INVESTIGATIONS IN FISH CONTROL

32. Toxicity of Hyamine 3500 to Fish

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United States Department of the Interior
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife

INVESTIGATIONS IN FISH CONTROL

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Continued on inside back cover--

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Washington, D.C. • October 1969

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IN THIS COVER

	Pages
Toxicity of Hyamine 3500 to Fish, by James W. Hogan.	1-9
Voidance Time for 23 Species of Fish, by Thomas H. Lane and Howard M. Jackson	1-9
Laboratory Studies on Possible Fish-Collecting Aids, With Some Toxicities for the Isomers of Cresol, by Robert M. Howland . . .	1-10

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32. Toxicity of Hyamine 3500 to Fish

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CONTENTS

	Page
Abstract	3
Materials and methods	3
Results	4
Trout	4
Warmwater fish	5
Effects of temperature	5
Effects of water quality	5
Discussion	5
Conclusions	7
References	9

TOXICITY OF HYAMINE 3500 TO FISH

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ABSTRACT.--Toxicity of Hyamine 3500 to three species of trout and 11 species of warmwater fish was determined in static bioassays. Twenty-nine lots of fish from nine sources were used in water at various levels of pH, temperature, and total hardness. Hyamine 3500 is more toxic in alkaline or acidic water than in water with a pH of 7. In general, toxicity varies directly with temperature and inversely with total hardness. Dilute solutions of Hyamine 3500 appear to degrade rapidly in open vessels.

Because of its efficacy as a microbicide and its relatively low toxicity to mammals (Rohm and Haas Co., 1965), Hyamine 3500 is being considered as a disinfectant in fish culture (Warren, 1964). Before it can be considered for general use, its toxicity to fish must be known. The purpose of this study was to determine the toxicity of Hyamine 3500 to several species of fish in waters of various qualities.

MATERIALS AND METHODS

The active ingredients in Hyamine 3500 are a selected blend of alkyl (C_{14}, 50 percent; C_{12}, 40 percent; and C_{16}, 10 percent) dimethyl benzyl ammonium chlorides having an average molecular weight of 358 g /mole. Two forms of Hyamine 3500 are available: an 80-percent concentrate in ethanol, and a 50-percent aqueous solution. We used the latter. The other ingredients in the aqueous solution include 40 percent of water and 10 percent of ethanol. This solution is pale yellow, has a mild odor, congeals at low temperatures, and is miscible in all proportions with water, lower alcohols, and ketones.

The species, lot numbers, weights, and sources of fish are listed in table 1. Most of the lots were from National Fish Hatcheries, but two lots of goldfish were from a private producer, and one lot of channel catfish was from a Georgia State Fish Hatchery, and one lot of trout was from a Wisconsin State Fish Hatchery. The term "lot" refers to a particular group of fish of a species received from a hatchery in a single shipment.

Routine bioassays were conducted in accordance with the methods outlined by Lennon and Walker (1964), with slight modifications. In general, the formulation of water of various levels of total hardness, alkalinity, and pH was accomplished by the methods outlined by Marking (1966, 1967). Water temperatures in vessels were maintained at $12^{o}\pm 1^{o}$, $17^{o}\pm 2^{o}$, or $22^{o}\pm 1^{o}$ C.

Immediately before each bioassay, concentrated stock solutions of Hyamine 3500 were prepared with a deionized water-acetone (50/50, v/v) solvent. Concentrations were calculated on the basis of product rather than active ingredient. Aliquots of these stock solutions were measured directly into bioassay vessels to produce desired concentrations. The amount of acetone per bioassay vessel never was in excess of the volume tolerated by the test fish.

The data from each bioassay were analyzed by the method outlined by Litchfield and

Wilcoxon (1949). By this method, LC_{50} values (concentrations producing 50-percent mortality) and their 95-percent confidence intervals were calculated.

RESULTS

Trout.--The 24-hour LC_{50} values at 12° C. indicate that rainbow trout were less sensitive to Hyamine 3500 than lake trout and brown trout (table 2). The 96-hour LC_{50} values for the three species ranged from 1.90 to 2.45 p p m ; rainbow trout were still the least sensitive. The 95-percent confidence intervals of lake trout and brown trout overlap, indicating no statistically significant differences in the sensitivity of these two species.

TABLE 1.--The 14 species of fish used in toxicity tests of Hyamine 3500

Species	Lot	Average weight and range (grams)	Source
Rainbow trout, Salmo gairdneri	368	0.8(0.5 - 1.1)	Manchester NFH, Ia.
Brown trout, Salmo trutta	309	1.2(0.7 - 1.9)	McNenny NFH, S. Dak.
Lake trout, Salvelinus namaycush	346	1.9(1.5 - 2.2)	St. Croix Falls SFH, Wis.
Goldfish, Carassius auratus	W107	1.3(1.1 - 2.2)	Welaka NFH, Fla.
Do	W116	1.3(0.8 - 2.1)	Tallassee, Ala.
Do	W133	1.4(1.0 - 1.8)	Do.
Do	W153	1.1(0.7 - 1.5)	Marion NFH, Ala.
Carp, Cyprinus carpio	W162	1.0(0.9 - 2.1)	Do.
Fathead minnow, Pimephales promelas	W110	0.7(0.6 - 1.0)	Do.
Do	W113	1.0(0.5 - 2.0)	Do.
Do	W124	0.8(0.6 - 1.4)	Do.
Do	W139	0.8(0.6 - 1.4)	Do.
Smallmouth buffalo, Ictiobus bubalus	W163	1.1(0.5 - 1.4)	Do.
Brown bullhead, Ictalurus nebulosus	W148	1.1(0.5 - 1.2)	Do.
Channel catfish, Ictalurus punctatus	W115B	2.2(1.6 - 3.3)	Cordele SFH, Ga.
Green sunfish, Lepomis cyanellus	W157	0.9(0.5 - 2.1)	Marion NFH, Ala.
Do	W164	0.9(0.6 - 1.1)	Do.
Bluegill, Lepomis macrochirus	W109	0.8(0.7 - 1.0)	Do.
Do	W111	1.1(0.7 - 1.4)	Do.
Do	W119	1.1(0.7 - 1.4)	Do.
Do	W128	1.2(0.8 - 1.6)	Do.
Redear sunfish, Lepomis microlophus	W112	1.4(1.1 - 2.4)	Do.
	W117	1.1(0.8 - 1.5)	Do.
Smallmouth bass, Micropterus dolomieui	W146	0.7(0.5 - 0.9)	Mammoth Springs NFH, Ark.
Largemouth bass, Micropterus salmoides	W140	0.8(0.6 - 1.2)	Welaka NFH, Fla.
Do	W141	0.6(0.4 - 0.8)	Warm Springs NFH, Ga.

TABLE 2.--Toxicity of Hyamine 3500 to three species of trout at 12° C. at 24, 48, and 96 hours of exposure

Species	Lot	LC_{50} and 95-percent confidence interval (ppm) at --		
		24 hours	48 hours	96 hours
Rainbow trout	368	3.65 (3.38 - 3.94)	2.80 (2.59 - 3.02)	2.45 (2.19 - 2.74)
Brown trout	309	2.61 (2.14 - 3.18)	2.13 (1.79 - 2.53)	1.95 (1.65 - 2.30)
Lake trout	346	2.74 (2.51 - 2.99)	2.35 (2.12 - 2.61)	1.90 (1.71 - 2.11)

Warmwater fish.--The 96-hour LC_{50}'s of Hyamine 3500 for 10 species of warmwater fish range from 0.32 to 2.25 p p m at 17° C. (table 3). The response of the fish varied somewhat for different exposure periods. For instance, 3-hour LC_{50} values show that smallmouth bass were the most sensitive, while 96-hour data indicate that bluegill were the most sensitive. Similarly, 3-hour LC_{50}'s show that brown bullhead were the most resistant, but 96-hour data indicate that green sunfish were the most resistant. Thus, some species having a relatively high resistance in short exposures may have a comparatively low resistance in longer exposures, and for other species the converse is true.

The toxicity varied among lots of a given species, and within the same lot. For example, the 96-hour LC_{50} of lot W111 of bluegills was twice that of lot W109. Two tests on goldfish from lot W116 gave 3-hour LC_{50} values of 6.55 and 10 p p m, but results generally were consistent and predictable.

