h
12	Soft	7.5	1.10 0.904-1.34	0.181 0.123-0.266	0.112 0.0893-0.140
17	Soft	7.5	0.552 0.499-0.610	0.222 0.168-0.293	0.0940 0.0860-0.103
22	Soft	7.5	0.400 0.331-0.483	0.0691 0.0576-0.0831	0.0535 0.0442-0.0647
12	Very soft	8.0	0.600 0.440-0.818	0.106 0.0935-0.120	0.0750 0.0555-0.101
12	Soft	8.0	1.30 1.01-1.67	0.285 0.232-0.350	0.117 0.0972-0.140
12	Hard	8.0	1.72 1.23-2.41	0.284 0.229-0.351	0.142 0.115-0.176
12	Very hard	8.0	1.71 1.22-2.40	0.286 0.232-0.353	0.142 0.115-0.176
12	Soft	6.5	0.960 0.717-1.29	0.236 0.181-0.308	0.0975 0.0937-0.101
12	Soft	8.5	0.835 0.665-1.05	0.835 0.665-1.05	0.237 0.182-0.309
12	Soft	9.5	0.519 0.377-0.714	0.191 0.155-0.236	0.162 0.135-0.194

Table 5. *Toxicity of malachite green to fingerling bluegill at selected temperatures, hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC_{50} and 95% confidence interval (mg/l) at 3 h	6 h	24 h	96 h
12	Soft	7.5	6.00 4.41-8.17	2.19 1.66-2.89	0.231 0.184-0.290	0.0305 0.0218-0.0427
17	Soft	7.5	2.17 1.63-2.88	0.656 0.584-0.737	0.0920 0.0663-0.128	0.0340 0.0242-0.0477
22	Soft	7.5	0.860 0.737-1.00	0.238 0.184-0.308	0.0780 0.0594-0.102	0.0308 0.0221-0.0430
12	Very soft	8.0	2.30 1.72-3.08	2.00 1.54-2.59	0.117 0.0967-0.142	0.0413 0.0343-0.0497
12	Soft	8.0	>2.00	1.52 1.15-2.00	0.122 0.100-0.149	0.0400 0.0330-0.0486
12	Hard	8.0	>2.00	1.41 1.14-1.74	0.141 0.114-0.174	0.0450 0.0384-0.0528
12	Very hard	8.0	>2.00	1.42 1.10-1.83	0.141 0.114-0.174	0.0440 0.0370-0.0523
12	Soft	6.5	7.43 5.76-9.59	2.18 1.64-2.90	0.282 0.219-0.394	0.0780 0.0594-0.102
12	Soft	8.5	4.68 3.77-5.80	2.18 1.64-2.89	0.123 0.0955-0.158	0.0339 0.0241-0.0476
12	Soft	9.5	3.70 2.81-4.87	2.20 1.67-2.89	0.0810 0.0562-0.117	0.0340 0.0242-0.0477

Counteraction with Activated Carbon

Aqueous solutions of malachite green (2.0 mg/l) were filtered through a bed of activated carbon. In three runs the activated carbon adsorbed the chemical from 420, 401, and 425 liters of solution before the endpoint was reached (0.2 mg/l), an average of 23.4 mg of malachite green per gram of carbon. Activated carbon thus is an excellent means for removing this chemical from water.

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Toxicity of Furanace to Fish, Aquatic Invertebrates, and Frog Eggs and Larvae

