INVESTIGATIONS IN FISH CONTROL

18. Toxicity of 22 Therapeutic Compounds to Six Fishes
19. Toxicity of Bayer 73 to Fish
20. Toxicity of Dimethyl Sulfoxide (DMSO) to Fish
21. Labor-Saving Devices for Bioassay Laboratories

United States Department of the Interior
Fish and Wildlife Service
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INVESTIGATIONS IN FISH CONTROL

Investigations in Fish Control, published by the Bureau of Sport Fisheries and Wildlife, include reports on the results of work at the Bureau's Fish Control Laboratories at La Crosse, Wis., and Warm Springs, Ga., and reports of other studies related to that work. Though each report is regarded as a separate publication, several may be issued under a single cover, for economy.

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Continued on inside back cover--

INVESTIGATIONS IN FISH CONTROL

18. Toxicity of 22 Therapeutic Compounds to Six Fishes
By Wayne A. Willford (Resource Publication 35, p. 1-10)

19. Toxicity of Bayer 73 to Fish
By Leif L. Marking and James W. Hogan
(Resource Publication 36, p. 1-13)

20. Toxicity of Dimethyl Sulfoxide (DMSO) to Fish
By Wayne A. Willford (Resource Publication 37, p. 1-8)

21. Labor-Saving Devices for Bioassay Laboratories
By Robert J. Hesselberg and Ralph M. Burress
(Resource Publication 38, p. 1-8)

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CONTENTS

TOXICITY OF 22 THERAPEUTIC COMPOUNDS TO SIX FISHES

By Wayne A. Willford, Chemist
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ABSTRACT.--Of 22 therapeutic chemicals (18 parasiticides and 4 oral bacteriostats) tested by bioassays, 16 were toxic to fish and 6 were not. Tests were in 24- and 48-hour static bioassays on rainbow, brown, brook, and lake trout and bluegills at 12° C. and channel catfish at 17° C. The 16 toxic chemicals, in descending order, were malachite green, Trolene, CoRal, Tiguvon, Roccal, P.M.A., Acriflavine, amopyroquin dihydrochloride, merthiolate, methylene blue, Neguvon, Ruelene, TV-1096, nickel sulfate, formalin, and quinacrine hydrochloride; the 6 that did not appear to be toxic were erythromycin thiocyanate, quinine hydrochloride, Flagyl, sulfamerazine, sulfamethazine, and sulfisoxazole.

An objective of the Fish Control Laboratories is to develop chemical tools to prevent and control fish diseases. Although efficacious concentrations of many drugs have been determined, a thorough examination of their toxicity has not been reported. Prior to clearance of drugs, the Food and Drug Administration requires data on their toxicity. The purpose of this study was to define the toxicity of 22 therapeutic chemicals to six species of fish before further research is undertaken on their efficacy and residues.

Eighteen parasiticides of known or possible value as external treatments for fish were selected for investigation upon recommendations by other investigators. Four oral bacteriostats were tested to determine whether any toxicity to fish would result through leaching, or excretion, of the compounds into water.

MATERIALS AND METHODS

Six species of fish were obtained from various fish hatcheries (table 1). All were quarantined for 10 days, and those judged acceptable for bioassays were acclimated to conditions of the tests.

The static bioassays were made in facilities described by Lennon and Walker (1964). We used 5-gallon glass jars which contained 15 liters of reconstituted, deionized water at total hardness of 42 p.p.m., and a maximum of 1 gram of fish per liter of water. Each test included 10 concentrations of a chemical. Ten fish were exposed to each concentration, and 20 fish served as controls.

The 22 therapeutic compounds were tested at 12° C. against five species of fish at La Crosse, Wis. (table 2). Tests against channel catfish at 17° were made at the Southeastern Fish Control Laboratory, Warm Springs, Ga.

A concentrated stock solution of each compound, using acetone or deionized water or both as solvents, was usually prepared for addition to the test vessels immediately before each test. When solubility of the compound prevented preparation of concentrated stocks, the compound was added directly and allowed to dissolve in the test vessel.

Observations on survival and mortality were recorded at 24 and 48 hours. The data were then analyzed by plotting concentration against mortality on logarithmic normal

TABLE 1.--Fishes used in toxicity trials

Species	Lot	Average length (inches)	Average weight (grams)	Grading date	Source
Rainbow trout, Salmo gairdneri.....	159	1.5	0.5	1-21-65	National Fish Hatchery,
Do.....	159	1.8	0.9	2-15-65	Manchester, Iowa
Brown trout, Salmo trutta.....	177	1.7	0.8	3-16-65	National Fish Hatchery,
Do.....	177	1.9	1.2	4- 1-65	Lake Mills, Wis.
Brook trout, Salvelinus fontinallis....	161	1.5	0.4	1-21-65	State Fish Hatchery,
Do.....	161	1.6	0.6	2-15-65	St. Croix Falls, Wis.
Lake trout, Salvelinus namaycush.....	78	4.0	2.5	8-14-64	National Fish Hatchery,
Do.....	78	4.0	2.8	8-28-64	Jordan River, Mich.
Do.....	78	4.1	3.2	10- 7-64	
Channel catfish, Ictalurus punctatus...	W-70	2.1	1.2	7-21-65	National Fish Hatchery,
Do.....	W-74	2.2	1.5	8- 4-65	Marion, Ala.
Bluegill, Lepomis macrochirus.....	115	1.6	0.8	11- 5-64	National Fish Hatchery,
Do.....	131	1.4	0.7	11-17-64	Lake Mills, Wis.
Do.....	131	1.7	1.1	12- 1-64	

TABLE 2.--Common names and active ingredients of compounds tested

Common name	Grade or formulation	Active ingredient
Acriflavine (neutral).....	technical.....	3,6-diamino-10-methyl acridinium chloride and 3,6-diaminoacridine
Amopyroquin dihydrochloride.....	technical.....	4-(7-chloro-4-quinolylamino)-α-1-pyrrolidyl-o-cresol dihydrochloride
CoRal.....	technical.....	0,0-diethyl 0-3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl-phosphorothioate
Erythromycin thiocyanate.....	800 mcg/mg.....	erythromycin thiocyanate
Flagyl.....	technical.....	1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole
Formalin.....	U.S.P.....	37-percent formaldehyde gas in water
Malachite green.....	technical.....	bis-(p-dimethylaminophenyl) phenylmethane treated with HCL
Merthiolate.....	technical.....	sodium ethylmercurithiosalicylate
Methylene blue.....	technical.....	3,7-bis(dimethylamino) phenazathionium chloride
Neguvon.....	80-percent soluble powder.....	0,0-dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate
Nickel sulfate.....	analytical reagent.....	$NiSO_4 \cdot 6H_2O$
P.M.A.....	technical.....	pyridylmercuric acetate
Quinacrine hydrochloride (Atabrine).....	technical.....	3-chloro-7-methoxy-9- (1-methyl-4-diethylaminobutylamino) acridine dihydrochloride
Quinine hydrochloride.....	technical.....	quinine hydrochloride
Roccal.....	50-percent concentrate.....	alkyl dimethylbenzylammonium chlorides
Ruelene.....	227 mg/cc.....	4-tert-butyl-2-chlorophenyl methyl methylphosphoramidate
Sulfamerazine.....	U.S.P.....	N^1-(4-methyl-2-pyrimidyl) sulfanilamide
Sulfamethazine.....	U.S.P.....	N^1-(4,6-dimethyl-2-pyrimidinyl) sulfanilamide
Sulfisoxazole.....	U.S.P.....	N^1-(3,4-dimethyl-5-isoxazolyl) sulfanilamide
Tiguvon.....	300 mg/cc.....	0,0-dimethyl 0-[4-(methylthio)-m-tolyl] phosphorothioate
Trolene.....	technical.....	0,0-dimethyl 0-2,4,5-trichlorophenyl phosphorothioate
TV-1096 (Parke, Davis & Company).....	technical.....	Lg-threo-2-(5-nitro-2-furyl)-5-(p-nitrophenyl)-2-oxazoline-4-methanol

(probability) graph paper to define the concentration that produced 50-percent mortality (LC_{50}) as described by Litchfield and Wilcoxon (1949). Variance and the 95-percent confidence interval (C.I.) were also determined.

Most of the compounds tested were technical or U.S.P. materials, and the rest were formulated materials. To eliminate confusion, all results are reported in terms of p.p.m. of total material (formulated or technical) instead of active ingredient.

RESULTS

Of the 22 compounds, 16 were toxic to the six species of fish, and the LC_{50} values were determined (tables 3 to 8).

The most toxic compound, malachite green, is relatively uniform in toxicity to the six

TABLE 3.--Toxicity of 16 compounds to rainbow trout at 12° C.

Compound	At 24 hours		At 48 hours	
	LC_{50} (p.p.m.)	95-percent C.I.	LC_{50} (p.p.m.)	95-percent C.I.
Acriflavine	30.1	26.2-34.6	19.9	17.0-23.3
Amopyroquin	47.0	43.5-50.8	35.3	33.3-37.4
CoRal	2.60	2.28-2.96	0.55	0.51-0.59
Formalin	207	182-236	168	154-183
Malachite green	0.50	0.36-0.69	0.39	0.33-0.46
Merthiolate	60.5	53.5-68.4	21.2	18.6-24.2
Methylene blue	25.0	20.5-30.5	16.0	13.8-18.7
Neguvon	32.5	29.3-36.1	12.2	10.6-14.0
Nickel sulfate	320	302-339	160	150-171
P.M.A.	5.00	4.35-5.75	3.75	3.02-4.65
Quinacrine HCL	--	--	172	159-186
Roccal	3.24	2.92-3.60	2.57	2.16-3.06
Ruelene	35.0	31.5-38.8	32.0	30.5-33.6
Tiguvon	5.30	4.82-5.83	4.35	3.62-5.22
Trolene	1.17	0.89-1.53	0.74	0.64-0.86
TV-1096	24.2	22.8-25.7	16.1	14.9-17.4

TABLE 4.--Toxicity of 16 compounds to brown trout at 12° C.

Compound	At 24 hours		At 48 hours	
	LC_{50} (p.p.m.)	95-percent C.I.	LC_{50} (p.p.m.)	95-percent C.I.
Acriflavine	40.0	36.4-44.0	27.0	25.0-29.2
Amopyroquin	42.0	37.5-47.0	36.0	33.3-38.9
CoRal	0.92	0.84-1.00	0.73	0.62-0.86
Formalin	325	304-348	185	165-208
Malachite green	0.45	0.42-0.49	0.34	0.30-0.38
Merthiolate	110	75-160	54.0	47.8-61.0
Methylene blue	54.0	46.2-63.2	32.8	28.8-37.4
Neguvon	54.0	48.2-60.5	16.5	11.8-23.1
Nickel sulfate	400	345-464	270	241-302
P.M.A.	9.30	8.30-10.42	6.22	5.71-6.78
Quinacrine HCL	390	361-421	230	184-288
Roccal	2.95	2.46-3.54	2.05	1.74-2.42
Ruelene	26.2	24.7-27.8	25.7	24.2-27.2
Tiguvon	4.90	4.09-4.95	3.62	2.78-4.71
Trolene	0.53	0.38-0.74	0.39	0.30-0.51
TV-1096	--	--	--	--

TABLE 5.--Toxicity of 16 compounds to brook trout at 12° C.

Compound	At 24 hours		At 48 hours	
	LC_{50} (p.p.m.)	95-percent C.I.	LC_{50} (p.p.m.)	95-percent C.I.
Acriflavine	48.0	43.2-53.3	14.8	14.0-15.7
Amopyroquin	52.0	44.8-60.3	40.0	38.1-42.0
CoRal	1.06	0.87-1.29	0.80	0.70-0.91
Formalin	196	187-206	157	143-173
Malachite green	0.30	0.22-0.40	0.26	0.22-0.31
Merthiolate	89.5	85.2-94.0	74.5	71.0-78.2
Methylene blue	49.8	41.2-60.3	22.9	17.2-30.5
Neguvon	34.0	23.4-49.3	16.8	14.1-20.0
Nickel sulfate	--	--	242	224-261
P.M.A.	15.5	12.9-18.6	10.7	9.8-11.7
Quinacrine HCL	--	--	230	177-299
Roccal	4.13	3.79-4.50	3.40	3.09-3.74
Ruelene	36.8	34.4-39.4	35.0	31.5-38.8
Tiguvon	6.15	5.21-7.26	5.50	5.14-5.88
Trolene	0.59	0.44-0.78	0.39	0.26-0.59
TV-1096	29.3	26.4-32.5	19.0	16.8-21.5

TABLE 6.--Toxicity of 16 compounds to lake trout at 12° C.