Effects of temperature.--Usually, the toxicity of Hyamine 3500 increases with temperature as indicated by the LC_{50} values for lake trout (table 4), fathead minnow, and channel catfish (table 5). At 24 and 48 hours, the LC_{50}'s for lake trout at 17° are less than half those at 7° C. All of the 24-, 48-, and 96-hour LC_{50}'s for fathead minnow and channel catfish indicate increasing toxicity with an increase in temperature from 12° to 22° C. When both the 95-percent confidence interval and the LC_{50} value are examined, it is apparent that some of the differences in toxicity indicated by the LC_{50}'s alone are not statistically significant. Temperature changes appeared to have less effect on the toxicity of Hyamine 3500 to goldfish and bluegill than to the other two species tested. Some LC_{50} values indicate that toxicity increased with temperature, but other values show the opposite to be true. For instance, the 96-hour LC_{50} value for goldfish at 12° is 2.09 p p m and at 22° is 2.56 p p m, while the 3-hour LC_{50} at 12° is 13.30 p p m and at 22° it is 8.50.

Effects of water quality.--The toxicity of Hyamine 3500 to lake trout, fathead minnow, and bluegill is greater in soft water than in hard water (tables 6 and 7). The 96-hour LC_{50}'s in hard water are almost twice those in soft water.

The toxicity to goldfish and fathead minnow is greater at both acidic and alkaline pH levels than at neutrality (table 8). The effect of pH on the toxicity of Hyamine 3500 does not appear to be as great as the effect of water hardness.

DISCUSSION

In general, for at least the initial 24 hours, the toxicity of Hyamine 3500 to the 14 species of fish was dependent on the concentration of chemical and duration of exposure. Toxicity increased only slightly when exposures were continued from 24 to 96 hours. For instance, green sunfish from lot W164 had 3- and 6-hour LC_{50}'s of 8.00 and 4.50 p p m, respectively, while the 24-, 48-, and 96-hour LC_{50} values were 2.32, 2.25, and 2.25 p p m, respectively. For many species the 24- and 48-hour LC_{50}'s or the 48- or 96-hour LC_{50}'s were identical, or when slight differences existed they were not statistically significant.

There are many possible causes for the comparatively slow increase in toxicity when exposures were continued from 24 to 96 hours. Marking (1966) indicated that this slowness may be caused by absorption and metabolism of the toxicant by the test fish, and by natural degradation of the chemical in solution. The latter would seem to be especially true of Hyamine 3500 since Nicholes and Burton (1961) found that solutions of Hyamine 3500 deteriorated when stored in open vessels. Furthermore, they indicated that both the chemically titratable activity and the germicidal efficiency of Hyamine 3500 solutions decreased upon standing. This deterioration was found to increase rapidly if the storage temperature was higher than 22° C.

Bioassays showed that not only fish from different sources but fish from the same source and even fish from the same lot differ in their sensitivity to Hyamine 3500. Marking (1966) found that geographic location, water quality, pond fertilization, herbicide application, and feeding and handling during rearing

TABLE 3.--Toxicity of Hyamine 3500 to 11 species of fish at 17° C. at 3 to 96 hours of exposure

Species	Lot	LC_{50} and 95-percent confidence interval (ppm) at --				
		3 hours	6 hours	24 hours	48 hours	96 hours
Goldfish.....	W107	--	--	2.71 (2.36 - 3.12)	2.18 (1.82 - 2.62)	2.18 (1.82 - 2.62)
Do.....	W116	10.00 (8.47 - 11.80)	--	--	--	--
Do.....	W116	6.55 (5.74 - 7.47)	--	--	--	--
Do.....	W116	--	3.34 (3.06 - 3.64)	--	--	--
Do.....	W133	--	--	1.60 (1.30 - 1.97)	1.60 (1.30 - 1.97)	1.49 (1.16 - 1.91)
Carp.....	W162	4.40 (3.93 - 4.93)	2.18 (1.93 - 2.46)	1.85 (1.74 - 2.07)	1.80 (1.64 - 1.98)	--
Fathead minnow.....	W110	--	2.50 (2.31 - 2.70)	1.11 (0.98 - 1.25)	1.06 (0.91 - 1.23)	0.98 (0.89 - 1.08)
Do.....	W124	3.70 (3.33 - 4.11)	--	--	--	--
Smallmouth buffalo....	W163	3.62 (3.26 - 4.02)	2.20 (2.00 - 2.42)	--	--	--
Brown bullhead.....	W148	10.60 (9.06 - 12.40)	6.20 (5.00 - 7.69)	2.13 (2.01 - 2.26)	--	1.59 (1.48 - 1.70)
Do.....	W148	--	--	2.80 (2.35 - 3.33)	2.15 (2.05 - 2.26)	--
Channel catfish.....	W115B	--	--	1.38 (1.27 - 1.50)	1.05 (0.95 - 1.16)	0.95 (0.83 - 1.09)
Green sunfish.....	W157	--	--	--	1.80 (1.59 - 2.04)	1.75 (1.47 - 2.08)
Do.....	W164	8.00 (6.78 - 9.44)	4.50 (3.95 - 5.13)	2.32 (2.19 - 2.46)	2.25 (2.04 - 2.48)	2.25 (2.04 - 2.48)
Bluegill.....	W109	--	--	0.95 (0.85 - 1.06)	--	0.32 (0.22 - 0.47)
Do.....	W111	3.59 (3.15 - 4.09)	--	0.72 (0.59 - 0.88)	0.72 (0.59 - 0.88)	0.64 (0.55 - 0.75)
Do.....	W128	--	1.81 (1.63 - 2.01)	--	--	--
Redear sunfish.....	W112	7.00 (3.91 - 12.53)	--	--	--	--
Do.....	W117	3.50 (2.92 - 4.20)	2.22 (1.82 - 2.71)	1.03 (0.86 - 1.22)	0.74 (0.58 - 0.95)	0.74 (0.56 - 0.98)
Smallmouth bass.....	W146	3.82 (3.54 - 4.12)	--	1.83 (1.73 - 1.94)	--	1.66 (1.54 - 1.79)
Do.....	W146	2.68 (2.50 - 2.87)	1.96 (1.85 - 2.08)	1.60 (1.51 - 1.70)	1.40 (1.35 - 1.46)	1.37 (1.29 - 1.45)
Largemouth bass.....	W140	--	3.15 (3.00 - 3.31)	1.52 (1.41 - 1.64)	1.21 (1.10 - 1.33)	1.13 (1.07 - 1.20)
Do.....	W141	2.95 (2.52 - 3.45)	--	--	--	--

TABLE 4.--Toxicity of Hyamine 3500 to lake trout at three temperatures at 24, 48, and 96 hours of exposure.

Temperature (°C.)	Lot	LC_{50} and 95-percent confidence interval (ppm) at --		
		24 hours	48 hours	96 hours
7	346	4.00 (3.31 - 4.84)	2.45 (2.15 - 2.79)	1.80 (1.58 - 2.05)
12	346	2.74 (2.51 - 2.99)	2.35 (2.12 - 2.61)	1.90 (1.71 - 2.11)
17	346	1.36 (0.91 - 2.04)	1.22 (0.86 - 1.73)	1.00 (0.74 - 1.36)

may influence the relative tolerance of specimens to a toxicant. Also, water quality and feeding and handling during the holding time immediately before testing may have a direct influence on the physiological condition of the test fish. The 24-, 48-, and 96-hour LC_{50} values obtained with lots of goldfish from Welaka National Fish Hatchery and Tallassee, Ala., indicated that the fish had statistically significant differences in their tolerance to Hyamine 3500. The 24-hour LC_{50} values from separate tests conducted on different occasions with brown bullheads from lot W148 and with smallmouth bass from lot W146 show that there were statistically significant differences in resistance among fish from the same lot.

It is interesting that the 24-hour LC_{50} values did not vary over a wide range among the 14 species tested. The range for warmwater fishes was from 0.72 p p m for bluegill to 2.71 p p m for goldfish at 17° C. As a group the three species of trout were more resistant than warmwater fishes to Hyamine 3500 at 12° C. Of the four species of warmwater fish tested at 12° C., only goldfish were as resistant as the trout.

In the only field data currently available, Drake[1] reported on the use of Hyamine 3500 for control of gill disease at Pendills Creek National Fish Hatchery in Michigan. In these tests, lake trout held in lentic water were exposed to Hyamine 3500 for 1 hour and observed for 24 hours following exposure to determine mortality. The tests were conducted in two similar types of water having the following physical and chemical characteristics: (1) raceway water having temperatures ranging from 2° to 5° C., a pH of 7.2, and a total hardness of 51 p p m , and (2) tap water with temperatures ranging from 6° to 10° C., a pH of 7.5, and a total hardness of 51 p p m. Under the conditions listed, a 1-hour exposure to 2 to 5 p p m of Hyamine 3500 killed all fish within 45 minutes following the test. Similar exposures to concentrations of 1.50 to 1.75 p p m killed half the fish, while exposure to concentrations ranging from 1.10 to 1.40 p p m resulted in 0- to 80-percent mortality. Drake stated that mortality was greater when water temperature exceeded 4° C. He concluded that Hyamine 3500 was not suitable for treatment of gill disease in lake trout at Pendills Creek National Fish Hatchery.

