The Toxicity of Four Insecticides to Four Salmonid Species*

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Introduction

Information on the acute toxicity of certain organic pesticides to fish appears in a large number of publications. Examination of this information shows that a variety of environmental chemical and physical conditions existed while toxicity data were being obtained. Water quality, water temperature and fish body weight (or age) play major roles in the determination of acute toxicity of any toxicant to the particular fish species being used for analysis. The problem of comparing acute toxicity for a single compound to a single species of fish from published data is extremely difficult. Comparison of the relative toxicity of a specific compound to several species of fish from published information is usually impossible.

A study was conducted to determine the relative toxicity of four commonly used insecticides to four salmonid species commonly used for experimentation. Experiments were designed so that all species of fish were held under similar environmental conditions during the experimental period. This eliminated the effect of water chemistry and physical factors.

Another objective of the study was to determine the relative toxicity of the four insecticides to each species of fish at two different body weights. This was not always possible to accomplish because the numbers of fish of the correct body size were not always available.

The four insecticides used in the study included DDT and endrin (as chlorinated hydrocarbon insecticides), carbaryl (as a carbamate insecticide) and malathion (as an organophosphate insecticide).

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The four species of salmonids used in the study included a brook trout (Salvelinus fontinalis), rainbow trout (Salmo gairdnerii), cutthroat trout (Salmo clarki) and coho salmon (Oncorhynchus kisutch.

Materials and Methods

Water used to support experimental fishes is a most important consideration for a toxicological study. The water used in these experiments was supplied from a well. The water was delivered to the laboratory through a polyvinyl chloride pipeline. A flow of approximately 45 gpm was available to hatch and rear experimental fishes and for water to be used in static bioassay procedures. The water temperature ranged from 13.6 to 14.6 C. Water chemistry was monitored throughout the experimental period. Table 1 gives the minimum and maximum range for each physical or chemical factor, ion or element, monitored during the study.

Table 1

Range of Chemical and Physical Data on the Water Supply During the Year Long Experimental Period

Constituent	Constituent Range	
	Minimum	Maximum
Total Dissolved Solids (ppm	576	694
Dissolved Oxygen (ppm	5.9	6.0
Total Alkalinity (ppm as $CaCO_3$)	276	348
Total Hardness (ppm as $CaCO_3$)	318	348
Calcium Hardness (ppm)	200	235
Magnesium Hardness (ppm)	105	120
Chloride (ppm)	12	15
Sulfate (ppm)	140	150
Copper (ppm)	*	*
Manganese (ppm)	*	*
Iron (ppm)	*	*
Chromium (ppm)	*	*
pH	7.2	7.6
Temperature (^{o}C)	13.6	14.6

* Elements undetectable

Experimental fish were obtained from several sources. The Wigwam (Drew) strain of rainbow trout were obtained as eyed eggs from the Wyoming Game and Fish Commission. Eggs were obtained from fish spawning in December.

The Snake River strain of cutthroat trout were obtained as eyed eggs from the Wyoming Game and Fish Commission. Eggs were obtained from fish spawning in February.

Coho salmon were obtained as eyed eggs from the Oregon State Fish Commission. Their parental source is not known. The eggs were obtained in October from fall spawning fish.

The Wyoming, Soda Lake strain of brook trout, were obtained as small fingerlings from the Wyoming Game and Fish Commission. These fish were obtained in May from the previous fall's spawn.

Eyed eggs were hatched and reared in the same water supply used for bioassay procedures. Brook trout fingerlings were held in the well water supply for two weeks prior to being used for bioassay analysis.

All fish were fed a commercial dry ration prepared for trout prior to being used in bioassays. Feeding procedures prescribed by the manufacturer were carefully controlled to avoid nutritional deficiencies. Fresh ration was obtained at 60 day intervals. Rations were stored in a -20 C freezer until used.

Technical grade insecticidal compounds were used for all bioassay procedures. Formulations of these compounds were as follows:

DDT (Technical Grade)	= 77.2% P.P' isomer 22.8% inert ingredient
Endrin (Technical Grade)	= 95% active ingredient 5% inert ingredient
Malathion (Technical Grade)	= 95% active ingredient 5% inert ingredient
Carbaryl	= 98% active ingredient 2% inert ingredient

Stock solutions of each compound were prepared in acetone based on active ingredient weight. Acetone controls were used during bioassay procedures to account for any toxic effect which may have occurred from the acetone.