by

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Abstract

Furanace (6-hydroxymethyl-2[2-(5-nitro-2-furyl)vinyl]pyridine), a nitrofuran originally designated as furpirinol, nifurpirinol, or P-7138, is effective against certain bacterial infections in fish, especially myxobacteriosis. The toxicity of the drug to fish, frog eggs and larvae, and aquatic invertebrates was determined in standardized laboratory toxicity tests and in use pattern exposures. Additional tests were conducted in aged solutions of Furanace to determine the persistence in water. This research was done to broaden existing toxicity data and to help fulfill drug registration requirements. Furanace was not toxic to any test species in use pattern exposures of 1 mg/l for 1 h daily for up to three treatments. In 96-h exposures, LC_{50}'s ranged from 0.820 to 3.00 mg/l for six species of fish and from 1.13 to 20 mg/l for six species of invertebrates, and was 0.770 mg/l for larvae of the leopard frog *(Rana pipiens)*. Toxicity increased with elevated temperatures in tests with rainbow trout *(Salmo gairdneri)*, channel catfish *(Ictalurus punctatus)*, and green sunfish *(Lepomis cyanellus)*. Increased water hardness and pH decreased toxicity to rainbow trout, but did not influence toxicity to channel catfish and green sunfish.

Furanace (6-hydroxymethyl-2[2-(5-nitro-2-furyl) vinyl]pyridine), a nitrofuran originally designated as P-7138, furpirinol, or nifurpirinol, has proved to be therapeutic against certain fish diseases. It was developed as a fish bactericide by Dainippon Pharmaceutical Co., Ltd. in Japan (Shimizu and Takase 1967) and was tested further against fish diseases in the United States (Amend and Ross 1970; Ross 1972). A review of literature on the drug was provided by Herman (1974). U.S. studies were conducted with the intention of registering the drug for fishery use as outlined by Lennon (1967).

Amend and Ross (1970) demonstrated that experimentally induced columnaris disease in coho salmon *(Oncorhynchus kisutch)* can be controlled with Furanace, that the drug is readily absorbed and eliminated, and that it is nontoxic to juvenile salmon. Additional studies by Amend (1972) showed that furunculosis in coho salmon was partially controlled by 8 or 10 mg/l of Furanace with two daily 1-h baths, but not by feeding the drug because fish refused to eat the medicated feed. He also found that one or two 1-h treatments of 1 mg/l of Furanace in static baths resulted in excellent control of myxobacteriosis and that the drug had low toxicity to fish and posed no residue problems 9 days after the last treatment.

We expanded the information on Furanace toxicity by (1) including more species of fish and additional test conditions, (2) including six species of aquatic invertebrates, and frog eggs and larvae, (3) determining the toxicity of use pattern exposures, and (4) determining the persistence of Furanace in water. The sequence of test procedures and conditions were standardized as suggested in the toxicological protocol of Marking (1975).

Materials and Methods

Purified Furanace, furnished by Abbott Laboratories, North Chicago, Ill., was dissolved in dimethylformamide or acetone to prepare stock solutions, portions of which were then added to static test chambers to yield desired concentrations. Toxicity was calculated on the basis of 100% activity for the added quantity of drug.

The static test procedures were those recommended by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Ten fish were exposed to each concentration in glass jars containing 15 liters of oxygenated, reconstituted water prepared from deionized water (Marking 1969). Test waters were of

four different total hardnesses (mg/l as $CaCO_3$ in parentheses): very soft (10), soft (44), hard (170), and very hard (300). In separate tests, chemically buffered solutions (Marking 1975) were used to assess the effects of pH on toxicity. Test temperatures were regulated by immersing the test jars in constant temperature water baths.

Fish weighing 1 to 1.5 g each were obtained from National fish hatcheries and maintained according to standardized procedures of the Fish Control Laboratory (Hunn et al. 1968). Fish were acclimated to the test conditions before they were exposed to the drug. Mortalities were recorded at 1, 3, and 6 h on the first day of exposure and daily thereafter for the remainder of the test. Scientific names of the fishes are listed in Table 1.

Six species of invertebrates (scientific names listed later in Table 5) and eggs and larvae of the leopard frog *(Rana pipiens)* were collected from the wild. These organisms were exposed to Furanace in a manner similar to that for exposing fish except that exposures were in 3 liters of limed spring water (21 mg/l of total hardness) in glass jars. Frog eggs (2- to 8-cell stage) were placed in concentrations of Furanace ranging from 0.01 to 5.0 mg/l and allowed to complete their development. After 7 days in the test vessels, the various stages of development were assessed. Invertebrates and frog larvae were exposed for 96 h to Furanace concentrations ranging from 0.1 to 20 mg/l or to the use pattern concentration of 1.0 mg/l. Higher concentrations of Furanace were not used because such solutions approached saturation and often precipitated. The observation period was extended to 60 days for a group of clams that were exposed to Furanace for 4 and 24 h and then placed in floating cages in a pond.