Compound	At 24 hours		At 48 hours	
	LC_{50} (p.p.m.)	95-percent C.I.	LC_{50} (p.p.m.)	95-percent C.I.
Acriflavine	37.5	34.7-40.5	28.0	24.4-32.2
Amopyroquin	15.5	12.1-19.8	14.0	10.8-18.1
CoRal	6.80	4.00-11.56	4.00	1.25-12.80
Formalin	220	200-242	167	160-174
Malachite green	0.57	0.49-0.66	0.40	0.33-0.49
Merthiolate	13.0	9.6-17.6	2.13	1.06-4.26
Methylene blue	35.0	29.4-41.6	34.0	29.3-39.4
Neguvon	41.0	38.7-46.5	9.00	7.20-11.25
Nickel sulfate	170	139-209	75.0	55.6-101.2
P.M.A.	12.5	11.8-13.2	7.60	6.33-9.12
Quinacrine HCL	28.0	18.8-42.0	21.0	12.4-35.7
Roccal	2.70	2.41-3.02	1.95	1.68-2.26
Ruelene	27.0	25.0-29.2	27.0	23.9-30.5
Tiguvon	6.50	6.08-6.96	5.30	4.91-5.72
Trolene	0.73	0.62-0.86	0.62	0.53-0.72
TV-1096	32.0	28.6-35.8	16.5	13.2-20.6

TABLE 7.--Toxicity of 16 compounds to channel catfish at 17° C.

Compound	At 24 hours		At 48 hours	
	LC_{50} (p.p.m.)	95-percent C.I.	LC_{50} (p.p.m.)	95-percent C.I.
Acriflavine	43.5	39.9-47.4	33.2	31.0-35.5
Amopyroquin	19.8	17.7-22.2	12.5	11.8-13.2
CoRal	6.80	5.81-7.96	--	--
Formalin	137	129-145	96.0	90.6-101.8
Malachite green	0.21	0.17-0.27	0.20	0.16-0.26
Merthiolate	7.50	6.41-8.78	5.65	4.79-6.67
Methylene blue	120	110-131	104	93-116
Neguvon	80.0	72.7-88.0	32.0	24.8-41.3
Nickel sulfate	368	334-405	165	129-211
P.M.A.	3.22	2.66-3.90	2.89	2.60-3.21
Quinacrine HCL	198	169-232	70.0	59.3-82.6
Roccal	1.28	1.16-1.41	1.12	1.03-1.22
Ruelene	39.5	37.6-41.5	34.8	32.5-37.2
Tiguvon	5.90	4.50-7.73	5.90	4.50-7.73
Trolene	1.76	1.54-2.01	1.26	1.09-1.46
TV-1096	27.0	24.8-29.4	20.3	19.3-21.3

TABLE 8.--Toxicity of 16 compounds to bluegills at 12° C.

Compound	At 24 hours		At 48 hours	
	LC_{50} (p.p.m.)	95-percent C.I.	LC_{50} (p.p.m.)	95-percent C.I.
Acriflavine	18.0	16.8-19.3	13.5	12.6-14.4
Amopyroquin	33.0	23.6-42.2	18.5	16.7-20.5
CoRal	10.5	8.1-13.6	8.00	6.11-10.48
Formalin	185	156-220	140	127-154
Malachite green	0.26	0.22-0.31	0.11	0.09-0.14
Merthiolate	110	87-139	64.5	57.6-72.2
Methylene blue	51.0	40.2-64.8	33.0	26.2-41.6
Neguvon	78.0	64.5-94.4	71.0	55.9-90.2
Nickel sulfate	--	--	495	450-544
P.M.A.	20.0	18.0-22.2	16.0	13.4-19.0
Quinacrine HCL	120	73-198	79.0	54.1-115.3
Roccal	2.10	1.94-2.27	1.68	1.56-1.81
Ruelene	36.0	34.3-37.8	35.0	33.0-37.1
Tiguvon	15.7	13.2-18.7	8.90	7.67-10.32
Trolene	2.50	2.25-2.78	1.00	0.67-1.50
TV-1096	37.0	33.3-41.1	28.2	25.9-30.7

species, and LC_{50} values range from 0.11 to 0.40 p.p.m. at 48 hours. Clemens and Sneed (1958a) reported its LC_{50} to channel catfish as 0.14 p.p.m. in 24 and 48 hours at 25° C. Our results show the LC_{50} values to be 0.21 and 0.20 p.p.m. in 24 and 48 hours respectively at 17°. This variation between results may be due to differences in test temperatures.

Following malachite green in decreasing order of toxicity are Trolene, CoRal, and Tiguvon, all of which have the basic structure of phosphorothioate. In the same general range of toxicity are Roccal and P.M.A., with Roccal the more toxic of the two. Roccal, like malachite green, exhibits relatively uniform toxicity, and LC_{50} values range from 1.12 to 3.40 p.p.m. at 48 hours for all species.

P.M.A. exhibits a much wider range of toxicity with LC_{50} values of 2.9 to 16.0 p.p.m. at 48 hours. Clemens and Sneed (1958a) reported its LC_{50} to channel catfish as 3.8 p.p.m. in 24 hours at 24° C. In a later publication, these authors (1958b) reported the LC_{50} of P.M.A. to channel catfish as 2.96 and 2.81 p.p.m. in 24 and 48 hours, respectively, at 16.5°. Both reports compare favorably with our LC_{50} values of 3.22 and 2.89 p.p.m. for 24 and 48 hours at 17°.

Acriflavine, amopyroquin dihydrochloride, merthiolate, methylene blue, Neguvon, Ruelene, and TV-1096 fall into an intermediate toxicity range with LC_{50} of 10 to 100 p.p.m. Only Ruelene exhibits a uniform LC_{50} range of 25.7 to 35.0 p.p.m. for six species in 48 hours. The other compounds of this group demonstrate a relatively wide range of toxicity to the different species.

TV-1096 is soluble only to approximately 30 p.p.m. in water. Amounts above this level produce a saturated solution with a precipitate on the bottom of the test vessel. Brown trout fail to succumb to concentrations below 30 p.p.m. and for this reason, LC_{50} values could not be derived for the species. Also, amounts of TV-1096 in excess of a saturated solution are nontoxic to brown trout. In contrast, LC_{50} values of TV-1096 for the other species range from 16.1 to 28.2 p.p.m. at 48 hours.

Nickel sulfate, formalin, and quinacrine hydrochloride are the least toxic of the compounds analyzed. Formalin exhibits a fairly uniform LC_{50} range of 96 to 185 p.p.m. at 48 hours. The other two have a much wider range.

Clemens and Sneed (1958a) reported the LC_{50} values of formalin on channel catfish to be 87 and 69 p.p.m. in 24 and 48 hours, respectively, at 25° whereas we found them to be 137 and 96 p.p.m. in 24 and 48 hours, respectively, at 17° C. This variation in results seems to indicate that the toxicity of formalin may be increased by an increase in temperature. The observation is supported by our results which show that formalin is more toxic to channel catfish at 17° than it is to four species of trout and to bluegills at 12°.

Erythromycin thiocyanate and quinine hydrochloride were tested at an arbitrary level of 100 p.p.m. Their solubility would have permitted higher concentrations but preliminary tests indicated little toxicity. At 100 p.p.m., the substances were not toxic to the fish.

The poor solubility of Flagyl, sulfamerazine, sulfamethazine, and sulfisoxazole prevented the determination of LC_{50} values. Solutions were saturated before lethal levels could be reached. The arbitrary concentration of 100 p.p.m. was selected then for tests. This resulted in saturated solutions with excess chemical remaining on the bottom of the bioassay vessels. None of them was toxic to the six species of fish.

DISCUSSION

Malachite green has been in use for many years as a fungicidal dip for fish (Foster and Woodbury, 1936). Recently, Amlacher (1961) recommended it for prolonged treatment of fish in ponds to combat Ichthyophthirius, Chilodenella, and Costia. He applied 0.15 p.p.m. and allowed it to dissipate in the water. Concentrations of 0.11 to 0.40 p.p.m. in our bioassays proved toxic within 48 hours to the six species of fish tested. Thus, there is a risk with concentrations over 0.11 p.p.m. in long-term treatments.

Trolene, CoRal, and Tiguvon are under consideration as prolonged treatments for control of Ichthyophthirius. Tiguvon is the least toxic of these organophosphates to fish. This indicates that it may prove the most valuable of the group if minimum concentrations required for control of "Ich" are approximately the same for all three.

Roccal has been in use as a bactericide for many years (Fish, 1947). Putz (1964) reported its possible value in prolonged, indefinite treatments at 0.25 to 0.50 p.p.m. for Ichthyophthirius. In treatments such as this, the chemical attacks the free-living stages of "Ich". He did not say which formulation of the chemical he used, but 10-percent active is the formulation commonly used in hatcheries (Davis, 1956). We used 50-percent active, and upon converting from 10-percent active to 50-percent active, the treatment levels could be reduced to 0.05 and 0.10 p.p.m. This permits a comparison between treatment levels and toxicity which shows a 10-fold difference in concentrations.

P.M.A. has been of considerable value in combating bacterial and protozoan diseases (Davis, 1956). Evidence of its greater toxicity to rainbow trout than other trouts has been reported over the years (Foster and Olson, 1951; Rodgers et al., 1951; Wolf, 1951; Hammer, 1960). Allison (1957) reported variations in the toxicity of P.M.A. from lot to lot of chemical. We used only one lot of P.M.A. in this study, and the results support the earlier findings that it has greater toxicity to rainbow trout. For example, it was up to three times as toxic to rainbow trout as to brook trout. Channel catfish appear to be sensitive to the compound at 17° C.

Snieszko and Friddle (1948) used merthiolate (sulfo) as a disinfectant for rainbow trout eggs. Van Duijn (1956) cautioned against use of merthiolate as a fish bath since the compound is a mercurial and is certain to be toxic to fish in contact with it for some period. We find an extreme variation in its toxicity to different species. This is especially true at 24 hours where LC_{50} values range from 7.5 p.p.m. for channel catfish to 110.0 p.p.m. for brown trout. This variation diminishes somewhat at 48 hours, and lake trout become the most sensitive to the chemical. The LC_{50} at 48 hours for lake trout is 2.1 p.p.m. in contrast with 74.5 p.p.m. for brown trout. Variations in resistance such as this may make merthiolate extremely difficult to work with in routine treatments of several species.

Acriflavine, amopyroquin dihydrochloride, methylene blue, Neguvon, Ruelene, and TV-1096 are under consideration as prolonged, indefinite treatments for control of Ichthyophthirius. Putz (1964) reported that 3 p.p.m. of acriflavine shows promise against the parasite. Our results indicate that bluegills are the most sensitive to the compound with LC_{50} values of 18.0 and 13.5 p.p.m. at 24 and 48 hours respectively. Channel catfish are the most resistant with LC_{50} values of 43.5 and 33.2 p.p.m. at 24 and 48 hours.

Clemens and Sneed (1958a) reported the LC_{50} values of acriflavine on channel catfish at 24 and 48 hours to be 11.5 and 6.8 p.p.m., respectively, at 20° C. Our finding, is that it is only about one-fourth as toxic as that. Possible causes for the discrepancy are many. Among them are differences in water quality and temperature, differences in the condition of fish, and purity of the compound used. In addition to this unexplained variation in the toxicity of acriflavine, another factor warrants serious consideration in its use. Van Duijn (1956) reported sterility in both egg-laying and live-bearing aquarium fish. This is a temporary situation and normal fertility is restored after several months.