Bioassay procedures were carried out by standard methods described in the Standard Methods for the Examination of Water and Waste Water (2). A series of concentrations based on progressive bisection of intervals on a logarithmic scale were used (4). Replicate analyses were completed on each fish species for each insecticide except when numbers of experimental animals were unavailable.

Exploratory tests were run for each insecticide to determine the range of final concentrations of each toxicant to be used. One-gallon glass jars containing two liters of water and 3 to 5 fish per jar were used for the exploratory tests. Exploratory tests were run for 24 hours with the jars placed in a constant temperature water bath at 12.9 C (55 F).

Final bioassay analyses were run using two replicates of each toxicant concentration and experimental controls. Ten liters of water in five-gallon glass jars were used. The jars were held in a constant temperature water bath of 12.9 C (55 F) for 96 hours. A series of 5 or 6 toxicant concentrations, an experimental control and an acetone control were used for each test. Five fish were placed in each of two jars, except for tests with malathion. Because of the rapid breakdown of malathion, fresh malathion solutions were prepared at 24 hour intervals throughout the 96 hour bioassay test. Fish were transferred to these freshly prepared solutions each 24 hours. Therefore, the ten experimental fish could be held in the same jar without causing serious depletion of dissolved oxygen.

Mortality data were collected and recorded at 24 hour intervals for the 96 hour test period. These data were subjected to probit analysis as described by Litchfield and Wilcoxon (10).

Results

Results of bioassay are given in Tables 2 through 5. TLm values and the confidence limits (C.L.) at 48, 72, and 96 hour intervals are given for each species of fish to each insecticide at two different body weights. Table 6 demonstrates the relationship between the mean 96 hr TLm values of the four insecticides to the four salmonid species.

Table 2

TLm Values in ppb of the Toxicant and TLm Value Confidence Limits for Brook Trout

Toxicant	Fish Body Weight (g)	Time (hr)	TLm (ppb)	Confidence Limits
DDT	1.15	48	7.35	6.08 - 8.89
	1.15	72	7.4	6.08 - 9.03
	1.15	96	7.4	6.07 - 9.03
	2.13	24	23.1	18.48 - 28.88
	2.13	48	12.75	10.49 - 15.49
	2.13	72	11.9	10.59 - 13.38*
	2.13	96	11.9	10.59 - 13.38*
Endrin	1.15	24	1.28	1.02 - 1.61
	1.15	48	0.59	0.432 - 0.805
	1.15	72	0.42	0.343 - 0.514
	1.15	96	0.355	0.317 - 0.398*
	2.04	48	1.2	0.906 - 1.590*
	2.04	72	0.675	0.500 - 0.911
	2.04	96	0.59	0.464 - 0.743
Malathion	1.15	72	160.0	144 - 182
	1.15	96	130.0	110 - 154
	2.13	72	150.0	104 - 216
	2.13	96	120.0	96 - 153
Carbaryl	1.15	24	1,830	1,441 - 2,324
	1.15	48	1,500	1,176 - 1,913
	1.15	72	1,150	927 - 1,426
	1.15	96	1,070	905 - 1,263
	2.04	72	1,640	1,247 - 2,157
	2.04	96	1,450	1,047 - 2,008

* One replicate only

Table 3

TLm Values in ppb of the Toxicant and TLm Value Confidence Limits for Cutthroat Trout

Toxicant	Fish Body Wt. (g)	Time (hr)	TLm (ppb)	Confidence Limits
DDT	0.33	48	1.63	1.333 - 2.006
	0.33	96	0.85	0.368 - 1.530*
	1.25	96	1.37	1.28 - 1.454
Endrin	0.37	48	0.297	0.192 - 0.459
	0.37	72	0.147	0.117 - 0.184
	0.37	96	0.113	0.095 - 0.138
	1.30	48	0.235	0.218 - 0.255
	1.30	96	0.192	0.167 - 0.223
Malathion	0.33	72	200	163 - 245*
	0.33	96	150	133 - 170*
	1.25	96	201	175 - 231
Carbaryl	0.37	72	2,000	1,399 - 2,860
	0.37	96	1,500	1,176 - 1,913
	1.30	96	2,169	2,067 - 2,276

* One replicate only

Table 4

TLm Values in ppb of the Toxicant and TLm Value Confidence Limits for Rainbow Trout