Computations of LC_{50}'s (concentrations calculated to produce 50% mortality) and 95% confidence intervals were computed according to the methods of Litchfield and Wilcoxon (1949). All data reported fulfilled the chi-square test requirement for acceptability. LC_{50}'s were considered significantly different when their confidence intervals did not overlap.

Residues of Furanace in water were determined by Abbott Laboratories, North Chicago, Ill., by gas chromatography (R.E. Crutcher and J. T. Manneback, in preparation).

Table 1. *Toxicity of Furanace to selected species of fish in soft water at 12 C.*

Species	LC_{50} and 95% confidence interval (mg/l) at	
	24 h	96 h
Atlantic salmon *(Salmo salar)*	4.50 3.84-5.28	1.41 1.14-1.74
Rainbow trout *(Salmo gairdneri)*	4.32 3.64-5.13	1.00 0.824-1.21
Fathead minnow *(Pimephales promelas)*	4.39 3.96-4.87	0.820 0.717-0.938
Channel catfish *(Ictalurus punctatus)*	4.27 3.40-5.36	1.07 0.854-1.34
Green sunfish *(Lepomis cyanellus)*	6.60 6.16-7.07	2.48 2.07-2.97
Bluegill *(Lepomis macrochirus)*	— —	3.00 2.45-3.68

Results

Toxicity to Fish

The toxicity of Furanace was not significantly different in 24-h exposures at 12 C to Atlantic salmon, rainbow trout, fathead minnows, and channel catfish; the 24-h LC_{50}'s ranged only from 4.27 to 4.50 mg/l (Table 1). The 96-h LC_{50}'s for green sunfish and the 96-h LC_{50} for bluegills were significantly greater than those for the other species.

Table 2. *Toxicity of Furanace to rainbow trout at selected water temperatures, hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC_{50} and 95% confidence interval (mg/l) at	
			24 h	96 h
7	Soft	7.5	9.60 8.02-11.5	1.73 1.23-2.44
12	Soft	7.5	4.32 3.64-5.13	1.00 0.824-1.21
17	Soft	7.5	3.69 3.11-4.38	0.795 0.661-0.956
12	Very soft	8.0	2.45 1.90-3.16	0.569 0.450-0.719
12	Soft	8.0	2.83 2.29-3.50	0.618 0.490-0.779
12	Hard	8.0	2.82 2.28-3.99	0.760 0.615-0.939
12	Very hard	8.0	2.82 2.28-3.49	1.18 0.913-1.52
12	Soft	6.5	3.28 2.76-3.90	0.489 0.432-0.554
12	Soft	8.5	3.82 3.20-4.55	0.800 0.696-0.917
12	Soft	9.5	3.29 2.77-3.90	1.42 1.05-1.92

Elevated temperatures increased the toxicity of Furanace to some species. The LC_{50}'s were significantly different at the lowest and highest water temperatures at which rainbow trout and channel catfish were exposed (Tables 2 and 3).

Furanace was significantly less toxic to rainbow trout at 96 h in very hard than in soft or very soft water (Table 2). However, the toxicity to channel catfish and green sunfish at 96 h was not significantly different at the four different water hardnesses (Tables 3 and 4).

Toxicity of Furanace to rainbow trout was influenced by pH in 96-h exposures, and the drug was more toxic at pH 6.5 than at pH 8.5 and at pH 8.5 than at pH 9.5 (Table 2). However, toxicity of Furanace at 96 h to channel catfish and green sunfish was not affected by pH (Tables 3 and 4).

