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

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TOXICITY OF 22 THERAPEUTIC COMPOUNDS TO SIX FISHES

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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, Manchester, Iowa
Do...............................	159	1.8	0.9	2-15-65	
Brown trout, *Salmo trutta*.............	177	1.7	0.8	3-16-65	National Fish Hatchery, Lake Mills, Wis.
Do...............................	177	1.9	1.2	4- 1-65	
Brook trout, *Salvelinus fontinalis*....	161	1.5	0.4	1-21-65	State Fish Hatchery, St. Croix Falls, Wis.
Do...............................	161	1.6	0.6	2-15-65	
Lake trout, *Salvelinus namaycush*.......	78	4.0	2.5	8-14-64	National Fish Hatchery, Jordan River, Mich.
Do...............................	78	4.0	2.8	8-28-64	
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, Marion, Ala.
Do...............................	W-74	2.2	1.5	8- 4-65	
Bluegill, *Lepomis macrochirus*..........	115	1.6	0.8	11- 5-64	National Fish Hatchery, Lake Mills, Wis.
Do...............................	131	1.4	0.7	11-17-64	
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	4.50	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_{50s} 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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