Toxicant	Fish Body Wt. (g)	Time (hr)	TLm (ppb)	Confidence Limits
DDT	0.41	24	6.9	4.059 - 11.73
	0.41	48	3.05	2.678 - 3.474*
	0.41	72	2.25	1.837 - 2.756*
	0.41	96	1.72	1.416 - 2.090*
Endrin	1.24	48	0.695	0.568 - 0.850*
	1.24	72	0.53	0.451 - 0.623*
	1.24	96	0.405	0.327 - 0.502*
Malathion	0.41	24	240	198 - 291
	0.41	48	196	165 - 223
	0.41	72	175	146 - 209
	0.41	96	122	98 - 153
Carbaryl	1.24	96	1,470	980 - 2,205

* One replicate only

Table 5

TLm Values in ppb of the Toxicant and TLm Confidence Limits for Coho Salmon

Toxicant	Fish Body Wt. (g)	Time (hr)	TLm (ppb)	Confidence Limits
DDT	0.5	24	25.0	18.4 - 34.0
	0.5	48	12.5	9.9 - 15.7
	0.5	72	11.7	9.4 - 14.5
	0.5	96	11.3	9.7 - 13.2
	1.65	48	30.0	27.2 - 32.9
	1.65	72	24.0	21.3 - 26.6
	1.65	96	18.5	15.9 - 21.1
Endrin	1.50	24	2.85	2.355 - 3.449
	1.50	48	1.44	1.116 - 1.858
	1.50	72	0.91	0.674 - 1.229
	1.50	96	0.76	0.643 - 0.898
Malathion	1.70	24	300	211 - 346
	1.70	96	265	208 - 388
Carbaryl	1.50	24	2,950	2,201 - 3,953*
	1.50	48	2,700	1,929 - 3,780*
	1.50	72	1,690	1,341 - 2,129
	1.50	96	1,300	1,074 - 1,573

* One replicate only

Table 6

Relationship Between Mean 96 hr TLm Values of Four Insecticides and Four Species of Salmonid Fishes

Toxicant	Species	Fish Body Weight (g)	TLm (ppb)
DDT	Brook Trout	1.15*	7.4
	Brook Trout	2.13	11.9
	Cutthroat Trout	0.33	0.85
	Cutthroat Trout	1.25	1.37
	Rainbow Trout	0.41	1.72
	Coho Salmon	0.50	11.3
	Coho Salmon	1.65	18.5

Table 6 (Cont.)

Toxicant	Species	Fish Body Weight (g)	TLm (ppb)
Endrin	Brook Trout	1.15	0.355
	Brook Trout	2.04	0.59
	Cutthroat Trout	0.37	0.113
	Cutthroat Trout	1.30	0.192
	Rainbow Trout	1.24	0.405
	Coho Salmon	1.50	0.77
Malathion	Brook Trout	1.15	130
	Brook Trout	2.13	120
	Cutthroat Trout	0.33	150
	Cutthroat Trout	1.25	201
	Rainbow Trout	0.41	122
	Coho Salmon	1.70	265
Carbaryl	Brook Trout	1.15	1,070
	Brook Trout	2.04	1,450
	Cutthroat Trout	0.37	1,500
	Cutthroat Trout	1.30	2,169
	Rainbow Trout	1.24	1,470
	Coho Salmon	1.50	1,300

* Relationship between insecticide toxicity and size of fish is apparent where two fish sizes were available.

Discussion

Considerable research has been done in recent years to determine the acute toxicity and residual effects of a number of the more commonly used insecticides to fishes. Four of the more commonly used insecticidal compounds are those used in the study reported here. DDT and endrin, being quite nondegradable in the ecosystem will probably be used less commonly in the future. Compounds which show shorter toxicity degradability time, such as malathion and carbaryl, will probably find greater use as insecticidal agents. Certain toxicity data on the four insecticidal compounds given above to fishes have been published (1,3,5,6,7,8,9, 12).

The 96 hr TLm values obtained in the present study differed markedly from published data. As an example, rainbow trout in the present study yielded a 96 hr TLm value for DDT of approximately 1.7 ppb. The 96 hr TLm value shown by Katz and Chadwick (8) for DDT to rainbow trout was 42 ppb. This discrepancy may be explained by stating that water quality, physical factors in the water, fish body weight and fish strain all have entered into the final results. Comparison of these data bring forth the reason for the confusion surrounding published data on acute toxicity of specific compounds to fish species. Not enough effort has been made in past publications to set down the conditions under which the tests were accomplished.