Considering Drake's report, and the fact that warmwater fish are as sensitive as trout to Hyamine 3500, it appears that more field data are required before any recommendations can be made regarding Hyamine 3500 for control of fish diseases.

[1] Letter from Peter G. Drake, Manager, Pendills Creek NFH, Brimley, Mich., 1966.

CONCLUSIONS

1. Decreasing mortality among fish indicates rapid degradation of dilute solutions of Hyamine 3500 in open vessels.
2. Toxicity of Hyamine 3500 to fish decreases as water hardness increases.
3. In general, toxicity of Hyamine 3500 to fish decreases as water temperature decreases.
4. Hyamine 3500 is more toxic at acid and alkaline pH than at pH 7.
5. The three species of trout tested are as resistant to Hyamine 3500 as are warmwater fish.

TABLE 5.--Toxicity of Hyamine 3500 to fish at three temperatures at 3 to 96 hours of exposure

Species	Lot	Temp. (°C.)	LC_{50} and 95-percent confidence interval (ppm) at --				
			3 - hours	6 - hours	24 - hours	48 - hours	96 - hours
Goldfish.....	W107	12	--	6.10 (5.45 - 6.83)	2.72 (2.32 - 3.18)	2.19 (1.90 - 2.52)	2.09 (1.82 - 2.40)
Do.....	W116	12	13.30 (12.43 - 14.23)	--	--	--	--
Do.....	W107	17	--	--	2.71 (2.36 - 3.12)	2.18 (1.82 - 2.62)	2.18 (1.82 - 2.62)
Do.....	W116	17	10.00 (8.47 - 11.80)	--	--	--	--
Do.....	W116	17	6.55 (5.74 - 7.47)	--	--	--	--
Do.....	W116	17	--	3.34 (3.06 - 3.64)	--	--	--
Do.....	W133	17	--	--	1.60 (1.30 - 1.97)	1.60 (1.30 - 1.97)	1.49 (1.16 - 1.91)
Do.....	W107	22	8.50 (6.97 - 10.39)	4.29 (3.97 - 4.63)	2.90 (2.64 - 3.19)	2.67 (2.43 - 2.98)	2.56 (2.24 - 2.92)
Fathead minnow.....	W110	12	7.59 (6.72 - 8.58)	--	1.53 (1.28 - 1.82)	1.18 (1.04 - 1.34)	1.13 (1.00 - 1.28)
Do.....	W113	12	--	4.00 (3.50 - 4.60)	--	--	--
Do.....	W110	17	--	2.50 (2.31 - 2.70)	1.11 (0.98 - 1.25)	1.06 (0.91 - 1.23)	0.98 (0.89 - 1.08)
Do.....	W124	17	3.70 (3.33 - 4.11)	--	--	--	--
Do.....	W110	22	2.75 (2.62 - 2.89)	--	0.86 (0.78 - 0.95)	0.86 (0.77 - 0.96)	0.83 (0.77 - 0.90)
Do.....	W113	22	3.20 (2.91 - 3.52)	--	--	--	--
Do.....	W118	22	2.53 (2.39 - 2.68)	1.42 (1.24 - 1.62)	--	--	--
Channel catfish.....	W115B	12	--	--	1.80 (1.64 - 1.98)	1.40 (1.28 - 1.53)	0.98 (0.88 - 1.09)
Do.....	W115B	17	--	--	1.38 (1.27 - 1.50)	1.05 (0.95 - 1.16)	0.95 (0.83 - 1.09)
Do.....	W115B	22	--	--	1.01 (0.91 - 1.12)	0.87 (0.74 - 1.02)	--
Bluegill.....	W109	12	--	--	--	0.92 (0.83 - 1.02)	0.64 (0.54 - 0.76)
Do.....	W111	12	6.79 (5.56 - 8.28)	3.70 (3.27 - 4.18)	1.31 (1.11 - 1.54)	--	--
Do.....	W109	17	--	--	0.95 (0.85 - 1.06)	--	0.32 (0.22 - 0.47)
Do.....	W111	17	3.59 (3.15 - 4.09)	--	0.72 (0.59 - 0.88)	0.72 (0.59 - 0.88)	0.64 (0.55 - 0.75)
Do.....	W128	17	--	1.81 (1.63 - 2.01)	--	--	--
Do.....	W109	22	--	--	0.83 (0.75 - 0.92)	0.79 (0.71 - 0.88)	0.79 (0.71 - 0.88)
Do.....	W111	22	2.76 (2.58 - 2.95)	1.29 (1.11 - 1.50)	--	--	--

TABLE 6.--Toxicity of Hyamine 3500 to lake trout (lot 346) at various levels of total hardness at two temperatures

Temperature (°C.)	Total hardness as $CaCO_3$ (ppm)	LC_{50} and 95-percent confidence interval (ppm) at --		
		24 hours	48 hours	96 hours
12	10	2.85 (2.57 - 3.16)	1.82 (1.69 - 1.92)	1.49 (1.37 - 1.62)
12	42	2.74 (2.51 - 2.99)	2.35 (2.12 - 2.61)	1.90 (1.71 - 2.11)
12	170	4.18 (3.67 - 4.77)	3.15 (2.89 - 3.43)	2.83 (2.53 - 3.17)
17	10	0.59 (0.43 - 0.81)	0.50 (0.36 - 0.69)	0.42 (0.31 - 0.59)
17	42	1.36 (0.91 - 2.04)	1.22 (0.86 - 1.73)	1.00 (0.74 - 1.36)
17	170	0.96 (0.74 - 1.25)	0.70 (0.59 - 0.84)	0.70 (0.59 - 0.84)

TABLE 7.--Toxicity of Hyamine 3500 to fish in waters of various qualities at 17° C.

Species	Lot	Average pH	Total hardness as $CaCO_3$ (ppm)	96-hour LC_{50} and 95-percent confidence interval (ppm)
Fathead minnow	W113	6.99	11.0	0.82 (0.73 - 0.92)
Do	W113	7.54	41.3	1.29 (1.08 - 1.55)
Do	W113	8.15	150.0	1.67 (1.52 - 1.84)
Bluegill	W119	6.99	11.0	0.43 (0.33 - 0.56)
Do	W119	7.54	41.3	0.50 (0.38 - 0.65)
Do	W119	8.15	150.0	0.82 (0.65 - 1.03)

TABLE 8.--Toxicity of Hyamine 3500 to fish at three pH levels at 17° C.

Species	Lot	pH	LC_{50} and 95 percent confidence interval (ppm) at --		
			24 - hours	48 - hours	96 - hours
Goldfish	W153	5.0 ± 0.1	1.89 (1.60 - 2.23)	1.80 (1.56 - 2.07)	--
Do	W153	7.0 ± 0.1	2.17 (1.92 - 2.45)	2.00 (1.74 - 2.30)	1.83 (1.59 - 2.10)
Do	W153	9.0 ± 0.1	1.85 (1.62 - 2.11)	1.73 (1.57 - 1.90)	--
Fathead minnow	W139	5.0 ± 0.1	--	0.73 (0.64 - 0.84)	0.60 (0.49 - 0.74)
Do	W139	5.0 ± 0.1	0.74 (0.67 - 0.81)	0.62 (0.55 - 0.69)	0.37 (0.27 - 0.50)
Do	W139	7.0 ± 0.1	1.35 (1.24 - 1.47)	1.18 (1.07 - 1.30)	1.10 (1.00 - 1.21)
Do	W139	9.0 ± 0.1	1.04 (0.96 - 1.12)	0.93 (0.85 - 1.01)	0.93 (0.85 - 1.00)

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INVESTIGATIONS IN FISH CONTROL

33. Voidance Time for 23 Species of Fish

By Thomas H. Lane and Howard M. Jackson

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Washington, D.C. • October 1969

CONTENTS

	Page
Abstract	3
Methods and materials	4
Results	5
Discussion	7
Conclusions	8
References	8

VOIDANCE TIME FOR 23 SPECIES OF FISH

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ABSTRACT.--Observations on 23 species of fingerling-size bioassay fish indicated that voidance time (time required for food residues to pass through the alimentary canal) ranged from 12 to 108 hours.