Amopyroquin dihydrochloride also shows promise as a prolonged, indefinite treatment at 0.05 to 0.10 p.p.m. for control of Ichthyophthirius (Putz, 1964). Our results show that its toxicity to all trout tested, with the exception of lake trout, is between 35 and 40 p.p.m. for 48 hours. Also, bluegills at 12° C. and channel catfish at 17° are approximately as sensitive as lake trout. A treatment level of 0.1 p.p.m. would include a safety margin in use of more than a hundredfold even against these three species.

Van Duijn (1956) recommended methylene blue as a satisfactory control for Ichthyophthirius in aquariums. He used 2 to 4 p.p.m.

of it in a permanent bath at temperatures between 21 and 27° C. We found that rainbow trout are the most sensitive to the dye, and the LC_{50} is 16 p.p.m. at 48 hours. The most resistant species is channel catfish with an LC_{50} of 104 p.p.m. at 48 hours. The remaining species are intermediate in sensitivity with a 48-hour LC_{50} range of 22.9 to 34.0 p.p.m. Comparison of the use levels with toxicity levels indicates a good safety margin.

Neguvon and Ruelene are of approximately the same toxicity except in one very important respect. Neguvon has a marked difference between the 24-hour and 48-hour LC_{50}. The most striking example of this involves lake trout with LC_{50} values of 41 p.p.m. at 24 hours and 9 p.p.m. at 48 hours. Differences between the 24- and 48-hour LC_{50} values by a factor of at least two are common except with bluegills. For some unknown reason the difference with bluegills is only 78 to 71 p.p.m.

Ruelene provides a contrast with Neguvon because it exhibits approximately the same toxicity at 24 and 48 hours. In the case of lake trout, the 24- and 48-hour LC_{50} values are identical at 27 p.p.m. It is possible that Ruelene degrades very rapidly in the test vessel to a nontoxic level.

TV-1096 has toxicity comparable to that of Neguvon and Ruelene. Like Neguvon, it does not appear to degrade as rapidly as Ruelene.

Nickel sulfate is under consideration as a prolonged, indefinite treatment for control of Ichthyophthirius. Our results show that it is relatively low in toxicity when compared with the other compounds tested, but it has a fairly wide range of toxicity among the species tested. The LC_{50} values at 48 hours range from 75 p.p.m. for lake trout to 495 p.p.m. for bluegills. Twenty-four-hour tests of 50 to 275 p.p.m. on brook trout and 200 to 500 p.p.m. on bluegills did not cause death.

Allison (1957) reported use of formalin as a parasiticide in long-term treatments in ponds. He suggested 5 p.p.m. for Gyrodactylus and 15 p.p.m. for Trichodina and Ichthyophthirius. Our results show that formalin is relatively and uniformly low in toxicity when compared with the other compounds tested. It does appear to increase in toxicity as temperatures rise from 17° to 25° C. Even with this increase in toxicity, the compound retains a safety margin of at least sixfold at recommended use levels.

Van Duijn (1956) recommended use of quinacrine hydrochloride in treatment of stubborn cases of "Ich" in aquarium fish. The treatment consists of three applications of 1 p.p.m. at 48-hour intervals. This totals 3 p.p.m. if no degradation of the compound occurs. He also stated that this treatment should not be extended over long periods and that 8 to 10 days should be sufficient.

Our results show that lake trout are approximately 10 times as sensitive to quinacrine hydrochloride as the other trout and 3 or 4 times as sensitive as bluegills and channel catfish. The sensitivity is complicated by the fact that the toxicity to lake trout is quite erratic and some deaths occur over a wide range of concentrations. The 48-hour LC_0 of quinacrine hydrochloride for lake trout is approximately 10 p.p.m., the LC_{50} is 21 p.p.m., and the LC_{100} is 110 p.p.m. Some fish succumb to the chemical quickly and at comparatively low concentrations whereas the rest survive for long periods. Further evidence of this lingering is shown by the slight difference between the 24-hour LC_{50} of 28 p.p.m. and the 48-hour LC_{50} of 21 p.p.m. In contrast, there is a considerable difference between the 24- and 48-hour LC_{50} values obtained for the other species. A possible explanation is that there is considerable variation in resistance among lake trout individuals.

Van Duijn (1956) recommended use of quinine hydrochloride for treatment of Ichthyophthirius in aquarium fish. The treatment consists in adding 1 p.p.m. on 3 successive days, a final treatment level of 3 p.p.m. He cautioned against use of the treatment for long periods because of possible fertility problems. Our results show that 100 p.p.m. of the chemical in water are not toxic within 48 hours to the species tested.

Erythromycin thiocyanate has been used as a food additive for control of kidney disease in rainbow trout at 4.5 grams per 100 pounds of

fish per day for 21 days (Piper, 1961). Warren (1963) reported that it is toxic to rainbow trout at 500 mg. per kg. Our results show that 100 p.p.m. of the antibiotic in water is not toxic to the species tested within 48 hours.

Flagyl has been used in medicine as an antiprotozoal agent (Cutting, 1962). Putz (1964) reported experimental use of it at 1.5 p.p.m. for control of Ichthyophthirius. Our results show that Flagyl is nontoxic at 100 p.p.m. The finding is qualified somewhat since the compound is not immediately soluble at 100 p.p.m. It dissolves slowly, however, and is completely in solution within 48 hours.

Snieszko and Bullock (1957) reported use of sulfamerazine, sulfamethazine, and sulfisoxazole as food additives in the treatment of furunculosis at 8 to 10 grams per 100 pounds of fish per day for 10 to 20 days. Van Duijn (1956) recommended use of the sodium salt of sulfamerazine at 1 part per thousand as an effective cure for worm cataract in aquarium fish. In our water, sulfamerazine, sulfamethazine, and sulfisoxazole are not soluble at 100 p.p.m., and saturated solutions are not toxic to the six species within 48 hours.

All of our results were obtained with fish which were, to the best of our knowledge, healthy. They showed no signs of disease or physical injuries. The toxicity of these compounds to fish which are sick or in poor condition might be significantly different.

None of the compounds reported herein are cleared by the Food and Drug Administration and the Department of Agriculture for use on fish destined for human consumption. The data and discussion presented in this paper should not be construed as recommendations for use.

CONCLUSIONS AND SUMMARY

The toxicities of 22 therapeutic compounds to rainbow trout, brown trout, brook trout, lake trout, and bluegills at 12°C. and channel catfish at 17°were determined in 24- and 48-hour static bioassays.

LC_{50} values for malachite green, the most toxic compound tested, range from 0.1 to 0.4 p.p.m. for all species tested. CoRal, P.M.A., Roccal, Tiguvon, and Trolene are less toxic than malachite green, but still rank relatively high in toxicity. Their LC_{50} values range from approximately 0.5 to 10 p.p.m. for all species.

Acriflavine, amopyroquin dihydrochloride, merthiolate, methylene blue, Neguvon, Ruelene, and TV-1096 are intermediate in toxicity. The LC_{50} values range from approximately 10 to 100 p.p.m. for all species. Merthiolate has wide variations in toxicity to various species.

Formalin, nickel sulfate, and quinacrine hydrochloride have relatively low toxicities. The LC_{50} values are usually above 100 p.p.m. Quinacrine hydrochloride is substantially more toxic to lake trout than to the other species.

No tests of erythromycin thiocyanate and quinine hydrochloride were made at concentrations above 100 p.p.m. They are not toxic within 48 hours at this concentration. Saturated solutions of Flagyl, sulfamerazine, sulfamethazine, and sulfisoxazole are also not toxic within 48 hours.

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19. Toxicity of Bayer 73 to Fish

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CONTENTS

TOXICITY OF BAYER 73 TO FISH

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ABSTRACT.--Bayer 73, a molluscicide sold commercially as Bayluscide, is highly toxic to 18 species of freshwater fish. Various temperatures and water qualities in static bioassays do not influence the toxicity greatly, but pH variations in chemically buffered solutions do. The biodegradability, efficacy, and relative safety of Bayer 73 in aquatic situations indicate possible usefulness as a general fish toxicant.

Nitrosalicylanilides display intense biological activity against a broad spectrum of organisms. Taborsky et al. (1959) and Taborsky and Starkey (1962 and 1963) reported antimicrobial activity of salicylanilides. They found various levels of activity of nitro- and halo-substituted salicylanilides against fungi and bacteria, and concluded that certain positions on the salicylanilide molecule are more active than others.

Taborsky and Starkey (1962) also reported antitumoral activity of substituted salicylanilides. Molnar and Baron (1964) and Vinson et al. (1961) stated that salicylanilides possess germicidal properties and are efficacious as hospital sanitizers.

The molluscicidal activity of Bayer 73, 2', 5-dichloro-4'-nitrosalicylanilide, was first recognized by Gönnert and Schraufstätter (1958). Since then it has been tested thoroughly in the laboratory and field against snails which are intermediate hosts for schistosomiasis (Gönnert, 1961; Schraufstätter et al., 1961; Schraufstätter, 1962; Strufe and Gönnert, 1962; Foster, 1962; and Webbe, 1963). In addition, Gillet and Braux (1962) determined in laboratory tests with snails that Bayer 73 is ovicidal and cercaricidal, which enhances its effectiveness in breaking the cycle of schistosomes. Meyling et al. (1962) found that it killed 100 percent of the snail eggs at 1.0 p.p.m. in 4 to 5 hours.

Bayer 73 is relatively nontoxic to mammals. Hecht and Gloxhuber (1962) reported that dogs tolerated 0.1 g./kg. orally. Duhm (1963) indicated that humans and animals are not harmed by drinking water containing molluscicidal concentrations of Bayer 73. Foster (1962) and Holz and Hwa (1963) reported survival of plants exposed to molluscicidal concentrations. Shiff and Garnett (1961) reported reduced planktonic life immediately after application of 1.0 p.p.m. of Bayer 73, but the effect was short lived, and 32 days later populations were back to normal.

Gönnert (1962) and Webbe (1963) reported fish kills with Bayer 73 at a concentration of 0.3 p.p.m. Howell et al. (1964) found that Bayer 73 is highly toxic to sea lamprey larvae and rainbow trout. They further discovered that Bayer 73 synergized 3-trifluoromethyl-4-nitrophenol, and potentiated the compound as a selective lamprey larvicide.

In research for fish control chemicals, Walker et al. (1966) described the structure-activity relation of substituted salicylanilides to fish. Starkey and Howell (1966) showed that a number of substituted salicylanilides are more toxic to larval sea lampreys than rainbow trout. Marking et al. (in press) defined the toxicity of 3'-chloro-3-nitrosalicylanilide to larval sea lamprey and many species of

fish in the laboratory under standard test conditions and also in simulated stream environments. The compound is highly toxic to all species but most toxic to larval lamprey and several species of rough fish.

In view of the possible wide use of Bayer 73 in acquatic environments for controlling schistosomiasis and the piscicidal activity previously reported, there is a need to define its toxicity to fish and evaluate its potential in controlling fish populations. Accordingly, Bayer 73 was bioassayed at the Fish Control Laboratories at La Crosse, Wis., and Warm Springs, Ga., to determine its effects on fish in various laboratory environments.

MATERIALS AND METHODS

The sample (control No. C910) was obtained from Chemagro Corporation, Kansas City, Mo. as a technical formulation containing 99 percent 2-aminoethanol salt of 2', 5-dichloro-4-nitrosalicylanilide. Its structure is illustrated in figure 1. The material is a bright yellow, crystalline powder.

Test fish were obtained from National, State, and private fish hatcheries (table 1) and were acclimated to test conditions according to procedures described by Lennon and Walker (1964).

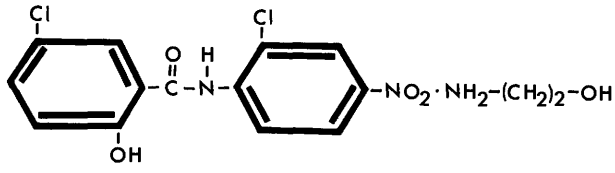

Figure 1.--Structure of 2-aminoethanol salt of 2', 5-dichloro-4'-nitrosalicylanilide.