Toxicity of Use Pattern Exposures to Fish

The suggested use pattern of Furanace as a fish therapeutant is a 1-h exposure daily to a 1-mg/l solution for up to 3 consecutive days, as necessary. Accordingly, rainbow trout were exposed to concentrations ranging from 0 to 3.0 mg/l of Furanace for 3 consecutive days and then observed for 10 additional days. No mortality occurred and no stress was observed during the 13-day period at any of the exposure concentrations.

Exposure of leopard frog larvae to concentrations of 0.2 to 20 mg/l of Furanace for 3 consecutive days resulted in no mortality after 7 days. Many of the larvae were immobilized in the 10- and 20-mg/l concentrations during the exposure, but they recovered when placed in fresh water. Furanace in the use pattern exposure was nontoxic to fish, frog larvae, and aquatic invertebrates.

Toxicity to Invertebrates

Invertebrates were more resistant than fish to Furanace; the 96-h LC_{50}'s ranged from 1.13 mg/l for snails to more than 20 mg/l for water fleas, freshwater prawns, and backswimmers (Table 5).

Asiatic clams that were exposed for 4 or 24 h and placed in floating cages in a pond also survived concentrations of Furanace greater than the therapeutant treatment level. The 4-h exposure produced less mortality than the 24-h exposure. Mortality from most of the exposures increased during the 60-day holding period. However, there was some survival among those exposed to concentrations as high as 20 mg/l.

Table 3. *Toxicity of Furanace to channel catfish at selected water temperatures, hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC_{50} and 95% confidence interval (mg/l) at 6 h	24 h	96 h
12	Soft	7.5	32.6 23.4-45.5	6.19 5.78-6.63	1.72 1.48-2.00
17	Soft	7.5	— —	5.25 4.48-6.15	1.90 1.55-2.33
22	Soft	7.5	14.2 11.5-17.6	2.90 2.41-3.49	1.22 1.05-1.42
12	Very soft	8.0	27.6 22.5-33.9	2.90 2.62-3.21	0.945 0.791-1.13
12	Soft	8.0	24.6 20.6-29.4	4.00 3.59-4.46	1.00 0.821-1.22
12	Hard	8.0	24.6 20.6-29.4	4.00 3.57-4.48	0.960 0.784-1.18
12	Very hard	8.0	22.8 18.2-28.6	4.38 3.96-4.84	0.969 0.810-1.16
12	Soft	6.5	— —	8.25 7.76-8.77	1.82 1.62-2.05
12	Soft	8.5	— —	7.59 7.20-8.01	1.80 1.60-2.03
12	Soft	9.5	— —	6.55 5.95-7.21	1.42 1.15-1.76

Table 4. *Toxicity of Furanace to green sunfish at selected water temperatures, hardnesses, and pH's.*

Temp (°C)	Water hardness	pH	LC_{50} and 95% confidence interval (mg/l) at 24 h	LC_{50} and 95% confidence interval (mg/l) at 96 h
12	Soft	7.5	6.30 5.55-7.16	2.42 2.04-2.86
17	Soft	7.5	5.88 5.29-6.54	2.00 1.59-2.52
22	Soft	7.5	4.25 3.84-4.70	1.42 0.980-2.04
12	Very soft	8.0	7.00 6.10-8.04	2.44 2.06-2.89
12	Soft	8.0	6.28 5.88-6.71	2.20 1.79-2.70
12	Hard	8.0	6.60 6.16-7.07	2.48 2.07-2.97
12	Very hard	8.0	7.85 7.32-8.42	2.50 2.20-2.84
12	Soft	6.5	6.00 5.38-6.69	1.73 1.23-2.43
12	Soft	8.5	7.00 6.71-7.31	2.00 1.10-3.63
12	Soft	9.5	4.64 3.53-6.09	2.00 1.08-3.69

Effects of Furanace on Frog Eggs and Larvae

Fertilized eggs at the 2- to 8-cell stage were exposed to Furanace at concentrations of 0.2 to 20 mg/l for 1 h on 3 consecutive days and then held in fresh water. After 7 days, there was no apparent effect of 0.2 to 4.0 mg/l of Furanace on the development of embryos. At concentrations of 6.0 to 20 mg/l, however, fewer embryos survived; some developed a head and tail and then died, and others appeared to develop normally but failed to escape the egg mass.