Many species of fish are used in bioassays of pesticides, pollutants, and other chemicals (Douglas, 1960; Ward and Irwin, 1961; and Gould and Irwin, 1965). Guidelines for the acquisition, acclimatization, and evaluation of bioassay fish were defined by Hart, Doudoroff, and Greenbank (1945) and Doudoroff et al. (1951). They recommended feeding specimens during the 10-day pretest holding period, but they advised that food be withheld for 1 to 2 days before bioassays and during bioassays. Lennon and Walker (1964) advocated withholding food from test fish for as long as 4 days before bioassay, depending on the life stage and species of fish. The objective of the withholding is to empty the digestive tract of food wastes before the fish is introduced into bioassay.

Ward and Irwin (1961) stated that fish in good condition may not be greatly affected by short periods without food, but they cited evidence that the resistance of fish to chemicals may decrease with longer periods of starvation. In any case, the period off feed should be held to a minimum. Accordingly, the purposes of this investigation were to measure voidance times for various freshwater fishes and to determine whether voidance time is constant for a given species at one or two temperatures.

Some information concerning digestion rates, food passage rates, nutrition, and feeding behavior of fish is scattered throughout the literature. Results are diverse for a species, even though size of fish, water temperature, and other variables are similar. Many of the variables characteristically found in this type of investigation are either referred to or briefly discussed by Darnell and Meierotto (1962) in their study on digestion rate in a population of small black bullheads. According to these authors, digestion rate and rate of food passage through the digestive tracts of fishes have been shown to vary with temperature, age of experimental animal, and type and amount of food. They also point out that digestion rates are affected by the general activity of the animals which in turn may be influenced by the light regimen.

Phillips et al. (1960) in a test using brook trout averaging 6 grams observed no significant change in rate of food passage which could be ascribed to a temperature reduction of 2.8° C., from 11.1° to 8.3°. Earlier studies showed a deceleration in food passage in fingerling brook trout when the temperature was reduced 8.3° C., from 10.5° to 2.2° (Phillips et al., 1956). Markus (1932) and Baldwin (1957) demonstrated in experiments with warm- and cold-water fish that a temperature change of 4° C. can appreciably affect digestion rate.

Phillips et al. (1956) observed that 6-gram brook trout showed a significant change in food passage rate when fed different types of food, that is, meat and a mixture of meat and dry meal.

METHODS AND MATERIALS

The observations on voidance time were made at the Fish Control Laboratory at La Crosse, Wis. and the Southeastern Fish Control Laboratory at Warm Springs, Ga. The water in the indoor holding facilities at La Crosse is supplied from a deep well. It is hard (220 to 330 parts per million as $CaCO_3$), and its temperature during the test was $12^{o} \pm 1^{o}$ C. The holding and test temperatures were so similar that no acclimation was necessary when transferring fish into test facilities.

The water at Warm Springs for outdoor holding facilities and indoor testing is supplied by a spring and is hardened with lime to 35 to 45 ppm as $CaCO_3$. Its temperature is 17^{o} C. Test temperatures ranged from 12^{o} to 25^{o}, and experimental fish were carefully acclimated to them.

Most of the fish were acquired from Federal and State hatcheries. Golden shiners and brown bullheads were obtained from private ponds. The list of species is given in table 1.

The fish were fed routinely during the holding period, with special care taken within the last 24 hours before trials to provide as much food as the fish would eat. Carnivorous species were furnished with live foods such as daphnia and fish fry. Some lots of fish were supplied with dry food, but most lots were given a synthetic food originally formulated at the Southeastern Fish Cultural Laboratory, Marion, Ala., for use in studies on the nutrition of channel catfish (Harry K. Dupree and Kermit E. Sneed. Purified diets for channel catfish nutritional research. Manuscript.)

TABLE 1.--Fish used in voidance tests

Rainbow trout, Salmo gairdneri.
Lake trout, Salvelinus namaycush.
Northern pike, Esox lucius.
Goldfish, Carassius auratus.

Carp, Cyprinus carpio.
Golden shiner, Notemigonus crysoleucas.
Fathead minnow, Pimephales promelas.
White sucker, Catostomus commersoni.

Bigmouth buffalo, Ictiobus cyprinellus.
White catfish, Ictalurus catus.
Black bullhead, Ictalurus melas.
Yellow bullhead, Ictalurus natalis.

Brown bullhead, Ictalurus nebulosus.
Channel catfish, Ictalurus punctatus.
Green sunfish, Lepomis cyanellus.
Pumpkinseed, Lepomis gibbosus.

Bluegill, Lepomis macrochirus.
Longear sunfish, Lepomis megalotis.
Smallmouth bass, Micropterus dolomieui.
Largemouth bass, Micropterus salmoides.

White crappie, Pomoxis annularis.
Yellow perch, Perca flavescens.
Walleye, Stizostedion v. vitreum.

All voidance observations at La Crosse and initial tests at Warm Springs were conducted in 1-gallon wide-mouth jars. Later experiments were accomplished in funnel-type aquariums which proved more useful (fig. 1). Cover screens were necessary on the funnel aquariums to prevent escape of some species and to minimize the effect of the observer's movements. Bottom screens of epoxy-coated hardware cloth were installed to prevent coprophagy.

Each test vessel in a battery of four vessels per species contained 2.5 liters of reconstituted, deionized water which was continuously aerated (Lennon and Walker, 1964). The fish were loaded into vessels at a rate of about 8 grams per liter of water. Observations were made twice daily on the fish and feces; any dead fish were removed; and the presence or absence of fecal material was noted. At each observation, test vessels containing significant amounts of solid feces were scored with a plus (+). Those containing only one or two small deposits or none were scored with a minus (-).

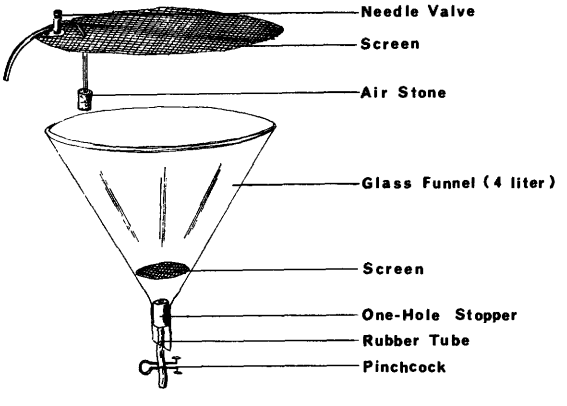

Figure 1.--Funnel-type aquarium used in voidance tests.

The tests of most species continued for 4 or 5 days. Voiding was judged to be complete at the end of the first interval during which two of the four test vessels were free of fecal deposits, that is, when a 50-percent reduction of + observations occurred. If three vessels were free of feces at the end of a period, voidance was listed as complete at the previous observation period, 12 hours earlier.

At La Crosse, the fish were transferred to clean jars at each observation period. At Warm Springs, about 10 percent of the water was removed from each jar each 12 hours and replaced with fresh reconstituted water.

Three techniques were used in identifying and enumerating feces: (1) Discrete fecal deposits were observed and counted; (2) Sudan 111, a red dye insoluble in water but soluble in oil, was added to the Marion catfish diet at 1 part per thousand to permit differentiation of food-waste feces from non-food feces which were found after last meals were completely voided; (3) A colorimetric method of detecting Sudan 111 in food-waste feces was employed.

The colorimetric method involved placing fecal samples in a Squibb separatory funnel after excess water was decanted. Glass beads and 20 milliters of chloroform were added to the funnel and shaken vigorously until the dye was extracted. The bottom layer of chloroform with dye was drawn off and read in a DB spectrophotometer at a wavelength of 510 mμ. as percent transmittance. The peak absorbance of this wavelength was determined previously for Sudan 111 which was extracted from fecal material with chloroform. Readouts for each of four replications were averaged and plotted for each observation period (fig. 2).

During preliminary colorimetric experiments, reference blanks of solvent-extracted feces from fish fed the same synthetic diet without dye and blanks of 100-percent solvent were compared. The transmittance of the blanks was not significantly different, and the chloroform blank was used, therefore, in tests plotted in figure 2.

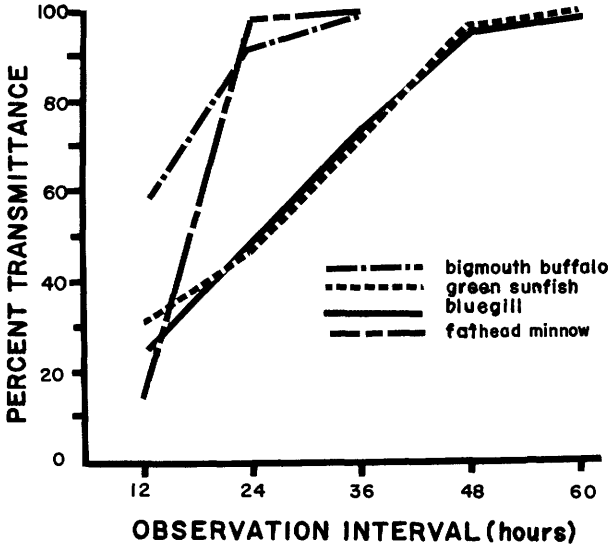

Figure 2.--Relative concentrations of Sudan 111 in fecal voidings of four fishes.