Preliminary screening was in 15 liters of bioassay medium at 12° C. at La Crosse and at 17° C. at Warm Springs. The fish were exposed to 0.1, 1.0, and 10.0 p.p.m. Bayer 73 to determine the general range of toxicity and the rate of reaction at the higher concentrations.

Delineative tests, including variations in test media such as temperature, water quality, and pH were conducted using 10 fish per concentration and at least 9 concentrations per test, plus a control. Temperature variations were maintained mechanically, and water quality variations were obtained by adding selected quantities of reconsitution salts to deionized water (table 2).

TABLE 1.--Sizes and sources of test fish

Species	Lot	Average length (inches)	Average weight (grams)	Source
Rainbow trout, Salmo gairdneri	279	1.9	1.0	Nevin SFH, Wis.
Brook trout, Salvelinus fontinalis	295	1.8	1.0	Osceola SFH, Wis.
Goldfish, Carassius auratus	W116	1.7	1.3	Tallassee PFH, Ala.
Do	W96A	1.6	1.3	Marion NFH, Ala.
Carp, Cyprinus carpio	W78	1.7	1.1	Marion NFH, Ala.
Do	W86	2.0	1.9	Marion NFH, Ala.
Do	W89	2.3	2.2	Marion NFH, Ala.
Do	367	1.5	0.8	Genoa NFH, Wis.
Fathead minnow, Pimephales promelas	W113	1.8	1.0	Marion NFH, Ala.
White sucker, Catostomus commersoni	381	2.0	1.2	Lake Mills NFH, Wis.
Bigmouth buffalo, Ictiobus cyprinellus	W79	2.0	1.5	Marion NFH, Ala.
Black bullhead, Ictalurus melas	393	1.9	1.3	Guttenberg NFH, Iowa
Brown bullhead, Ictalurus nebulosus	W148	1.8	1.1	Marion NFH, Ala.
Channel catfish, Ictalurus punctatus	W74A	3.0	2.8	Marion NFH, Ala.
Do	W87	2.8	3.0	Marion NFH, Ala.
Flathead catfish, Pylodictis olivaris	W154	2.2	1.1	Marion NFH, Ala.
Green sunfish, Lepomis cyanellus	W75	1.6	1.4	Marion NFH, Ala.
Bluegill, Lepomis macrochirus	W119	1.6	1.1	Marion NFH, Ala.
Do	340	1.5	0.6	Lake Mills, NFH, Wis.
Redear sunfish, Lepomis microlophus	W91	1.6	0.9	Marion NFH, Ala.
Smallmouth bass, Micropterus dolomieui	W146	1.6	0.7	Mammoth Springs NFH, Ark.
Largemouth bass, Micropterus salmoides	W140	1.6	0.8	Welaka NFH, Fla.
Yellow perch, Perca flavescens	382	1.7	0.6	Lake Mills NFH, Wis.
Tilapia, Tilapia mossambica	W156	1.5	0.8	Marion NFH, Ala.

TABLE 2.--Quality and composition of reconstituted water used at the Fish Control Laboratories

Classification of water	Salt added in mg. per liter				pH range	Concentration in p.p.m. $CaCO_3$ as total	
	$NaHCO_3$	$CaSO_4$	$MgSO_4$	KCl		hardness	alkalinity
Soft	12	7.5	7.5	0.50	6.4-6.8	10- 13	10- 13
Medium[1]	48	30.0	30.0	[1] 2.00	7.2-7.6	40- 48	30- 35
Hard	192	120.0	120.0	8.00	7.6-8.0	160-180	110-120

[1] Standard reconstituted water used in routine bioassays.

Different buffers to control the pH of test media were prepared from reagent grade chemicals. The normal and molar solutions and corresponding dilutions are listed in table 3. The pH's of solutions were checked daily and adjusted as necessary to yield approximately the desired pH level.

The delineative data were analyzed according to the methods of Litchfield and Wilcoxon (1949) to determine LC_{50} values, variation, slope functions, and 95-percent confidence intervals.

TABLE 3.--Volumes of buffer reagents (ml.) necessary to yield desired pH levels in 15 liters of test solution

Reagent and strength	Volume to yield pH of approximately--		
	5	7	10
Sodium hydroxide (NaOH).....1.0 N	10	10	20
Potassium acid phthalate ($KHC_8H_4O_4$).....0.1 M	510	--	--
Potassium phosphate (KH_2PO_4).....1.0 M	--	30	--
Boric acid (H_3BO_3).....1.0 M	--	--	21

RESULTS

PRELIMINARY SCREENING

Initial tests with Bayer 73 indicated a high level of biological activity in fish. In a concentration of 0.1 p.p.m., all rainbow trout succumbed in 15 minutes, flathead catfish died within an hour, and yellow perch died within 24 hours, but not all white suckers, black bullheads and bluegills died within the 96-hour bioassay. Exposure to a 1.0 p.p.m. concentration for 15 minutes caused deaths of all white suckers, flathead catfish, green sunfish, and yellow perch. Carp, black bullheads, and bluegills were more resistant, but died during a 15-minute exposure to a concentration of 10.0 p.p.m.

The response of fish to the chemical is noticeable immediately after exposure to the higher concentrations. They appear irritated, and become excited when exposed to sound or external movements. Their early reactions include rapid, irregular respiration. Swimming is aimless, and some fish skitter on the surface while losing equilibrium. Just before death, respiration and swimming actions gradually slow and become irregular, and finally are spastic. Often the opercula are extended on the dead fish.

DELINEATIVE SCREENING

General toxicity.--Bayer 73 is toxic to the 18 species of fish tested. The LC_{50} values, 95-percent confidence intervals, and mean slope functions for routine tests are given in table 4 for the 24- to 96-hour exposure periods. Exposure beyond 24 hours produces little additional mortality. In fact, the figures indicate no difference in the 24- and 48-hour LC_{50} values because the confidence intervals overlap for all species at the two exposure periods. The LC_{50} values for brook trout, one lot of carp, and brown bullheads remained the same from 24- to 96-hour exposures.

The 95-percent confidence intervals of 24- to 96-hour LC_{50}'s overlap for all species except brown bullhead, redear sunfish, smallmouth bass, largemouth bass and tilapia. Intervals are narrow in most cases, indicating consistent toxic effects with little range between concentrations resulting in survival and death.

TABLE 4.--Toxicity of Bayer 73 to selected species of fish

Species	Temp. C.	LC_{50} (p.p.m.) and 95-percent confidence interval			Mean slope function
		24 hours	48 hours	96 hours	
Rainbow trout	12°	0.052 0.049-0.056	0.052 0.049-0.056	0.050 0.047-0.054	1.133
Brook trout	12°	0.061 0.058-0.065	0.061 0.058-0.065	0.061 0.058-0.065	1.070
Goldfish	17°	0.279 0.243-0.321	0.279 0.243-0.321	0.230 0.201-0.263	1.147
Carp	12°	0.143 0.133-0.154	0.139 0.134-0.145	0.139 0.134-0.145	1.053
Do	17°	0.148 0.142-0.159	0.148 0.142-0.159	0.148 0.142-0.159	1.060
Do	17°	0.245 0.227-0.265	0.235 0.214-0.258	0.225 0.208-0.243	1.106
Fathead minnow	17°	0.106 0.097-0.116	0.103 0.096-0.111	0.102 0.089-0.117	1.106
White sucker	12°	0.084 0.073-0.097	0.081 0.075-0.088	0.081 0.076-0.086	1.084
Bigmouth buffalo	17°	0.080 0.059-0.108	0.064 0.056-0.073	-	1.245
Black bullhead	12°	0.104 0.084-0.123	0.096 0.087-0.106	0.088 0.078-0.098	1.156
Brown bullhead	17°	0.071 0.065-0.077	0.071 0.066-0.077	0.056 0.049-0.064	1.107
Channel catfish	17°	0.084 0.079-0.089	0.084 0.079-0.089	0.082 0.077-0.088	1.063
Flathead catfish	17°	0.043 0.040-0.046	0.043 0.040-0.046	0.043 0.040-0.046	1.060
Green sunfish	17°	0.158 0.145-0.172	0.115 0.101-0.130	0.100 0.094-0.107	1.097
Bluegill	12°	0.105 0.095-0.116	0.098 0.085-0.112	0.094 0.083-0.107	1.131
Do	17°	0.082 0.072-0.092	0.082 0.070-0.096	0.068 0.057-0.080	1.158
Redear sunfish	17°	0.157 0.148-0.167	0.153 0.142-0.165	0.088 0.086-0.090	1.069
Smallmouth bass	17°	0.089 0.085-0.099	0.089 0.085-0.099	0.060 0.048-0.074	1.223
Largemouth bass	17°	0.111 0.099-0.124	0.097 0.087-0.109	0.062 0.050-0.076	1.157
Yellow perch	12°	0.082 0.076-0.088	0.081 0.069-0.087	0.081 0.069-0.087	1.077
Tilapia	17°	0.180 0.159-0.203	0.150 0.133-0.170	0.109 0.090-0.132	1.157

The slope functions were consistent in exposures of 24, 48, and 96 hours, and they were averaged to yield the figures given in table 4. The values, ranging from 1.060 to 1.245, are low, the regression is nearly vertical, and there is little difference between concentration permitting complete survival and that producing complete mortality. In contrast, the slope function of p,p'-DDT for goldfish is 6.02 (Marking, 1966).

Comparative toxicity.--Bayer 73 is highly toxic to all species, with 96-hour LC_{50} values ranging from 0.043 to 0.230 p.p.m. Flathead catfish, rainbow trout, brown bullheads, smallmouth bass, brook trout, and largemouth bass in that order are the most sensitive species to Bayer 73 (fig. 2). Goldfish are the most resistant, followed by carp. Intermediate in sensitivity are white suckers, yellow perch, channel catfish, black bullheads, redear sunfish, bluegills, green sunfish, tilapia, and fathead minnows. Bayer 73 does not appear specific to any undesirable species of fish but rather is generally toxic to game and rough fish.

Short exposures.--Among the six species selected to determine effects of short-term exposures to Bayer 73, rainbow trout were most sensitive and carp most resistant (table 5). Rainbow trout, brook trout, and yellow perch respond rather quickly; LC_{50} values are only slightly greater at 1 hour than at 6 hours. The 95-percent confidence intervals overlap for each species in the 2-, 3-, and 6-hour exposures. Also, the values at 6 hours and 24 hours for rainbow trout and brook trout do not differ significantly (tables 5 and 6).

Carp, white suckers, and black bullheads respond somewhat more slowly at 1 hour, although their resistance is greater initially and the increments in LC_{50} values from 1- to 96-hour exposures are small and fairly uniform.

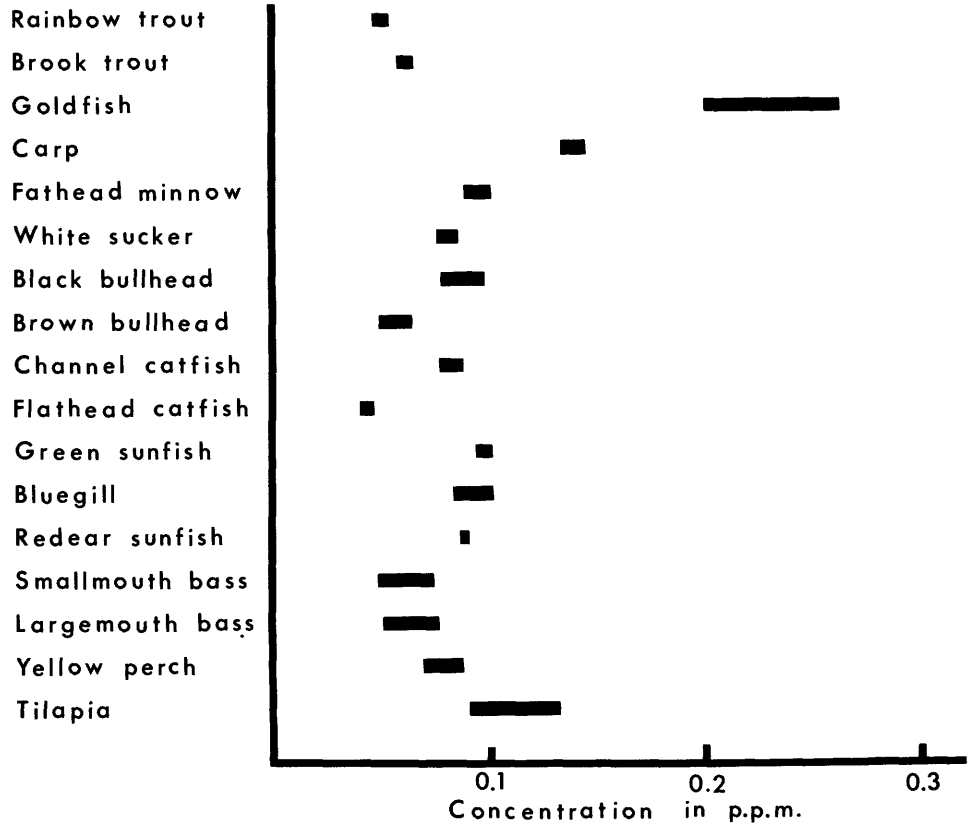

Figure 2.--95-percent confidence intervals for LC_{50} values of Bayer 73 to 17 species of fish. The data was taken from table 4 at 96-hour exposures.

