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UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
Washington, D.C. • 1977

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 e of certain water characteristics on the toxic formalin to fish, (5) the persistence of forma water, and (6) the feasibility of counteractin malin by oxidation or reduction, or removal water with activated carbon.

Materials and Methods

Stock solutions of commercial grade formali formaldehyde) obtained from North C Chemical Co., La Crosse, Wisconsin, were prepa water (the liquid formulation was mea volumetrically and diluted with water). All co trations listed are based on the formulated pr To prepare test solutions of the desired co trations, we pipetted portions of stock solution the test vessels, and stirred the resulting mixt ensure homogeneity. In flow-through toxicity required amounts of the stock solutions delivered by a solenoid-activated pipetting (Micromedic Systems Automatic Pipette Model

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 o aeration tests, the solutions were aerated stones for 24 h before fish were added. We c the 96-h LC_{50}'s with a reference standard t changes in toxicity. To determine if formalin removed from aqueous solutions, we fi concentrated solution (150 µl/l) at a flow 100 ml/min through a 15-cm column of a charcoal (Darco 20 × 40 mesh). Samples of were taken at selected volumes (0–200 800–1,000 ml). These samples and a samp stock solution were bioassayed against rainb and the 96-h LC_{50}'s compared.

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

Results

Toxicity of Formalin to Fish

The 96-h LC_{50}'s for formalin against nine s fish ranged from 62.1 µl/l for black bullhea µ l/l for green sunfish and Atlantic salmon (Toxicity of formalin increased with t bluegills, for example, the 3- and 96-h LC 2,290 µl/l and 100 µl/l, respectively. Ictalu twice as sensitive to formalin as the centra salmonids. Green sunfish were the most centrarchid exposed, followed by largemo smallmouth bass, and bluegills. Atlantic were the most resistant salmonid, foll rainbow trout and lake trout (Table 1).

The effects of temperature, water hardnes on toxicity were determined by exposing trout, channel catfish, and bluegills to for short exposures, formalin was significan toxic to these species at the higher temp however, at 96 h the differences were insi except in rainbow trout (Tables 2, 3, and hardness had no apparent effect on toxi rainbow trout and channel catfish, form more toxic in waters of pH 9.5 than in wat 6.5, 7.5, or 8.5 (Tables 2 and 3).

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

Toxicity of Formalin to Invertebrates

Invertebrates differed widely in their res formalin. The 96-h LC_{50}'s ranged from 1.0 seed shrimp to 835 µl/l for backswimmers the bivalve and snail—126 and 93 µl/l—we

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*

Temp. (°C)	Water hardness	pH	LC$_{50}$ and 95% confidence interval (μl/l) at 1 h	3 h	6 h	24 h	96
12	Soft	7.5	779 666-911	495 430-570	232 178-303	122 102-145	65 58.1-
17	Soft	7.5	600 523-689	350 299-409	282 228-349	119 102-138	65 58.2-
22	Soft	7.5	559 475-658	284 229-351	234 185-297	100 83.7-119	64 58.6-
12	Very soft	6.6	771 660-901	490 425-565	490 425-565	99.0 86.0-114	69 63.7-
12	Hard	7.8	1050 798-1382	450 360-563	355 298-422	117 100-137	49 43.3-
12	Very hard	8.2	872 703-1081	439 346-557	285 229-355	111 94.5-130	61 53.9-
12	Soft	6.5	779 666-912	424 356-505	282 228-349	118 103-135	62 54.4-
12	Soft	8.5	630 554-717	455 365-567	285 230-353	94.0 84.0-105	56 51.4-
12	Soft	9.5	559 474-659	282 228-349	235 185-298	63.9 54.7-74.7	42 36.2-

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

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 80.0-1
17	Soft	7.5	2300 1822-2904	780 670-908	189 153-234	73.5 63.5-8
22	Soft	7.5	1750 925-3312	469 403-545	142 115-176	91.0 81.2-1
12	Very soft	6.6	1800 1532-2115	1230 1071-1412	369 315-433	88.4 75.1-1
12	Hard	7.8	1720 1458-2029	1190 1027-1379	249 166-373	106 84.0-1
12	Very hard	8.2	1740 1499-2019	1310 1038-1654	233 181-300	117 101-1
12	Soft	6.5	2310 1961-2721	2290 1944-2697	335 284-395	125 89.1-1
12	Soft	8.5	2300 1822-2904	1650 1401-1943	230 182-290	86.2 72.6-1
12	Soft	9.5	2300 1950-2712	1055 883-1260	232 174-309	100 72.6-1

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

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

Aging period (weeks)	96-h LC_{50} (µl/l) and 95% confidence interval	Deactivatio 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

60.0 µl/l as compared with 107 µl/l for the forma reference (Table 7).

When the first and last 200-ml portions of t filtrate of a 150-µl/l formalin stock solution filter through a 15-cm column of activated carbon we bioassayed against rainbow trout along with sample of the stock solution, the 96-h LC_{50}'s we 210 µl/l for the first 200-ml sample, 132 µl/l for t 800- to 1,000-ml sample, and 121 µl/l for t reference solution. Although this difference indicat some removal of formalin, the removal was insigni cant when the relative amounts of formalin a carbon involved (1 mg formalin/1 g carbon) a considered (Table 8).

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 fing 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 p condition of the fish appear to influence the to formalin, variations in sensitivity within a may be due to genetic composition.

The effect of water chemistry on the tox formalin to fish is somewhat controversial. Bi and Avault (1971) reported that the toxi formalin to pompano *(Trachinotus carolinus)* affected by different salinity levels. Piper an (1973) reported that water chemistry has no e the toxicity of formalin to fish; however, th were based on questionnaires received from hatcheries rather than on experimental data ing et al. (1972) also reported that the tox formalin was not affected by water hardnes Bills (1974) first demonstrated that formal more toxic to fish and fish eggs in alkaline acid water. This conclusion is further suppo data from the present study, which show tha water formalin was more toxic to channel catf rainbow trout at pH 9.5 than at lower pH's.

Formalin is frequently used at concentratio to 25 μl/l for control of parasites on fish in ponds. Much information is available on the e of formalin as a parasiticide; however, the ef formalin on pond flora and fauna, particul aquatic invertebrates, have not been deter Schnick (1974) reported few data on the tox formalin to invertebrates. Our data show range of sensitivities for invertebrates; th LC_{50}'s ranged from 1.05 μl/l for seed shr 835 μl/l for backswimmers. Our data als formalin to be persistent under laboratory con and at use-pattern concentrations some invert could be affected. Present governmental cont the use of chemicals in the environment nece

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.

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 $\mu l/l$).
2. Atlantic salmon and green sunfish were the most resistant species (96-h LC_{50}'s = 173 $\mu l/l$).
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 $\mu l/l$ and 835 $\mu l/l$.
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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