RESULTS

At La Crosse, 30 of 32 groups of fish voided within 36 to 96 hours at 11° to 13° C. (table 2). Metabolism is lower at these temperatures, and fish generally require more time to complete voiding. One group of lake trout did not complete voiding for 108 hours. One lot of channel catfish voided in less than 36 hours but it had appeared to feed poorly during pretrial holding. Another lot of channel catfish, a lot of yellow perch, and one of bluegill fed only lightly during holding, and the intestines of several specimens from each lot were examined during the tests. Because of the comparatively small amounts of food found in them, the voiding results probably should be regarded as minimal values (table 2).

In tests at Warm Springs in warm water (19° to 25°), 13 of 17 groups of fish completed voiding within 36 to 60 hours (tables 3 and 4). One group each of goldfish and yellow bullheads required 72 hours to complete voiding, and one group each of fathead minnows and bigmouth buffalo completed voiding in less than 36 hours.

Three groups of bluegills from lot number 17 (table 4) were tested at 12°, 17°, and 22°C. to ascertain the effects of temperature on voiding. The voidance times were 84, 48, and 36 hours, respectively. The expected

TABLE 2.--Voidance time for fish held at 12°±1° C. at La Crosse, Wisconsin

Species	Total length (inches)		Weight (grams)		Number of fish	Number of replicates	Time to void (hours)
	Average	Range	Average	Range			
Rainbow trout.....	1.0	--	0.2	--	90	3	36
Do.....	--	1.6-2.4	1.2	--	30	3	60
Do.....	2.8	2.5-2.9	3.1	--	24	4	60
Lake trout.....	2.8	2.4-3.2	2.5	--	32	4	108
Do.....	3.7	3.4-4.1	5.6	4.4-6.5	12	4	60
Northern pike.....	1.5	1.3-1.7	0.4	0.2-0.8	40	4	[1] 72
Do.....	2.9	2.6-3.1	1.9	--	80	8	72
Goldfish.....	1.4	1.2-1.9	0.8	0.6-1.5	100	4	48
Do.....	1.8	1.5-2.7	2.5	--	74	7	36
Carp.....	2.0	1.6-2.5	2.5	2.0-3.6	80	8	60
Fathead minnow.....	2.1	1.7-2.5	1.7	0.9-3.1	48	4	36
White sucker.....	--	3.5-3.9	6.0	--	12	3	60
Black bullhead.....	2.2	1.6-2.5	2.1	0.9-3.2	36	4	84
Channel catfish....	--	--	2.1	1.4-3.7	36	4	[2] 24
Do.....	3.5	3.0-4.1	5.2	3.5-8.0	16	4	[2] 36
Green sunfish.....	1.6	1.3-2.0	1.4	0.5-1.7	72	4	60
Pumpkinseed.....	--	1.7-2.5	2.6	--	45	3	84
Bluegill.....	1.7	1.5-2.1	1.1	0.8-2.1	80	4	[2] 60
Do.....	--	1.2-2.0	1.2	--	60	3	84
Do.....	1.8	1.4-2.0	1.3	0.8-2.0	60	4	36
Longear sunfish....	1.6	1.4-2.0	1.0	0.7-1.8	125	7	72
Smallmouth bass....	1.3	1.1-1.6	0.5	0.3-0.7	40	4	[1] 72
Do.....	1.9	1.5-2.1	1.3	--	120	8	48
Largemouth bass....	1.4	1.2-1.4	0.5	--	160	4	84
Do.....	1.8	1.4-2.0	0.8	--	96	4	48
Yellow perch.....	1.4	1.2-1.7	0.5	--	160	4	60
Do.....	1.9	1.8-2.2	0.8	0.9-1.1	100	4	[2] 36
Walleye.....	1.6	1.2-2.0	0.5	--	360	12	60

[1] Observations made at 24-hour intervals.
[2] Fish may not have consumed sufficient food before being placed in test vessels.

TABLE 3.--Voidance time for fish held at 20°±1° C. at Warm Springs, Ga.

Species	Weight (grams)		Number of fish	Number of replicates	Time to void (hours)	
	Average	Range				
Goldfish.....	1.5	0.5-4.9	80	8	72	
Do.....	1.2	0.9-2.0	40	4	60	
Golden shiner.....	1.4	0.5-3.5	40	4	36	
Fathead minnow.....	0.9	0.6-1.9	40	4	12	[1] (24)
Bigmouth buffalo...	1.5	1.2-3.0	40	4	24	[1] (24)
Do.....	1.5	1.2-3.0	40	4	48	
White catfish.....	3.2	2.0-5.1	40	4	48	
Brown bullhead.....	0.6	0.6-0.9	40	4	60	
Green sunfish.....	1.2	0.7-2.0	40	4	48	[1] (48)
Bluegill.....	1.2	0.4-2.0	40	4	36	[1] (48)
Do.....	1.6	0.9-2.0	40	4	48	
Do.....	1.6	0.6-4.0	80	8	60	
Largemouth bass....	1.2	0.5-2.0	40	4	60	
White crappie.....	0.4	0.2-0.7	40	4	60	

[1] Values obtained by colormetric method.

TABLE 4.--Voidance time for fish held at various temperatures at Warm Springs, Ga.

Species	Lot number	Weight (grams)		Number of fish	Number of replicates	Average temp. (°C.)	Time to void (hours)
		Average	Range				
Goldfish.....	13	0.8	0.6-0.9	40	4	25.0	60
Carp.....	14	3.0	1.8-4.2	40	4	23.5	48
Do.....	14	3.6	2.4-5.6	40	4	22.5	48
Yellow bullhead	15	1.5	0.8-2.0	40	4	24.0	72
Bluegill.....	16	0.5	0.1-0.9	40	4	25.0	36
Do.....	17	0.6	0.4-1.5	20	2	22.0	36
Do.....	17	0.6	0.4-1.5	20	2	17.0	48
Do.....	17	0.6	0.4-1.5	20	2	12.0	84

physiological effects of increased temperature were apparent, especially in tests made at 12° and 17°.

Visual assessments of voidance time for four species were made concurrently on samples which were evaluated by using the transmittance-time curve of the colorimetric method (fig. 2), and they were well corroborated by the latter.

DISCUSSION

The 1-gallon test jars used at La Crosse and initially at Warm Springs had several disadvantages. They made it necessary to handle the fish at each 12-hour period, which resulted in unnecessary stress and stress-induced defecation. Several types of tests vessels were tried, but those which were funnel-like in design proved to be the most satisfactory. The design shown in figure 1 minimized handling of fish and permitted easy and efficient recovery of solid wastes which were concentrated in the neck of the funnel. According to Brockway (1950) excretory products in solution also tend to accumulate on the bottom in ponds and raceways because of their greater density. We presumed, therefore, that a large portion of liquid wastes at the bottom of an aquarium would be drawn off with the 250-milliliter sample of excrement and water.

The later feeding with the synthetic diet eliminated the early variance due to different types of foods. Some of the early variations in results with a species tested both at La Crosse and Warm Springs were due more to different types of food than to different temperatures. The synthetic diet also facilitated the use of a dye, Sudan 111, to detect the end point of voidance. It was acceptable, either with or without the dye, to all of the species tested except largemouth bass. Some species preferred it over several types of dry food. The gastric and intestinal mucosae of fish which were fasted for several days after eating dyed food were examined for evidence of dye absorption, but none was found.

The frequency of taking food varied with each test lot so that no pattern could be followed in feeding the fish. Food was offered frequently for 24 or more hours prior to testing, and each time to the point of satiation.

The fish used in the voiding experiments were exposed to a change in water hardness. The reconstituted deionized water has a calcium hardness of about 16 ppm as $CaCO_3$. Holding water at La Crosse has a calcium hardness range of 150 to 232 ppm, and that at Warm Springs ranges from 35 to 45 ppm. Phillips et al. (1954) observed that a diminution in water calcium was accompanied by an increase in metabolic activity in brook trout. A variable such as this may have influenced voidance times for fish at both laboratories.