TABLE 5.--Toxicity of Bayer 73 in short exposures at 12° C.

Species	LC_{50} (p.p.m.) and 95-percent confidence interval at--				Mean slope function
	1 hour	2 hours	3 hours	6 hours	
Rainbow trout.....	0.063 0.060-0.066	0.056 0.053-0.059	0.052 0.049-0.056	0.052 0.049-0.056	1.064
Brook trout.....	0.077 0.069-0.086	0.069 0.063-0.076	0.064 0.060-0.068	0.064 0.060-0.068	1.089
Carp.....	0.300 0.267-0.338	0.250 0.232-0.264	0.200 0.188-0.214	0.188 0.173-0.204	1.080
White sucker.....	0.180 0.177-0.183	0.108 0.092-0.127	0.105 0.091-0.122	0.100 0.088-0.114	1.126
Black bullhead....	0.275 0.230-0.329	0.210 0.181-0.243	0.156 0.138-0.177	0.156 0.138-0.177	1.156
Yellow perch.....	0.120 0.103-0.140	0.116 0.103-0.131	0.116 0.101-0.133	0.100 0.089-0.112	1.143

TABLE 6.-- Effects of temperature on toxicity of Bayer 73 to fish

Species	Temp. C.	LC_{50} (p.p.m.) and 95-percent confidence interval at--				
		3 hours	6 hours	24 hours	48 hours	96 hours
Rainbow trout.....	7°	0.059 0.053-0.066	0.050 0.044-0.057	0.048 0.042-0.055	0.048 0.042-0.055	0.047 0.043-0.052
Do.....	12°	0.052 0.049-0.056	0.052 0.049-0.056	0.052 0.049-0.056	0.052 0.049-0.056	0.050 0.047-0.054
Do.....	17°	0.054 0.048-0.059	0.053 0.048-0.058	0.052 0.048-0.057	0.052 0.048-0.057	0.050 0.048-0.054
Goldfish.....	12°	0.340 0.315-0.367	0.270 0.250-0.290	0.228 0.207-0.251	0.228 0.207-0.251	0.228 0.207-0.251
Do.....	17°	0.350 0.311-0.394	0.283 0.258-0.311	0.279 0.243-0.321	0.279 0.243-0.321	0.230 0.201-0.263
Do.....	22°	0.290 0.260-0.320	0.268 0.237-0.302	0.252 0.221-0.287	0.206 0.181-0.235	0.223 0.179-0.279
Channel catfish....	12°	0.065 0.060-0.070	0.056 0.053-0.059	0.052 0.048-0.056	0.050 0.046-0.054	0.049 0.045-0.053
Do.....	17°	-	-	0.084 0.079-0.089	0.084 0.079-0.089	0.082 0.077-0.088
Do.....	22°	0.058 0.055-0.061	0.058 0.055-0.061	0.055 0.052-0.058	0.055 0.052-0.058	0.042 0.035-0.051
Bluegill.....	12°	0.141 0.128-0.155	0.115 0.103-0.129	0.106 0.094-0.120	0.092 0.085-0.099	0.092 0.085-0.099
Do.....	17°	0.120 0.107-0.135	0.096 0.082-0.110	0.082 0.072-0.092	0.082 0.070-0.096	0.068 0.057-0.080
Do.....	22°	0.100 0.093-0.108	0.092 0.080-0.106	0.082 0.065-0.103	0.066 0.058-0.076	0.054 0.046-0.063

The values for all 6 species vary from 0.063 to 0.300 p.p.m. in 1 hour to 0.050 to 0.139 in 96 hours. The figures indicate that fish die rather quickly at concentrations only slightly greater than those required to kill at 96-hour exposures.

The average slope functions for 1-, 2-, 3-, and 6-hour exposures are low and similar to those for longer exposures (table 5). The figures indicate a steep regression where small increases in concentration produce considerable additional mortality.

Effects of temperature.--Bayer 73 was tested with rainbow trout, goldfish, channel catfish, and bluegills at 7°, 12°, and 17° C. in 3-, 6-, 24-, 48-, and 96-hour exposures (table 6). These changes in temperature had no significant effect on the toxicity of Bayer 73 to rainbow trout.

Goldfish, although more resistant than rainbow trout, reacted similarly at 12°, 17°, and 22° C. The difference is not significant at any one temperature, but the data indicate greater toxicity at 22° than at 12° or 17° C. The confidence intervals overlap for the 6-, 24-, 48-, and 96-hour exposures but not for the 3-hour exposure.

The channel catfish tested at 17° were different from the lot tested at 12° and 22° C. The results indicate greater resistance at 17°, but the difference may be attributable to the source and physical condition of the fish. Another lot of channel catfish tested in 3- and 6-hour bioassays indicated little or no difference at 17°. The responses at 12° and 22° obtained from the same lot of catfish show no significant differences.

Bluegills respond to increases in temperature more than the other species (table 6). Bayer 73 is more toxic at 22° than at 12° or 17°. The confidence intervals do not overlap at 12° and 22° at exposures of 3, 48, and 96 hours. The increase in toxicity at longer exposures also is more noticeable with bluegills than with the other three species.

Effects of water quality.--Bayer 73 was tested in soft, medium, and hard water prepared according to table 2. At all exposure periods, the chemical is more toxic to rainbow trout in soft water and less toxic in hard water (table 7). The greater decrease in toxicity is between medium and hard water and suggests degradation of Bayer 73 at higher pH values and higher alkalinities.

Carp respond much the same as rainbow trout, although the confidence intervals overlap at the extreme water quality variations (table 7). The carp tested in water of medium hardness were from a different lot of fish. The data indicate greater sensitivity in water of medium hardness than in soft water, but the difference is not significant.

The toxicity of Bayer 73 to channel catfish decreases significantly in hard water but changes very little from soft to medium hard water. The toxic effects are uniform within each hardness, and do not increase significantly from the 3-hour to the 96-hour exposure (table 7).

Bluegills also are more susceptible to Bayer 73 in softer waters (table 7). The LC_{50} values in hard water are 2 or more times those in medium water. Confidence intervals for medium and hard water do not overlap at any exposure.

Effects of pH.--Variations in pH were accomplished by adding buffering agents to standard test water according to table 3. There were significant differences in the toxicity of Bayer 73 to goldfish at pH 5, 7, and 10 (table 8). The tests at pH 7 conform somewhat to tests in standard bioassays although these goldfish are from a different lot and appear more sensitive to Bayer 73 than the goldfish tested in standard water.

TABLE 7.--Effects of water quality on toxicity of Bayer 73 to fish

Species	Water quality	LC_{50} (p.p.m.) and 95-percent confidence interval at--				
		3 hours	6 hours	24 hours	48 hours	96 hours
Rainbow trout....	soft	0.046 0.043-0.049	0.046 0.043-0.049	0.046 0.043-0.049	0.046 0.043-0.049	0.046 0.043-0.049
Do.....	medium	0.054 0.050-0.059	0.053 0.049-0.055	0.053 0.049-0.055	0.053 0.049-0.055	0.053 0.049-0.055
Do.....	hard	0.082 0.075-0.090	0.082 0.075-0.090	0.082 0.075-0.090	0.082 0.075-0.090	0.070 0.066-0.074
Carp.....	soft	-	-	0.197 0.168-0.230	0.188 0.181-0.196	0.191 0.170-0.214
Do.....	medium	-	-	0.169 0.163-0.178	0.169 0.163-0.178	0.169 0.163-0.178
Do.....	hard	-	0.342 0.309-0.374	0.229 0.210-0.250	0.229 0.207-0.242	0.224 0.207-0.242
Channel catfish..	soft	0.082 0.075-0.090	-	0.070 0.066-0.074	0.070 0.066-0.074	-
Do.....	medium	0.064 0.058-0.071	-	0.062 0.056-0.068	0.062 0.056-0.068	0.060 0.054-0.067
Do.....	hard	0.079 0.091-0.104	-	0.093 0.085-0.101	0.093 0.085-0.101	0.093 0.085-0.101
Bluegill.....	soft	0.092 0.084-0.101	0.083 0.074-0.093	0.083 0.074-0.093	0.073 0.060-0.088	0.064 0.055-0.074
Do.....	medium	0.120 0.106-0.135	0.096 0.082-0.110	0.082 0.072-0.092	0.082 0.070-0.096	0.068 0.057-0.080
Do.....	hard	0.200 0.183-0.219	0.190 0.170-0.212	0.178 0.152-0.209	0.154 0.136-0.175	0.117 0.100-0.137

TABLE 8.--Effects of pH on the toxicity of Bayer 73 to goldfish at 12° C.

pH	LC_{50} (p.p.m.) and 95-percent confidence interval at--			
	3 hours	24 hours	48 hours	96 hours
5...	4.600 3.880-5.460	3.520 3.120-3.980	3.500 3.100-4.000	3.500 3.100-4.000
7...	-	0.180 0.163-0.198	0.180 0.163-0.198	-
10...	-	6.420 5.780-7.130	6.150 5.690-6.640	3.190 2.850-3.580

DISCUSSION

Bayer 73 is toxic to fish in very brief exposures and does not become proportionately more toxic with increased exposure time. In several cases the LC_{50} differed only little between 1 and 96 hours. This suggests rapid degradation of the toxicant either by the water or the fish. Duhm et al. (1963) found that rats fed 50 mg./kg. 5,2'-dichloro-4'-nitro-salicic-anilide excrete most of the material as 5,2'-dichloro-4-aminosalicylic-anilide in a conjugated form. The amino-structures excreted are very unstable and decompose when the excrement is left to stand for several hours. They concluded that the chemical is reduced and detoxified by metabolism in rats. Quite possibly, fish are able to detoxify Bayer 73 by a reduction process similar to that in rats. This may explain to some extent why the comparatively high initial activity of the compound is followed by minimum time-concentration effects.

Strufe and Gönnert (1962) reported that water temperature influences the efficacy of Bayer 73 in destroying snails. They found that 0.3 p.p.m. in 24-hour exposures is sufficient to kill all the snails at 20°, but 0.5 p.p.m. is necessary to yield 100-percent mortality at 5° C. They also indicated that solutions of Bayer 73 exposed to diffused daylight at 20°

to 50° C. for 9 hours show no significant loss of active ingredient. Our data indicate greater biological activity at higher temperatures, and the results probably would be more significant at extreme temperatures.

Gönnert (1962) reported that environmental factors such as salt content and pH of the water do not decisively influence activity of Bayer 73 against snails during the required exposure time. Strufe and Gönnert (1962) stated that the biological action of Bayer 73 against snails is not influenced to any measurable degree by salty waters containing up to 500 p.p.m. of calcium and magnesium. The laboratory tests with fish indicate less activity of the compound in harder water but, as other scientists report on snails, the difference is not considerable.