Frog larvae were considerably more sensitive to Furanace than were frog eggs, invertebrates, or fish. The 96-h LC_{50} was 0.770 mg/l in limed water at 16 C (Table 5).

Persistence of Furanace in Water

The persistence of Furanace in water was measured biologically by determining the toxicity to rainbow trout of solutions that had been aged for periods as long as 5 weeks. Additionally, Furanace residues in the aged solutions were determined analytically. Aging for 5 weeks reduced the toxicity of Furanace (i.e., increased the 96-h LC_{50}) by about 50% (Table 6). The same waters showed a similar loss of drug as determined by gas chromatographic analysis. Therefore the half-life of biological activity corresponded with the half-life of chemical integrity, i.e., about 5 weeks (Table 7).

Table 5. *Toxicity of Furanace to selected species of aquatic invertebrates and frog larvae in limed water at 16 C.*

Species	LC_{50} and 95% confidence interval (mg/l) at 24 h	LC_{50} and 95% confidence interval (mg/l) at 96 h
Snail *(Physa* sp.*)*	8.00 6.98-9.17	1.13 0.860-1.47
Asiatic clam *(Corbicula leana)*	>20.0	11.6 8.75-15.4
Water flea *(Daphnia magna)*	>20.0	>20.0
Amphipods *(Hyalella azteca)*	16.0 11.3-22.7	13.6 9.43-19.6
Freshwater prawn *(Palaemonetes kadiakensis)*	>20.0	>20.0
Backswimmer *(Notonecta* sp.*)*	>20.0	>20.0
Leopard frog (larva) *(Rana pipiens)*	6.90 5.55-8.57	0.770 0.590-1.01

Table 6. *Deactivation of Furanace in soft water at 12 C, as determined by changes in 96-h LC_{50}'s of rainbow trout.*

Aging period (weeks)	96-h LC_{50}'s (mg/l) and 95% confidence interval	Deactivation index[a]
0	0.705 0.570–0.872	1.0
1	0.810 0.677–0.969	1.1
2	0.900 0.763–1.06	1.3
3	0.900 0.763–1.06	1.3
4	1.33 1.19–1.48	1.9
5	1.46 1.31–1.62	2.1

[a] Deactivation index = $\frac{LC_{50} \text{ of aged solution}}{LC_{50} \text{ of fresh solution}}$

Table 7. *Concentrations of Furanace (mg/l) detected by colorimetry and gas chromatography in water solutions aged for 0 to 5 weeks.*

Concentration before aging (calculated)	Aging period (weeks)	Concentration after aging	
		Colorimetric	GC-eC[a]
1.0	0	1.0	1.15
3.0	0	3.11	2.56
1.0	1	0.67	0.654
3.0	1	2.89	1.82
1.0	2	0.67	0.602
3.0	2	2.89	0.807
1.0	3	0.78	0.575
3.0	3	2.22	2.52
1.0	4	0.56	0.466
3.0	4	2.56	1.74
1.0	5	0.78	0.485
3.0	5	2.50	1.58

[a] Gas chromatography-electron capture—average of two samples.

Discussion and Conclusions

The toxicity of Furanace was relatively low to fish and aquatic invertebrates. Saturated solutions (about 10-20 mg/l) usually did not cause sufficient mortality to permit the derivation of LC_{50}'s in exposures shorter than 24 h. No frog eggs or rainbow trout succumbed after use pattern exposures of 1 mg/l for 1 h daily for up to three treatments, and none of the invertebrates died after a single use pattern treatment. In fact, most fish and invertebrates survived 96-h exposures to 1 mg/l of Furanace.

Toxicity of Furanace was influenced by certain physical and chemical characteristics of water. Toxicity increased with increases in water temperatures in tests with rainbow trout and channel catfish. Increasing water hardness and pH decreased the toxicity of Furanace to rainbow trout but had no effect on its toxicity to channel catfish and green sunfish.

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