Of the factors shown to affect digestion rates and food passage rates in fish (Darnell and Meierotto, 1962), temperature and those factors associated with diets and feeding probably are the most important contributors to the variance in voidance times. Large differences were found between voidance times of fish of the same species and size from different hatchery lots which were tested at the same temperature. This observation leads us to believe that interpopulation differences may be significant in causing variation in voidance time. The test results for different lots of fish of the same species and size were not averaged so that the differences would remain apparent. Even so, sufficient data are not available to evaluate effects which might result from population differences, and the question remains unanswered.

The problem of distinguishing between fecal deposits which contain food residues and those which do not became evident in early experiments with green sunfish, carp, goldfish, and bigmouth buffalo. According to Hawk, Oser, and Summerson (1954), feces resulting from food digestion and absorption are composed of (1) food residues, (2) the remains of intestinal and digestive secretions, (3) substances secreted into the intestinal tract, principally salts, (4) bacterial flora and their metabolic end products, (5) cellular elements, and (6) abnormal elements. During fasting, some species apparently void large quantities of any one, or possibly all, of the elements mentioned above except food residues. In one test not included in the tables, bigmouth buffalo voided for 156 hours without any noticeable decrease in the quantity or change in the

appearance of the fecal deposits. However, two tests with dyed food were performed with fish from the same lot. The results showed that voidance of food residues was complete in 24 and 48 hours (table 3) even though deposition of fecal material continued as before. Thus, the value of the dye method is clearly demonstrated.

The colorimetric method, although time-consuming, was used as a check on the visual method. It is considered more accurate since all of the excreted dye can be extracted and measured.

CONCLUSIONS

1. All but 2 of the 32 groups of fish tested at La Crosse at 11° to 13° C. completed voiding in 36 to 96 hours. One group of lake trout required 108 hours, and one group of channel catfish, which fed poorly, completed voiding in less than 36 hours.

2. All but 4 of 17 groups of fish tested at Warm Springs at 19° to 25° C. completed voiding in periods of 36 to 60 hours. One group each of goldfish and yellow bullheads required 72 hours, and one group each of fathead minnows and bigmouth buffalo completed voiding in less than 36 hours.

3. Solid feces formed as a result of feeding and those formed during fasting could not be discriminated in green sunfish, carp, goldfish, and bigmouth buffalo without use of a dye marker. Voidance times determined using the dye, Sudan 111, were corroborated by the colorimetric method.

4. The period of time that fingerling-size fish should be held off feed before use in bioassays depends principally on the species, type of food, and temperature. If contamination of bioassays by food-waste feces is to be minimized, food should be withheld from most of the 23 species studied for 2 to 3 days before bioassays.

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INVESTIGATIONS IN FISH CONTROL

34. Laboratory Studies on Possible Fish-Collecting Aids With Some Toxicities for the Isomers of Cresol

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Washington, D.C. · October 1969

CONTENTS

	Page
Abstract	3
Methods and materials	4
Chemicals	4
Toxicity	4
Efficacy	4
Results	5
Toxicity	5
Observations during toxicity tests	6
Efficacy	7
Discussion	8
Conclusions	9
References	9

LABORATORY STUDIES ON POSSIBLE FISH-COLLECTING AIDS, WITH SOME TOXICITIES FOR THE ISOMERS OF CRESOL

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ABSTRACT.--The relative merits of quinaldine (2 methylquinoline), McNeil-JR-7464 (dl-1-(1-phenyl-ethyl)-5-(propoxy-carbonyl)-imidazole hydrochloride), and three isomers of cresol (p-methylphenol, o-methylphenol, and m-methylphenol) as collecting agents were determined in a lotic system under laboratory conditions at 12°C. The toxicity of the three cresol isomers to rainbow trout, brown trout, and brook trout also was measured in bioassays conducted in standard constituted water, and LC50 values were calculated for exposures of 6, 24, 48, and 96 hours. The toxicity of para-cresol was also established for carp, fathead minnow, black bullhead, channel catfish, bluegill, and yellow perch. Conclusions of these toxicity and efficacy tests were as follows: (1) Para-cresol is the most active of the three isomers of cresol in inducing total incapacitation of rainbow, brown, and brook trout; (2) The cresols are too harsh physiologically to warrant further development as fish-collecting agents; (3) MS-222 is not well suited as a fish-collecting tool; it does not induce surfacing and a high concentration is necessary for total incapacitation; (4) Quinaldine may have some usefulness for collecting fish in small streams with rapid flow but its physiological effects need elucidation; (5) McNeil-JR-7464 does not induce surfacing but is effective at low concentrations and appears to have desirable characteristics as a fish-collecting agent which merits further investigation.

Chemical compounds are needed for collecting fish without harming them. Such compounds as rotenone, cresol, quinaldine, aqualin, and tranquilizers have come under scrutiny (Wilkins, 1955; Louder and McCoy, 1962; Blanchard, 1965; Penfold, 1965; Tate, Moen, and Severson, 1965). Chemically induced surfacing of fish has been considered a desirable characteristic by some investigators (Loeb, 1962; Blanchard, 1965). Commercial cresol evoked a degree of surfacing, according to Wilkins (1955), when used as a stream sampling aid by the Tennessee Game and Fish Commission.

Because of the performance of cresol in past trials (Embody, 1940; Wilkins, 1955), we decided on further laboratory studies on the chemical. We used static bioassay to determine the toxicities of three isomers of cresol to rainbow trout, brown trout, and brook trout. The toxicity of para-cresol was also determined on carp, fathead minnows, black bullheads, channel catfish, bluegill sunfish, and yellow perch. The efficacies of ortho-cresol, meta-cresol, and para-cresol as collecting aids were measured with rainbow trout. Subsequently, quinaldine, MS-222, and McNeil-JR-7464 were investigated along with the

most effective isomer of cresol, against rainbow trout, to compare their relative merits as collecting agents in a lotic system.

METHODS AND MATERIALS

Chemicals

The isomers of cresol were a practical grade of para-cresol (p-methylphenol) and a purified grade of ortho-cresol (o-methylphenol) and meta-cresol (m-methylphenol). The quinaldine (2-methylquinoline) was practical grade. The McNeil-JR-7464 is dl-1-(1-phenyl-ethyl)-5-(propoxy-carbonyl)-imidazole hydrochloride.

Toxicity

Static bioassays with the isomers of cresol were with acclimated 2-inch fish in 5-gallon glass jars (Lennon and Walker, 1964). We exposed 10 fish to each concentration, and 20 served as controls. The statistical methods of Litchfield and Wilcoxon (1949) were used for determining LC50 values, slope functions, and 95-percent confidence intervals. Observations were made on surfacing and signs of physiological stress.

Efficacy

The lotic system for constant-flow experimentation was a modified alumium hatchery through 14 feet long (fig.1). A head-box

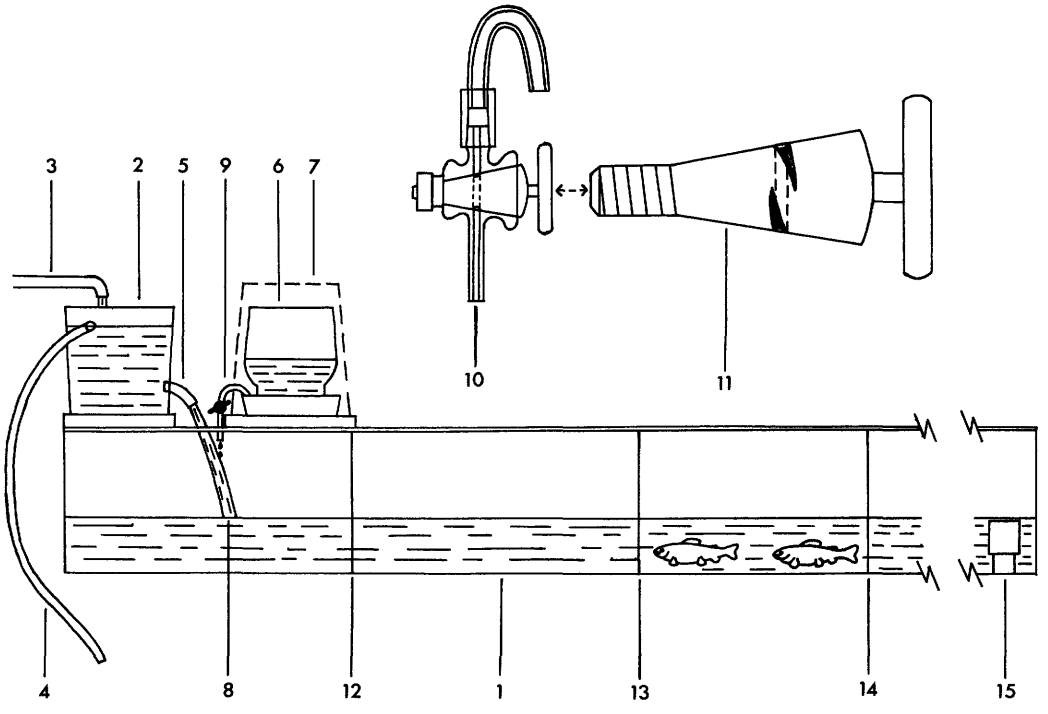

Figure 1.--A lotic system for constant-flow experimentation: (1) standard aluminum hatchery trough, (2) head-box, (3) water supply, (4) overflow tube, (5) spout, (6) 1-gallon poultry waterer, (7) inverted-plastic-pail cover to reduce evaporation and noxious fumes, (8) admixture of inflowing water and chemical solution, (9) siphon tube, (10) enlargement of siphon tube, (11) modified teflon stopcock, notched at each hole with a shallow tapering v-groove to increase flow adjustability, (12) diffusion screen, (13 and 14) test chamber screens, (15) outlet standpipe with adjustable rubber sleeve for depth regulation.