TLm values for the other insecticides obtained in the present study also differ from published data. Those obtained for malathion and carbaryl were higher than those obtained by other researchers. Part of the discrepancies for malathion may be attributed to the high alkalinity of the diluent water. Some malathion could have been destroyed in experimental mixtures even though insecticide-test water mixtures were prepared at 24 hour intervals.

No explanation can be given for decreased toxicity of carbaryl. Higher toxicity for this insecticide was expected than is shown in published literature because Burdick et al. (3) found it to be more toxic in hard, alkaline water. The diluent water used in the present study was classified as a very hard, highly alkaline water.

The relationship of toxicity of the four insecticides to the four species of salmonids used indicate definite species difference when comparable fish body weight is considered and environmental conditions are similar.

The 96 hour TLm value for DDT indicate that DDT is 13.5 times more toxic to Snake River cutthroat trout and 2.5 times more toxic to Soda Lake brook trout than it was to the Oregon strain of coho salmon used in these tests when considering fish of comparable body weight. Likewise, endrin was approximately 4.0 times more toxic to Snake River cutthroat trout, 2.1 times more toxic to Soda Lake brook trout, 1.9 times more toxic to Wigwam (Drew) rainbow trout than to the Oregon strain of coho salmon of comparable body weight.

The species difference in the toxicity of malathion to the four salmonids was not as great as was shown by DDT and endrin. These results indicated that malathion was approximately 2.0 times more toxic to Soda Lake brook trout and 1.3 times more toxic to Snake River cutthroat trout than to the Oregon strain of coho salmon of comparable body weight.

Carbaryl was generally the least toxic of the four insecticides. Carbaryl was 2.0 times more toxic to Soda Lake brook trout, 1.7 times more toxic to the Oregon strain of coho salmon and 1.5 times more toxic to Wigwam (Drew) rainbow trout than to Snake River cutthroat trout of comparable body weight.

Body weight of fish had a definite relationship to toxicity of the four insecticides. Generally the 96 hour TLm values obtained were significantly different when the two body weights of fish were compared. DDT was 61% more toxic to 0.33 g cutthroat trout than to 1.25 g cutthroat trout, 61% more toxic to 1.15 g brook trout than to 2.13 g brook trout and 64% more toxic to 0.50 g coho salmon than to 1.65 g coho salmon. Endrin was 66% more toxic to 1.15 g brook trout than to 2.04 g brook trout and 70% more toxic to 0.37 g cutthroat trout than to 1.25 g fish.

Body weight did not have as great an effect on toxicity of malathion to brook trout and cutthroat trout as did endrin or DDT. Malathion yielded a similar 96 hour TLm value to 1.15 and 2.04 g brook trout, the value obtained on the smaller body weight fish being 130 ppb and the larger body weight fish being 120 ppb with confidence limits overlapping. This insecticide showed approximately 34% higher toxicity to 0.33 g cutthroat trout than to 1.25 g fish.

Relationship of body weight to toxicity of carbaryl was determined on brook trout and cutthroat trout only. This insecticide was 45% more toxic to 0.37 g cutthroat trout than to 1.30 g fish and 36% more toxic to 1.15 g brook trout than to 2.04 g fish.

Mortalities which occurred during bioassay were observed closely in an attempt to determine, by symptomatology, that deaths were indicative of the toxic effect of the insecticides. Symptoms of poisoning followed two general patterns. The symptoms of chlorinated hydrocarbon poisoning were similar to those described by Mayhew(11).

The second pattern was an example of poisoning by malathion and carbaryl. Fish became irritable and moved sluggishly when the jar was tapped. Fish experienced muscular spasms and convulsions as exposure time increased. Fish usually lost equilibrium and became completely paralyzed. They usually died with opercula open and the body bent at right angles.

The data reported here on the 96 hour TLm values for DDT, endrin, malathion and carbaryl to four species of salmonids are for one water temperature (12.9 C or 55 F). Controlling this variable, as well as variables associated with chemical and physical factors in the diluent water, yielded results which are assumed to be a more true measurement of the relative acute toxicity of each toxicant to the four fish species.

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