The pH variations in different water qualities which ranged from 6.4 to 8.0, did not influence toxicity drastically; the buffered pH 5 and 10 solutions did. Data from the latter test indicate less toxicity at low and high pH values and little difference at pH 7. The probable cause of decreased toxicity at extreme pH's may be a solubility or ionization factor in which less active chemical is available to the fish. Recently, Meyling and Pitchford (1966) found that as the pH of Bayer 73 solutions increase from 6.0 to 8.0 the solubility increases from less than 1.0 to 5.0 p.p.m. They did not define the solubility at pH 10. Strufe and Gönnert (1962) concluded from irradiation tests that up to 15 percent of the molluscicide is inactivated in neutral and weakly alkaline conditions, and up to 30 percent in the acid range of pH 5 to 6. Our tests at the extreme pH values of 5 and 10 confirm their findings.

Fox et al. (1963) reported that at pH 9.7 to 9.9 about 4 times as much Bayer 73 is required to kill snails as at pH 7.0 to 9.2. They suggest that snails require continuous stimulation by chlorine in order to bring into play their detoxifying mechanisms.

Meyling et al. (1962) reported that concentrations of Bayer 73 in hard water exposed to sunlight were reduced from 1.0 to 0.57 p.p.m. in 16 hours whereas in darkness the concentrations did not change after 312 hours. Apparently sunlight, hard water, high pH, and alkalinity contribute to the reduction of Bayer 73.

Bayer 73 is highly toxic to certain fish species resistant to other chemicals. Catfishes, for example, are readily susceptible to it but are resistant to such other toxicants as antimycin A (Walker et al., 1964) and rotenone (Henegar, 1966).

Gönnert (1961) summarizes the results of laboratory and field trials with Bayer 73 in a number of countries. Minimum lethal concentrations for snails and snail ova were found to be between 0.2 and 0.5 p.p.m. Applications of 1.0 p.p.m. of Bayer 73 destroyed snails in standing and flowing water. He also indicated that the toxicity of Bayer 73 to fish is very similar to that for snails.

Our tests in the laboratory show that Bayer 73 produces 50-percent mortality in 18 species of fish at concentrations of 0.043 to 0.230 p.p.m. in standard 96-hour bioassays. More than 1.0 p.p.m. was required to kill fish only when the bioassays were chemically buffered to pH 5 and 10. Thus, in the laboratory, molluscicidal concentrations of Bayer 73 are toxic to all species of fish tested. Applications of 1.0 p.p.m. are probably toxic to fish in natural environments provided that extreme pH values of 5 or 10 do not prevail.

CONCLUSIONS

Bayer 73, a molluscicide, is highly toxic to 18 species of fish in static bioassays. Concentrations lethal to snails in the laboratory are also toxic to fish.

Bayer 73 kills 50 percent of the rainbow trout, brook trout, brown bullheads, flathead catfish, smallmouth bass, and largemouth bass at 0.062 p.p.m. or less in 96-hour exposures. Fathead minnows, white suckers, black bullheads, channel catfish, green sunfish, bluegills, redear sunfish, yellow perch, and tilapia are intermediate in sensitivity, and LC_{50} values range from 0.81 to 0.109 p.p.m. in 96 hours. Goldfish and carp are more resistant species, with LC_{50} values of 0.230 and 0.148 p.p.m.

The toxicity of Bayer 73 to bluegills increased with temperature, but the toxicity to trout, goldfish, and catfish was not significantly affected. Various water qualities also do not influence the toxicity greatly. Extreme pH changes significantly reduce the toxicity of Bayer 73 to fish. This effect may be due to decreased ionization at extreme pH values or to a metabolic reduction of the compound through stimulation of the detoxifying system in fish.

Because of its biodegradability and safety in water, Bayer 73 deserves consideration as a general fish toxicant.

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20. Toxicity of Dimethyl Sulfoxide (DMSO) to Fish

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CONTENTS

TOXICITY OF DIMETHYL SULFOXIDE (DMSO) TO FISH

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ABSTRACT.--Toxicities of dimethyl sulfoxide (DMSO) to rainbow trout, brook trout, lake trout, carp, black bullhead, channel catfish, green sunfish, bluegill, and yellow perch were determined in 24-, 48-, and 96-hour static bioassays at 12° C. Toxicity was of low order, around 30 p.p.t. Water quality had little effect, but increased temperature increased the toxicity to rainbow trout. A preliminary test indicated that DMSO has little effect on the toxicity of antimycin to bluegill.

Dimethyl sulfoxide (DMSO) is the simplest of the homologous series of organic sulfoxides; it is prepared by oxidation of dimethyl sulfide. Its formula is $(CH_3)_2SO$, and the pure form is a hygroscopic, colorless, odorless liquid, melting at 18.45° and boiling at 189° C., with a specific gravity of 1.100 (Stecher, 1960).

DMSO was first synthesized in 1867 but remained a laboratory curiosity until the 1940's when it was successfully used as a solvent for the spinning of polyacrylonitrile fibers (Block, 1964). The first physiochemical data on the compound appeared in 1948, and since that time DMSO has been used extensively in several fields.

DMSO has been shown to possess remarkable potential as a solvent for many types of inorganic and organic compounds including gases (Willson et al., 1965). It has also been used as a preservative during freeze storage of red blood cells (Lovelock and Bishop, 1959), platelets (Geisler et al., 1964), spermatozoa (Sherman, 1964), mitochondria (Greiff, 1961), protozoa (Hwang, 1964), bone marrow (Ashwood-Smith, 1961a; Persidsky and Richards, 1963), cardiac muscle (Levy et al., 1962), and tissue culture cells (Dougherty, 1962; Porterfield and Ashwood-Smith, 1962). DMSO also exhibited radioprotective action against lethal doses of X-irradiation in mice (Ashwood-Smith, 1961b).

In the medical field, the compound is under investigation as a penetrant carrier, local analgesic, anti-inflammatory adjunct, bacteriostatic agent, diuretic, tranquilizer, and potentiator (Jacob et al., 1964a). It is even reported to be good for headaches (Jacob, 1965). The most promising of these areas appears to be the ability of DMSO to penetrate biological membranes and act as a carrier for other drugs (Horita and Weber, 1964; Jacob et al., 1964b; Stoughton and Fritsch, 1964; Stoughton, 1965).

In the agricultural field, DMSO has been shown to be of value as a solvent carrier for certain compounds used in the control of plant diseases (Bean, 1965; Keil et al., 1965). It also has some herbicidal activity when used by itself for the control of purple nutsedge (Anderson and Dunford, 1966).

Additional information and references on DMSO can be obtained from articles written by Rosenkrantz et al. (1963), and Kligman (1965a and 1965b).

These interesting properties of DMSO plus the fact that it is miscible in all proportions with water suggested that it might be useful

in fisheries as a nontoxic solvent in toxicity studies and for the administration of non-water-soluble drugs to fish. In addition, if DMSO enhances the absorption by fish of compounds dissolved in it, a major breakthrough in the fields of fish control and fish disease control would result. It is for these purposes that the following toxicity studies were undertaken.

MATERIALS AND METHODS

Samples of DMSO were obtained from Ayerst Laboratories, New York, N.Y. The formulation was 90 percent DMSO and 10 percent water.

The fish were obtained from several fish hatcheries (table 1), and were introduced to the static bioassays after routine acclimation as described by Lennon and Walker (1964).

Preliminary bioassays were conducted in 1-gallon glass jars each containing 3 liters of bioassay media and two fish. After the approximate level of toxicity had been established, delineative bioassays were conducted in 5-gallon glass jars each containing 15 liters of reconstituted deionized water and 10 fish. The proper volume of bioassay media was maintained by removal of a quantity of water equal to the aliquot of DMSO which was to be added. Each test included 5 to 9 concentrations of chemical and 50 to 90 test fish plus 10 fish for controls.

Various water qualities were obtained by adding selected concentrations of reconstituting salts to deionized water (table 2). Tests were maintained at 7°, 12°, or 17° C. by water baths.

Survival and mortality were recorded at 24, 48, and 96 hours. The data were analyzed by plotting concentration versus mortality on logarithmic normal (probability) graph paper to define the concentration which produced 50-percent mortality (LC_{50}), slope function, variation, and 95-percent confidence intervals (C.I.) as described by Litchfield and Wilcoxon (1949).

All results are reported in parts per thousand (p.p.t.), by volume, of total material added to the test vessel instead of active ingredient.

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

Species	Average length (inches)	Average weight (grams)	Source[1]
Rainbow trout, Salmo gairdneri	1.8	0.9	NFH, Manchester, Iowa.
Do.	1.8	1.0	Rainbow Ranches, Spokane, Wash.
Brook trout, Salvelinus fontinales	1.5	0.5	SFH, Osceola, Wis.
Lake trout, Salvelinus namaycush	1.9	0.7	SFH, St. Croix Falls, Wis.
Carp, Cyprinus carpio	1.6	1.0	NFH, Lake Mills, Wis.
Black bullhead, Ictalurus melas	2.0	1.7	NFH, New London, Minn.
Channel catfish, Ictalurus punctatus	2.0	1.1	NFH, Fairport, Iowa.
Green sunfish, Lepomis cyanellus	1.5	0.8	NFH, Lake Mills, Wis.
Bluegill, Lepomis macrochirus	1.3	0.5	Do.
Yellow perch, Perca flavascens	2.6	1.7	Do.

[1] NFH = National Fish Hatchery; SFH = State Fish Hatchery.

TABLE 2.--Composition and analysis of reconstituted, deionized water used in bioassays

Classification of water	Amount of salts added (mg./l.)				pH range	Range of total hardness as p.p.m. $CaCO_3$	Range of total alkalinity as p.p.m. $CaCO_3$
	$NaHCO_3$	$CaSO_4$	$MgSO_4$	KCL			
Soft	12.0	7.5	7.5	0.5	6.4 - 6.8	10-13	10-13
Medium[1]	48.0	30.0	30.0	2.0	7.2 - 7.6	40-48	30-35
Hard	192.0	120.0	120.0	8.0	7.6 - 8.0	160-180	110-120

[1] Standard reconstituted water used in routine bioassays.

RESULTS AND DISCUSSION

PRELIMINARY TESTING

Preliminary tests to determine the approximate level of DMSO toxicity were carried out with yellow perch as the test species. In order to preserve the supply of test material it was necessary to prepare a 3-liter solution containing 500 p.p.t. of DMSO. Subsequent concentrations were obtained by dilution of this solution.

Results in terms of the approximate time to death at each concentration were as follows:

500 p.p.t. - 10 minutes
250 p.p.t. - 30 minutes
125 p.p.t. - 1.5 hours
62 p.p.t. - 24 hours
31 p.p.t. - no mortality within 96 hours

From these data, the 24-, 48-, and 96-hour LC_{50} values appear to lie between 30 and 60 p.p.t., and concentrations were selected accordingly for the routine toxicity bioassays.

GENERAL TOXICITY

DMSO exhibited a consistent and nonselective toxicity of very low order to the nine species tested (table 3).

Probit analysis yielded slope functions on logarithmic paper which ranged from 1.06 to 1.20 with a mean of 1.11 on all tests. This indicates that as the level of acute toxicity is approached a minimal change in concentration is required to produce either 0- or 100-percent mortality.

The comparative resistance of the nine species was extremely close. The 96-hour LC_{50}'s ranged from only 32.3 to 43.0 p.p.t. for the various species.

The order of susceptibility of the nine species varied with the observation period. At 24 and 48 hours, for example, channel catfish were the most susceptible, with bluegill the most resistant at 24 hours and yellow perch the most resistant at 48 hours. By 96 hours, rainbow trout became the most susceptible and green sunfish the most resistant. This fluctuation in the comparative order of sensitivity further exemplifies the extremely nonselective toxicity to all fish tested.

Recent studies at the Western Fish Nutrition Laboratory with yearling coho salmon (Oncorhynchus kisutch) determined the median tolerance limit (TLm) to be 72, 55, and 46 p.p.t. at 24. 48, and 96 hours respectively.[1] Ball (1966) reported that the 48-hour LC_{50} of DMSO to goldfish (Carassius auratus) is 43 p.p.t. at 15° C. These results are in close agreement with the results at this laboratory and serve to further substantiate the consistent and nonselective toxicity of DMSO.

[1] Personal communication from Pete Benville, Jr., Chemist, Western Fish Nutrition Laboratory, Bureau of Sport Fisheries and Wildlife, Cook, Wash., 1966.