provided a uniform flow of water. Chemical-stock solution was held in a 1-gallon poultry waterer positioned below the head-box to allow admixture of chemical and inflowing water. Metering-in of the stock solution was accomplished with a glass siphon tube, equipped with an adjustable stopcock, modified from a standard burette. With this arrangement we could maintain a flow of 20 milliliters per minute with solutions of different viscosities, for the short periods involved in the experiments.

The trough was divided into compartments with screens 1 foot, 3.5 feet, and 5.5 feet from the point of inflow. The first screen served to enhance mixing and the following screens formed a test compartment for the fish. The inflow from the head-box was adjusted by using spouts of different diameters. The required amounts of chemicals and stock solutions were determined by the method described by Burrows (1949).

The stocks for quinaldine and the cresols were prepared with a 50:50 acetone:water solution. MS-222 and McNeil-JR-7464 dissolved readily in water. The progress of chemicals through the trough was simulated with 5 ppm (parts per million) of malachite green. Samples taken 1 minute apart at the head-screen of the fish compartment and analyzed by spectrophotometer provided a chronology of the increase toward maximum concentration. Thus, parameters were established for the subsequent exposure of test fish.

To appraise the value of these chemicals for collecting purposes, criteria of response were established in preliminary trials with cresol (mixture) and 3-inch rainbow trout:

1. Repellency minimal to reduce evasion in areas of weak concentration; surfacing desirable.

2. Total incapacitation, i.e. loss of equilibrium with only weak fin and directional movements remaining, within 10 minutes of contact.

3. Recovery not so rapid as to interfere with collecting.

4. No sign of physiological damage. We used twenty 3-inch rainbow trout to test each concentration for determining the most active isomer of cresol. Ten 9-inch rainbow trout were used at each concentration in the comparative testing of four compounds. Fish used in the lotic system were acclimated overnight. We kept a chronological record of fish responses for each trial. The flow of chemical was halted when all fish were in total incapacitation or when this stage was not attained within 15 minutes.

All tests were carried out at 12°C. in reconstituted water (Lennon and Walker, 1964). Table 1 lists the sources and sizes for test fish.

TABLE 1.--Species, sizes, and sources of test fish

Species	Average length (inches)	Source
Rainbow trout, Salmo gairdneri.....	2	Nevin, Wis., SFH.[1] Manchester, Iowa, NFH.[2]
Do.....	3	Troutlodge Springs, Soap Lake, Wash.
Do.....	9	Manchester, Iowa, NFH.
Brown trout, Salmo trutta.....	2	Do.
Brook trout, Salvelinus fontinalis..	2	Osceola, Wis., SFH.
Carp, Cyprinus carpio.....	2	Lake Mills, Wis., NFH.
Fathead minnow, Pimephales promelas.	2	Bud's Marina, La Crosse, Wis.; collected from Minnesota lakes
Black bullhead, Ictalurus melas.....	2	Necedah, Wis., NWR.[3]
Channel catfish, Ictalurus punctatus	2	Fairport, Iowa, NFH.
Bluegill, Lepomis macrochirus.....	2	Lake Mills, Wis., NFH.
Yellow perch, Perca flavescens.....	2	Do.

[1] State Fish Hatchery.
[2] National Fish Hatchery.
[3] National Wildlife Refuge.

RESULTS

Toxicity

The three isomers of cresol were toxic to trout (table 2). The 24-hour LC50's reveal the differences in toxicity most clearly: against rainbow trout the value for para-cresol was 9.2 ppm, for ortho-cresol 9.9 ppm, and for meta-cresol 10.4 ppm. Similarly, against brown trout, para-cresol produced a value of 4.4 ppm, ortho-cresol 7.2 ppm, and meta-cresol 8.6 ppm. The values against brook

TABLE 2.--Toxicities of the isomers of cresol to fish at 12° C.

Isomer	Species of fish	LC50 (ppm) and 95-percent confidence interval at				Mean slope function
		6 hours	24 hours	48 hours	96 hours	
ortho-cresol.....	Rainbow trout	11.0 9.6-12.7	9.9 8.8-11.1	8.6 7.6-9.8	7.0 6.1-8.0	1.400
Do.....	Brown trout	--	7.2 6.7-7.8	7.2 6.7-7.8	6.2 5.2-7.4	1.253
Do.....	Brook trout	14.1 10.4-19.0	7.9 5.3-11.8	7.8 5.9-10.3	7.2 6.7-7.7	2.135
meta-cresol.....	Rainbow trout	14.9 13.4-16.6	10.4 9.3-11.6	10.2 9.3-11.2	8.6 7.7-9.6	1.228
Do.....	Brown trout	11.0 9.7-10.6	8.6 7.5-9.8	8.4 7.4-9.6	8.4 7.8-9.6	1.288
Do.....	Brook trout	11.4 9.7-13.4	8.2 7.7-8.8	7.6 7.0-8.2	7.6 7.0-8.2	1.208
para-cresol.....	Rainbow trout	11.4 10.2-12.8	9.2 8.4-10.1	8.4 7.2-9.8	7.4 6.4-8.5	1.203
Do.....	Brown trout	4.7 4.1-5.3	4.4 3.9-4.9	4.4 3.9-4.9	4.4 3.9-4.9	1.230
Do.....	Brook trout	8.5 7.3-9.9	6.3 5.4-7.4	5.8 5.1-6.6	5.8 5.1-6.6	1.250
Do.....	Carp	--	22.0 21.8-22.2	15.0 14.9-15.1	13.3 13.2-13.4	1.182
Do.....	Fathead minnow	--	60.3 50.0-72.9	50.8 37.9-67.9	15.5 13.8-17.4	1.450
Do.....	Black bullhead	--	120.0 112.1-128.4	94.0 85.5-103.5	57.5 46.0-72.0	1.215
Do.....	Channel catfish	65.0 54.0-78.0	58.0 47.0-71.2	50.0 39.1-64.0	39.7 28.3-55.6	1.635
Do.....	Bluegill	--	7.9 6.5-9.3	7.1 6.0-8.4	7.1 6.0-8.4	1.473
Do.....	Yellow perch	19.5 14.7-26.0	12.3 10.5-14.4	10.0 8.1-12.3	10.0 8.9-11.2	1.505

trout were 6.3 for para-cresol, 7.9 for ortho-cresol, and 8.2 for meta-cresol. The data show that the isomers are only slightly more toxic to the salmonids at 96 hours than they are at 24 hours. Regarding all LC50's for the salmonids, a definite order of toxicity emerges for the three isomers: para-cresol is most toxic, followed by ortho-cresol, and finally meta-cresol.

Among the warm-water fish in bioassays with para-cresol, fathead minnows, black bullheads, and channel catfish proved to be most resistant. For these species as well as carp, the toxicity increased markedly in relation to time of exposure.

Observation during toxicity tests

During the first 10 minutes of the bioassays against brook trout at 6 to 20 ppm, the approximate incidences of surfacing were meta-cresol 20 percent, ortho-cresol 30 percent, and para-cresol 90 percent. The incidences for warm-water species exposed to para-cresol were carp 80 percent at 15 to 23 ppm, fathead minnows 30 percent at 30 to 150 ppm, bluegills 30 percent at 14 to 16 ppm, and yellow perch 50 percent at 12 to 18 ppm. Black bullheads and channel catfish did not surface at any concentration. Hemorrhages of the gills and at the base of the pectoral and/or pelvic fins were common in all bioassays.

Efficacy

Tests conducted at 10 ppm with the isomers of cresol in the lotic system confirmed the order of potency revealed in the static bioassays (fig. 2). Meta-cresol caused total incapacitation in 15 of 20 rainbow trout within 11.5 minutes, after which recovery to a higher level of activity occurred despite the continuing flow of chemical. Ortho-cresol induced total incapacitation in 17 of 20 fish within 15 minutes, with 3 fish remaining partially active. Para-cresol totally incapacitated 20 fish within 8.5 minutes.

