THE ACUTE TOXICITY OF SOME PESTICIDES TO FISH

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ABSTRACT

Acute static bioassays were conducted with 13 pesticides to determine their comparative toxicity to fish. There was a wide range in the toxicity of these compounds with 96-hour TL_m values ranging from 0.0033 to 4.0 mg/l. Of the compounds tested, Thiodan, a chlorinated hydrocarbon, and Thimet, an organic phosphorus pesticide, were the most toxic; and Bayer 29493, an organic phosphorus compound, and Fermate, a carbamate, were the least toxic. In about half of these static tests, toxicity increased significantly with an increase of exposure time from 24 to 96 hours. The toxicity of the organic phosphorus and chlorinated hydrocarbon compounds did not appear to be influenced by the water quality characteristics (pH, hardness, alkalinity) examined. The toxicity of two of the carbamates was influenced by water quality characteristics.

INTRODUCTION

In order to gain some idea of the relative toxicity of new pesticides, acute static toxicity studies have been conducted at the Robert A. Taft Sanitary Engineering Center. Such tests indicate those compounds that should be carefully monitored for potential hazards to fishes. This phase of the research program for the determination of the water quality requirements of fishes is now being terminated, and the final results of the studies are presented herewith. Emphasis is now being placed on long-term, continuous-flow studies to determine toxicological effects of common pollutants and concentrations that are neither lethal nor significantly harmful.

To date, most of the studies to evaluate the toxicity of pesticides to aquatic life have been static bioassays. Results of these and other studies have been summarized in McKee and Wolf (1963). Weiss (1964) has reviewed the literature on the use of fish to detect insecticides in water. The present study was undertaken to determine the acute toxicity of some new pesticides to warm-water fishes and to obtain information on the effects of certain water quality characteristics on their toxicity. The compounds bioassayed included carbamates, organic phosphorus, and chlorinated hydrocarbon pesticides.

METHODS

To provide a basis for evaluating and comparing the acute toxicity of these pesticides, 24-, 48-, and 96-hour median tolerance limit (TL_m) values were determined for each of them. The TL_m is a statistical estimate of the concentration of toxic material in water that kills 50 per cent of the test species under experimental conditions during a specified time interval. The TL_m was used because the concentration required to effect a response in 50 per cent of the test fish is more reproducible than any other value.

These static bioassays for determining acute toxicity were conducted according to the methods recommended by the American Public Health Association (1960). Laboratory facilities and equipment were similar to those described by Henderson and Tarzwell (1957).

Three species of fish were used: fathead minnows (*Pimephales promelas*), bluegills (*Lepomis macrochirus*), and guppies (*Lebistes reticulatus*). The first two species ranged in total length from 38 to 64 millimeters and in weight from 1 to 2 grams. Guppies were approximately 6 months old, ranging in length from 19

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[2]Manuscript received May 21, 1965.

to 25 millimeters, and in weight from 0.1 to 0.2 gram. The fathead minnows and bluegills were raised in ponds at the State Fish Hatchery at Newtown, Ohio, and the guppies were raised in our laboratories.

Ten fish were used in each test concentration and in the control. In bioassays with fathead minnows and bluegills, five fish were placed in each of two 5-gallon widemouth glass bottles, both containing 10 liters of test solution. Five guppies per duplicate sample were similarly added to 2-liter test solutions in 1-gallon glass bottles.

A soft diluent water, prepared in the laboratory by mixing five parts natural limestone spring water with 95 parts distilled, demineralized water, was used in most of the bioassay studies. Some bioassays were conducted using the spring water as the diluent. Before use, these dilution waters were adjusted in the constant- temperature room to the test temperature of 25°C and aerated for about 1 hour to bring dissolved gasses to near equilibrium with the atmosphere. At time of use, and before the introduction of the pesticide, the characteristics of these waters were as follows:

Dissolved Oxygen (mg/l)	pH	Alkalinity (mg/1	Hardness (EDTA) (mg/l)
Soft water 7.8	7.5	18	20
Hard water 7.8	8.2	300	360

These bioassays were designed so that oxygenation would not normally be required when toxicants having little or no oxygen demand were used. The addition of acetone as a carrier, however, increased bio-oxidation so that pure oxygen in the form of large bubbles had to be added at a slow rate in order to maintain dissolved oxygen at a satisfactory level. Physical and chemical determinations (dissolved oxygen, pH, alkalinity, and hardness) were made on each test concentration initially and after fish mortality or at the completion of the test.