TABLE 3.--Toxicity of 90-percent DMSO to nine species of fish at 12° C.

Species	At 24 hours		At 48 hours		At 96 hours	
	LC_{50} (p.p.t.)	95-percent C.I.	LC_{50} (p.p.t.)	95-percent C.I.	LC_{50} (p.p.t.)	95-percent C.I.
Rainbow trout	53.0	48.6 - 57.8	41.7	39.3 - 44.2	32.3	30.2 - 34.6
Brook trout	54.5	50.9 - 58.3	46.0	42.2 - 50.1	36.5	33.2 - 40.2
Lake trout	47.8	42.3 - 54.0	38.2	35.4 - 41.3	37.3	35.2 - 39.5
Carp	44.0	39.3 - 49.3	44.0	39.3 - 49.3	41.7	36.3 - 48.0
Black bullhead	42.5	37.9 - 47.6	39.2	35.3 - 43.5	36.5	33.8 - 39.4
Channel catfish	39.0	36.1 - 42.1	34.5	31.7 - 37.6	32.5	29.8 - 35.4
Green sunfish	65.0	61.3 - 68.9	52.5	47.7 - 57.8	43.0	35.8 - 51.6
Bluegill	72.0	63.2 - 82.1	56.0	51.9 - 60.5	33.5	29.9 - 37.5
Yellow perch	65.0	61.3 - 68.9	57.0	52.3 - 62.1	37.0	33.9 - 40.3

Rabinowitz and Myerson (1966) stated that a concentration of 19 p.p.t. of DMSO produced an approximate 48-hour LD_{50} with neon tetras (Paracheirodon innesi), platys (Xiphosphorus maculatus), mollies (Pescilia latipinna), and guppies (Poecilia reticulata). The LD_{50}'s for zebras (Brachydanio rerio) and catfish (Corydoras paleatus) were somewhere in excess of 25 p.p.t. These results were obtained in distilled water at 24° to 25° C. and the increased toxicity indicated may be the result of osmotic stress induced by the test media.

EFFECT OF WATER QUALITY AND TEMPERATURE ON TOXICITY

Changes in water quality at 12° C. had little or no effect upon the toxicity of DMSO (table 4). The LC_{50} confidence interval in any particular water quality overlaps the LC_{50}'s in other water qualities within the same observation period in all cases except one. This exception was in hard water at 96 hours. In general, it appears that DMSO is slightly less toxic in hard water than it is in waters of soft or medium hardness.

Changes in temperature at medium hardness exhibited a substantial influence on toxicity (table 4). An increase in toxicity in excess of 10 p.p.t. as the temperature increases from 7° to 17° C. was observed at all observation periods.

This increase in toxicity at warmer temperature is in agreement with observations made at the Western Fish Nutrition Laboratory.

EFFECT OF DMSO ON TOXICITY OF ANTIMYCIN

A preliminary test was performed to determine what, if any, influence DMSO has on the toxicity of antimycin to bluegill. Various concentrations of antimycin were added in combination with enough DMSO to produce 1.0 p.p.t. of DMSO in the test vessel. A comparison test was run using only acetone as solvent for the antimycin.

The 96-hour LC_{50} of antimycin and acetone alone was 0.089 parts per billion (p.p.b.), while antimycin in combination with 1.0 p.p.t. of DMSO produced a 96-hour LC_{50} of 0.084 p.p.b. These results reflect biological variation and indicate that DMSO has no effect on the toxicity of antimycin at 96 hours. It is possible that in a bioassay designed to yield toxicity with shorter exposures, DMSO could enhance the absorption of antimycin sufficiently to affect toxicity.

Ball (1966) compared the relative toxicity of 0.05 p.p.m. p,p'-DDT to goldfish when used in combination with 6 and 18 p.p.t. of either DMSO or acetone. His results indicated that DMSO does not significantly affect the median survival time of goldfish when compared to acetone. He further suggested that DMSO may be a better solvent than acetone for pesticide toxicity studies.

Rabinowitz and Myerson (1966) were unable to show a significant difference in the uptake by aquarium fish of radioactive labeled dyes when used in combination with 1.0 p.p.t. of DMSO.

TABLE 4.--Effect of water quality and temperature on toxicity of DMSO (90-percent) to rainbow trout

Temperature C.	Water quality	At 24 hours		At 48 hours		At 96 hours	
		LC_{50} (p.p.t.)	95-percent C.I.	LC_{50} (p.p.t.)	95-percent C.I.	LC_{50} (p.p.t.)	95-percent C.I.
7°.....	medium	65.5	57.0-75.3	46.0	41.8-50.6	41.5	37.7-45.6
12°.....	medium	53.0	48.6-57.8	41.7	39.3-44.2	32.3	30.2-34.6
17°.....	medium	41.5	37.7-45.6	35.0	31.8-38.5	27.7	25.0-30.7
12°.....	soft	53.5	49.1-58.3	42.3	38.4-46.5	33.5	30.7-36.5
12°.....	hard	57.0	51.8-62.7	44.8	41.5-48.4	38.0	35.8-40.3

All of these results indicate that DMSO, when used as a diluted constituent, does not affect the absorption of some chemicals by fish. It has been shown to be an excellent solvent, and is worthy of further investigation as such for certain chemicals used in fisheries. In addition, the potential of DMSO as a penetrant carrier of certain drugs used in human medicine suggests investigation of similar potential in the treatment of fish disease.

CONCLUSIONS

1. The acute toxicity of DMSO to fish is of a very low order.

2. When the level of acute toxicity is reached, DMSO is abruptly and nonselectively toxic to the nine species tested.

3. Various water qualities at 12° C. have little effect upon the toxicity of DMSO to rainbow trout.

4. Increases in temperature cause a definite increase in the toxicity of DMSO to rainbow trout.

5. Preliminary results indicate that 1.0 p.p.t. of DMSO has no effect on the toxicity of antimycin to bluegill at 96 hours.

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21. Labor-saving Devices for Bioassay Laboratories

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Fish and Wildlife Service, Clarence F. Pautzke, *Commissioner*
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CONTENTS

LABOR-SAVING DEVICES FOR BIOASSAY LABORATORIES

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ABSTRACT.--Three inexpensive pieces of labor-saving apparatus for bioassay laboratory use are described and illustrated. Construction features, material costs, and use of a jar rinser, automatic liquid measuring vessel, and jar emptier are discussed.

Nearly all of the indoor bioassays at the Fish Control Laboratories require the use of 5-gallon glass jars. Considerable work is involved in handling the scores of jars used daily, and much thought and effort have been given to developing methods to reduce the labor.

In recent months three labor-saving devices were developed and put into use: a rack for simultaneously rinsing 26 jars, a vessel for automatically measuring 15 liters of water into each jar, and a simple apparatus for emptying jars. Hundreds of man-hours of work are saved, and the hazards in handling heavy, slippery jars are reduced. The devices are not expensive and may be adapted to meet needs which differ from our own.

JAR RINSING RACK

DESCRIPTION

The essential features of the rack are shown in figure 1. It is about 15 feet 6 inches long, 28 inches wide, and 18 inches high, but dimensions may be varied according to needs. Materials are not of critical importance either, unless highly corrosive wastes will be encountered. In our case, availability of materials and the ease with which they could be worked were the chief factors considered. Two-inch lumber and large nails are used to construct load-bearing structures (sides, ends, and cross bracing), while the pipe supports and grate-retaining rim are of 1-inch material. All wooden parts were given two coats of high-grade enamel. The sections of aluminum grating which hold the jars were part of the covering for the floor trench over which the jar-rinsing rack is supported and into which waste water drains. Wooden crosspieces could be substituted for the metal gratings at considerable savings in material costs. Plumbing components include 3/4-inch plastic pipe and fittings (1/2-inch pipe would be adequate for a small rack), 3/4-inch valves (one for each pipe), 1/4-inch brass spray nozzles, and 1/4- by 1 1/8-inch all-thread nipples for attaching nozzles to the pipe. Total cost of materials exclusive of the gratings was $54.20.

USE

After the jars are emptied and removed from bioassay troughs, they are cleaned according to a procedure like that outlined by Lennon and Walker (1964). The rack not only provides jar support at a convenient working height during washing, but also cuts the time for washing and rinsing to less than half that required when jars were cleaned individually.

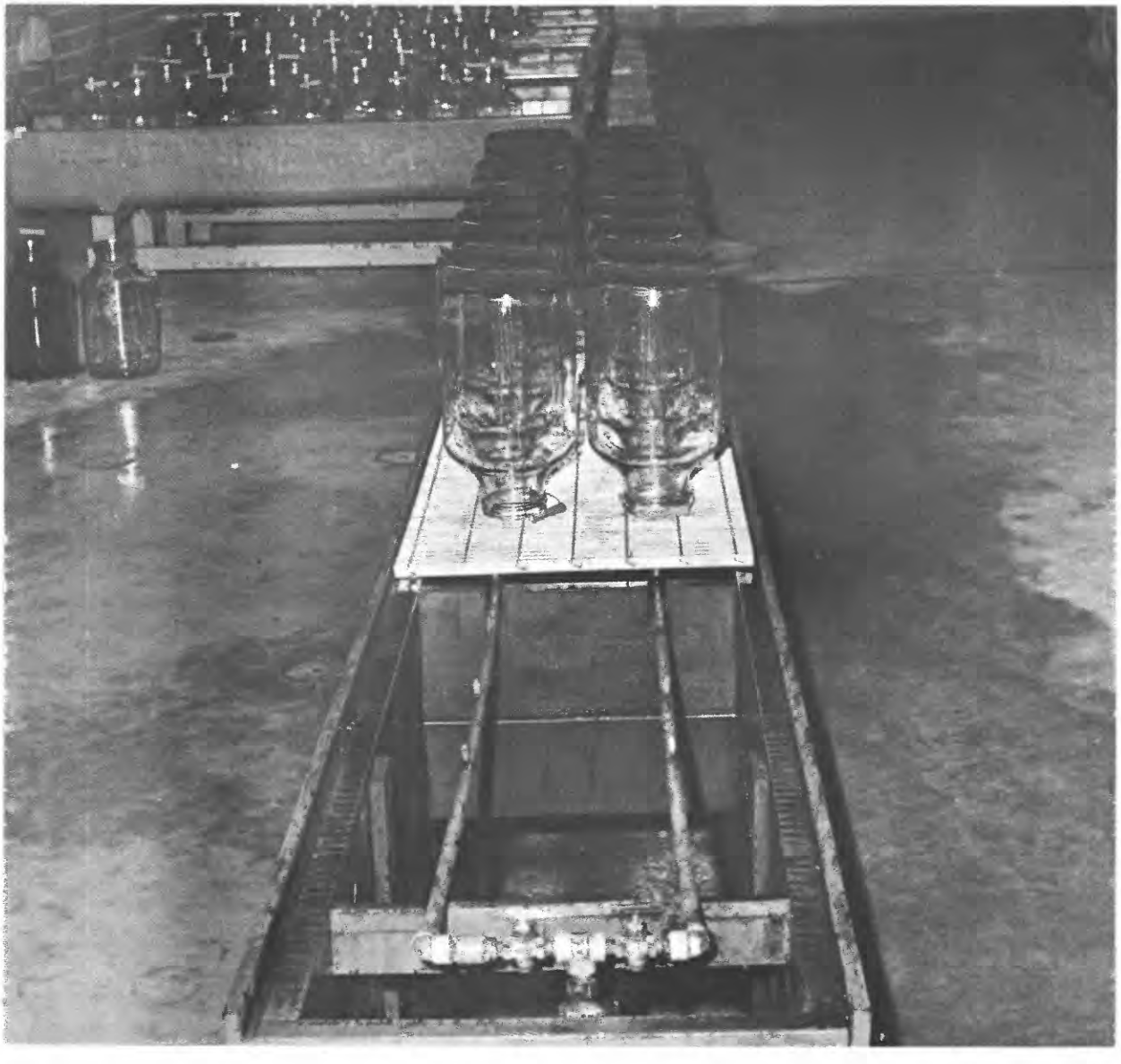

Figure 1.--Jar-rinsing rack with sections of grating removed to show construction detail.