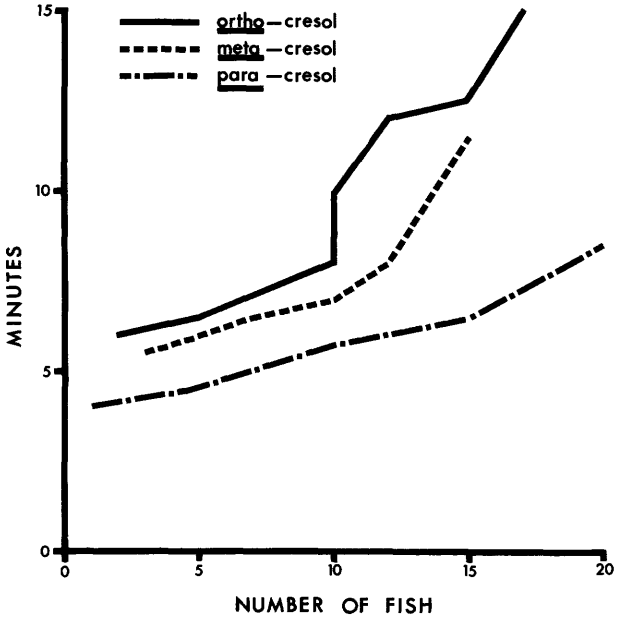

Figure 2.—Induction times and numbers of fish in total incapacitation from exposure to the isomers of cresol at 10 ppm.

Rainbow trout reacted violently upon contact with the isomers, as did all species tested in the static bioassays. Random lunging and thrashing occurred among the rainbows. Surfacing appeared during the throes preceding stupefaction but was not sustained. As the fish lost equilibruim, they became darker, the gular area reddened in some, and their bellies bloated as swimming activity diminished. Most fish settled to the bottom where blanched gills and several fin-base hemorrhages were noted. These responses were evoked quite uniformly by all three isomers.

The results of the flowing trials and static bioassays make it apparent that para-cresol is the prominent isomer for the incapacitation of rainbow, brown, and brook trouts. Thus it was decided to evaluate para-cresol in conjunction with certain compounds which are known to immobilize trout (Muench, 1958; Bové, 1962; Thienpoint and Niemegeers, 1965).

The concentrations of MS-222, quinaldine, and McNeil-JR-7464 which induce total incapacitation in 10 fish within specified times are presented in table 3. Flows and depths differed between these trials and those involving the isomers of cresol shown in figure 2. Therefore, the induction times of the two trials are not comparable. At least 7 minutes were required to attain the maximum concentration of a chemical in the test chamber, using malachite green as a tracer. Therefore, in some tests the fish never were exposed to the desired concentration, and in others they received the full dosage for only a portion of the time stated in the table. The calculated, ultimate concentrations achieved the desired effect in the times listed through buildup toward or to maximum level, as would be the case in a practical, stream application.

TABLE 3.--Concentration of four compounds necessary to induce total incapacitation in 9-inch rainbow trout within specified times

Time (minutes)	Number of fish	Concentration (ppm) of --			
		para-cresol	McNeil-JR-7464	Quinaldine	MS-222
4.5.....	10	--	3.0	37.5	110.0
5.....	10	--	2.5	35.0	95.0
6.....	10	--	2.3	25.0	80.0
7.....	10	30.0, 35.0, 40.0	2.2	20.0	65.0
8.....	10	22.4	2.0	17.3	56.3
9.....	10	15.0	1.8	15.0	--
10.....	10	14.0	1.7	--	--

Concentrations of para-cresol below 14.0 ppm required more than 10 minutes to induce total incapacitation; concentrations of 35 and 40 ppm did not improve on the induction time at 30 ppm (table 3). Repellency was strong at all concentrations. The incidence of surfacing was about 50 percent during partial incapacitation, but transitory with each fish. They settled to the bottom at the onset of total incapacitation.

The symptoms of stress, observed in the flowing trials of the isomers of cresol, were manifested again in these trials. Nine of 70 rainbow trout exposed to para-cresol developed hemorrhages at the base of pectoral and/or pelvic fins. Eight of 40 fish died as a result of the trials above 25 ppm. Recovery from incapacitation required 5.5 to 26.5 minutes, the longer times being associated with higher concentrations.

MS-222 was less efficacious than para-cresol and did not induce total incapacitation within 10 minutes at levels below 56.3 ppm (table 3). However, higher concentration were efficacious, although there was no improvement in reaction time at concentrations greater than 110 ppm. Neither repellency nor surfacing was observed, and no external signs of stress were visible. The time for recovery ranged between 4 and 10 minutes, and there were no deaths among 60 fish.

Quinaldine was effective in causing total incapacitation within 10 minutes at levels from 15.0 to 37.5 ppm (table 3). Concentrations below 15.0 ppm were not efficacious within 10 minutes; doses above 37.5 ppm did not improve significantly on reaction time. Moderate repellency was noted, and the rainbow trout darted about, shaking their heads and bodies. Only incidental surfacing occurred prior to total incapacitation. The only unusual manifestation in the stupefied fish was gyrating of the eyes. As recovery commenced, tremoring of the bodies was general. No deaths were recorded among 80 fish, and recovery required 5 to 14 minutes.

McNeil-JR-7464 was most effective between 1.7 and 3.0 ppm (table 3). There was no repellency and surfacing was only incidental before total incapacitation. Most of the fish assumed an inverted position while stupefied at the bottom. No signs of stress were evident at any stage of testing. The time for recovery ranged from 12 to 22 minutes, and no losses resulted among 50 fish.

DISCUSSION

Of the compounds tested, para-cresol fared poorly according to the criteria established for testing potential collecting compounds. It caused violent repellency before rapid incapacitation and deaths of more than 10 percent. The active surfacing induced by para-cresol is not conducive to collecting fish as would a passive surfacing. Cresol-induced surfacing is a constantly shifting phenomenon which appears to be a vague effort to escape the irritating solution during the paroxysms which precede stupefaction.

There was considerable evidence of physiological disturbance from contact with the cresols at effective concentrations. Violent lunging and gulping was followed by bloating of the abdomen in most cases. Some fish reddened in the gular area, and others developed hemorrhages at the bases of the pectoral and/or pelvic fins. When the fish settled to the bottom, opercula and mouths were agape, and the gills were considerably blanched. They retained this appearance until death. These symptoms are similar to those described by Jones (1964) for phenol and cresols against perch.

As for the physiological damage which occurs, a Polish investigator has provided detailed accounts of phenol-induced changes in the blood and tissues of the bream Abramis brama (L) (Waluga, 1966a, 1966b). Among his findings were quantitative changes in the blood, circulatory impairment, hemorrhages, cell necrosis leading to disintegration, and damage to the central nervous system; in summary, a general poisoning of the fish, damaging systems and organs essential to life.

The cresols are alkyl derivatives of phenol, and their systemic actions are so identical as not to warrant separation (Goodman and Gilman, 1965). It is apparent that the search for fish-collecting agents shoul be directed toward substances less harsh than the cresols.

MS-222 met the test criteria satisfactorily, although it did not induce surfacing. The relatively high concentrations required would make practical employment of this expensive drug unfeasible.

Quinaldine caused moderate irritation and repellency and no significant surfacing. This compound probably would be satisfactory as a collecting aid in small swiftly flowing streams,

where current action would wash the immobilized fish into a blocking seine. However, further data on the physiological effects of this compound are necessary.

McNeil-JR-7464 produced most of the desired effects on fish at very low concentrations. It should be effective for collecting in swift waters, and possibly in slower sections as well. The long recovery period and the inverted position of stupefied trout, exposing their light undersides, should enhance collection in nonturbid slow waters. This compound lacks only surfacing action to have excellent potential as a collecting tool. Its combination with a compound which induces only active surfacing might yield a valuable collecting tool.

CONCLUSIONS

1. Para-cresol is the most active of the three isomers of cresol in inducing total incapacitation of rainbow, brown, and brook trout.
2. The cresols are too harsh physiologically to warrant further development as fish-collecting agents.
3. MS-222 is not well suited as a fish-collecting tool; it does not induce surfacing, and a high concentration is necessary for total incapacitation
4. Quinaldine may have some usefulness for collecting fish in small streams with rapid flow. Its physiological effects need elucidation.
5. McNeil-JR-7464 does not induce surfacing but is effective at low concentrations and appears to have desirable characteristics as a fish-collecting agent. It merits further investigation.

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