When fewer than 26 jars are rinsed, a board is laid over the unused nozzles to deflect water into the floor trench.

AUTOMATIC LIQUID MEASURING VESSEL

DESCRIPTION

Most of the custom-made shell of the vessel was constructed of sections of polyethylene pipe having a wall thickness of one-fourth inch and inside diameters of 3, 4, and 8 inches (fig. 2). The main body of the vessel was made by heat-fusing a specially shaped top and bottom on a 17-inch-long section of 8-inch pipe. The convex top plate was made from a polyethylene block machined to a thickness of one-fourth inch, and the weight-bearing, concave lower plate was similarly machined to a thickness of one-half inch. An 8-inch-long piece of the 4-inch pipe was fused to the top plate to form the neck of the vessel, and a 1 1/2-inch-long piece of 3-inch pipe was fused to the bottom plate to serve as a drain (it also holds the vessel in place on the jar).

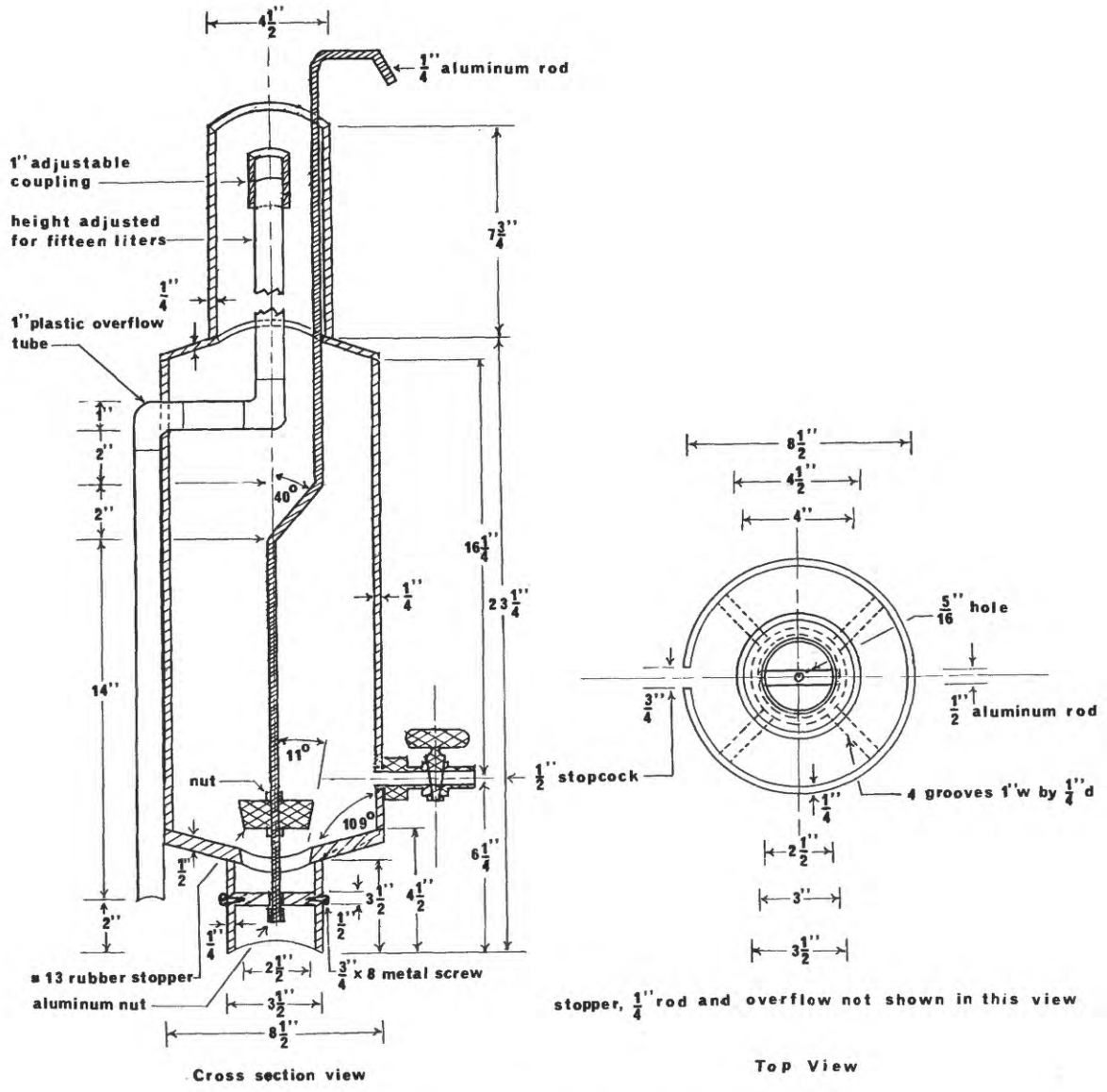

Figure 2.--Schematic illustration of automatic measuring vessel.

Special shaping of the upper and lower plates prevents air bubbles from being trapped under the upper plate, and results in rapid, complete drainage from the lower plate. The underside of the lower plate was made with four radial grooves one-half inch wide and one-fourth inch deep to permit rapid escape of air from the jar as it fills.

In filling the vessel, water enters through a 1/2-inch hard-rubber stopcock near the bottom, and any excess overflows through a 1-inch plastic pipe inserted through the side of the vessel near the top. The internal upper section of the overflow pipe extends upward into the neck to the required level, and the external lower section extends down to the bottom of the vessel where it is rigidly attached. A threaded plastic coupling at the top of the overflow allows for calibration of the volume measured.

The vessel is emptied through a drain hole in the bottom plate, the sides of which are tapered at an angle of 11°. A No. 13 rubber stopper and a 34-inch-long piece of aluminum rod serve as valve and handle, respectively. The lower 6 inches of the rod are threaded.

The stopper, which is located about 5 inches from the lower end of the rod, is held securely in place by means of aluminum washers and nuts above and below it. The lower end of the rod extends through a hole in a 3-inch-long piece of 1/2-inch aluminum rod which is held in place across the inside of the drain by small metal screws in each end. This guides the stopper into place, and when the vessel is emptied, upward travel of the rod is limited by the aluminum nut on its lower end. The midsection of the handle is bent to form a 2-inch offset which gives clearance around the upper end of the overflow pipe. The upper end of the rod extends slightly above the top of the vessel and is bent at a right angle to form a hand grip.

In the last vessel constructed, a stainless-steel rod was used for the handle, and a toilet-tank ball of soft, flexible rubber was used for a valve. The dimensions of the vessel could be changed easily to permit measurement of a wide range of volumes. The total cost of materials for the vessel was $37.20.

USE

To facilitate rapid filling of jars, two vessels are used concurrently. Water is supplied to each through sections of 5/8-inch plastic tubing connected to a single hose by a "Y" connector (fig. 3). Under our conditions it takes about 25 seconds to fill the measuring

Figure 3.--Automatic measuring vessels ready for use.

vessel with 15 liters of water, and less than half that long to empty it. Thus, a full vessel can be emptied and moved to another jar while the other vessel is filling. The plastic vessels are light, safe to handle, and quite durable.

JAR EMPTIER

DESCRIPTION

This tool consists of a U-shaped tube made of 1 1/2-inch plastic pipe, about a foot of which is inserted through a rubber seal (fig. 4). The seal is fashioned from the thick-walled upper section of a 2-way force cup (plumber's helper). A short section of 3/8-inch cooper tubing having a flared inner end also is inserted through the rubber cup to pass compressed air into the jar. The intake end of the 1 1/2-inch pipe is covered with a 1/8-inch mesh screen to prevent loss of fish as water is removed. The cost of materials was $2.35.

USE

The safe, low-pressure (5 p.s.i.) air supply used for aerating fish tanks provides the power to empty water from the bioassay jars. Once the screened end of the jar emptier and the rubber seal are inserted in the jar, compressed air seats the seal firmly against the

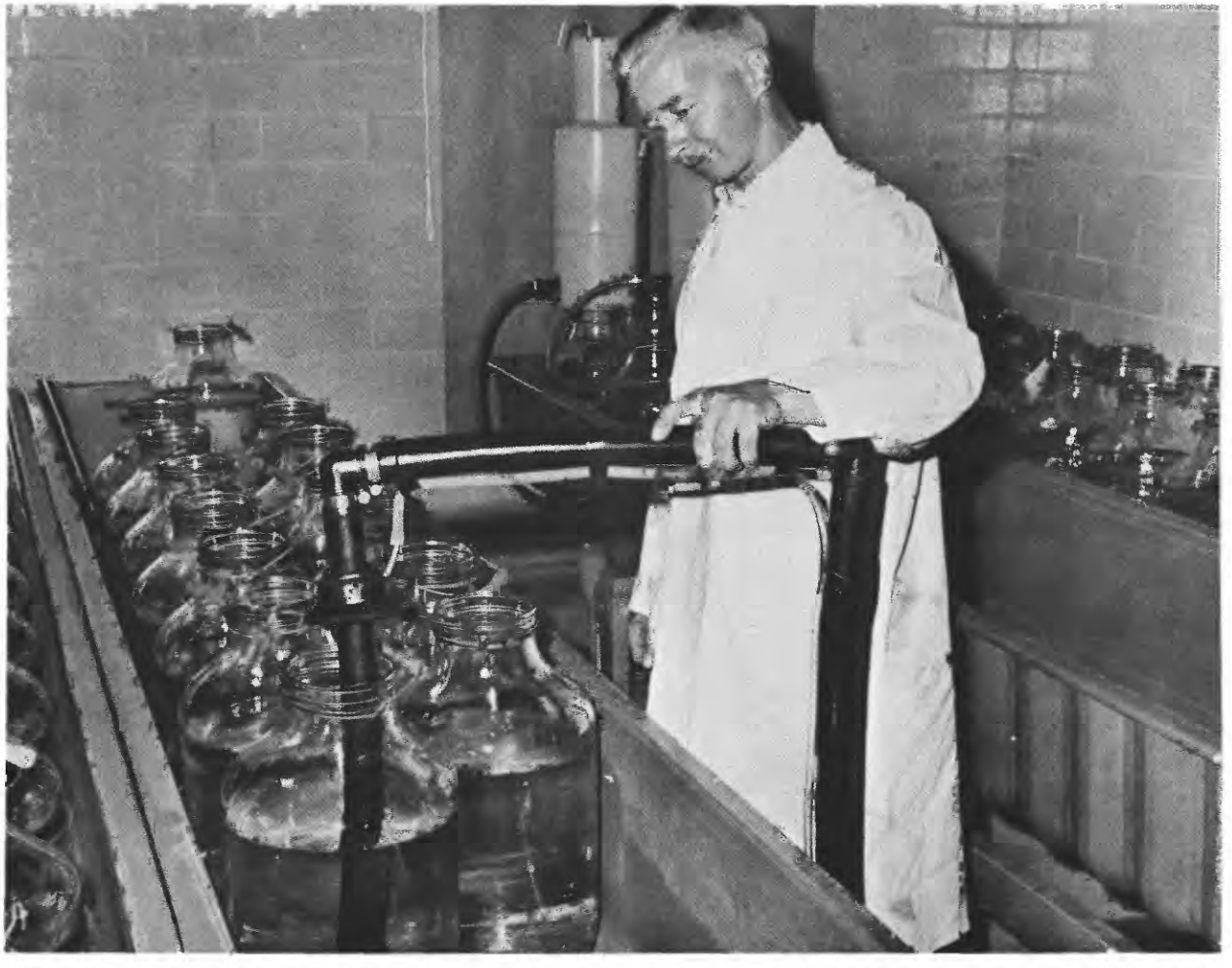

Figure 4.--Jar emptier partially inserted into jar; compressed air is supplied through the small tube while waste water is expelled through the 1 1/2-inch pipe.

neck. Nearly 15 liters of water are expelled in 11 seconds, and the resultant 30-pound reduction in weight makes the task of lifting jars from bioassay troughs much easier and safer. Tests were conducted to learn what would happen when the outlet screen was deliberately plugged and pressure was allowed to build up. In each case the seal was harmlessly forced out the neck of the jar, demonstrating that the necessary margin of safety was provided.

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