Different concentrations of test solution were prepared in a logarithmic series of numbers such as 10, 5.6, 3.2, 1.8, and 1.0 milligrams of the formulation per liter of water. Five test concentrations and a control were used for each bioassay. The reactions of the fish were recorded intermittently for a 96-hour period. From the mortalities in different concentrations of pesticide, 24-, 48-, and 96-hour TL_m values were calculated. All TL_m values are reported in terms of the initial amount of formulation of pesticide added to the dilution water. Chemical measurements were not made of the pesticides; undoubtedly the effective concentration became lower with time.

These acute toxicity studies were designed to give estimated TL_m values obtained by straight-line graphical interpolation from points representing per cent survival of fish and log concentrations of pesticide formulation that bracket the 50 per cent point (American Public Health Association, 1960). The data are also amenable, however, to use of the average-angle method (Harris, 1959). This method is free from assumption as to the precise type of fundamental toxicity curve or technique of fitting the curve and accounts for data other than those that bracket 50 per cent survival. This procedure also presents "exact" confidence limits and tests of significance.

An Automath program using the method of Harris was written for the Honeywell 400 computer to calculate TL_m values and their 95 per cent confidence limits, and to test the significance of difference between any two estimated TL_m values. Tests of significance of differences were made between all TL_m values for each pesticide. These results are the basis for this report.

BIOASSAY RESULTS

The pesticides tested, their grade, method of addition, and a summary of the acute toxicity results are given in Table 1. The 24-, 48, and 96-hour TL_m values

TABLE 1

Median tolerance limits and 95 per cent confidence limits expressed as mg/l of formulation

Compound	Solvent or Carrier	Dilution Water	Test Fish	24-hour			48-hour			96-hour		
				TL_m	Conf. limits		TL_m	Conf. limits		TL_m	Conf. limits	
Carbamates												
Vancide 51Z	Water	S	Fatheads	.41	.31	.52	.35	.24	.45	.35	.24	.45
Dispersion 50% active	Water	S	Bluegills	.85	.74	1.1	.85	.74	1.1	.85	.74	1.1
Ziram-Zn salt of	Water	S	Guppies	.59	.51	.70						
Di-Methyldithiocarbamic acid 46% Zinc salt of Mercaptobenzothiazole 4%	Water	H	Guppies	.51	.41	.67						
Manganese Salt of	Water blend	S	Fatheads	.91	.73	1.2	.83	.66	1.1	.83	.66	1.1
Vancide 51 85% Active	Water blend	H	Fatheads	.97	.77	1.3	.75	.58	.95	.71	.55	.90
Methyl Zimate	Water triton	S	Fatheads	.29	.22	.40	.25	.19	.33	.25	.19	.33
	Water trition	H	Fatheads	.26	.21	.33	.24	.19	.31	.24	.19	.31
Cumate	Water	S	Fatheads	NF(>1.0)			.21	.15	.26	.071	.054	.089
Dispersion 50% active	Water	H	Fatheads	NF(>3.2)			NF(>3.2)			.23	.16	.31
copper salt of Zimate	Water	S	Bluegills	NF			1.3	.58	1.9	.32	.27	.38
Fermate	Water blend	S	Fatheads	7.0	5.8	9.8	3.6	2.6	4.7	3.1	2.4	4.0
95% active iron salt	Water blend	H	Fatheads	3.0	2.4	3.9	1.3	1.0	1.7	1.3	.97	1.6
of Namate-90%	Water blend	S	Bluegills	6.2	5.2	7.9	3.7	2.3	5.2	3.6	2.3	5.2
Organic Phosphorus												
Thimet-Tech.	Acetone	S	Fatheads	.25	.17	NF	.25	.17	NF	.25	.17	NF
95% active	Acetone	S	Bluegills	.0065	.0050	.0082	.0047	.0037	.0060	.0047	.0037	.0060
	Acetone	H	Bluegills	.0089	.0070	.012	.0056	.0039	.0072	.0049	.0038	.0067
Ethion, Tech.	Acetone	S	Fatheads	NF			2.5	1.6	7.0	2.4	1.2	7.7
	Acetone	S	Blugeills	.21	.13	1.4	.13	.094	.19	.13	.080	.19
	Acetone	H	Bluegills	NF			.14	.099	.22	.13	.090	.20
	Acetone	S	Guppies	.19	.15	.25	.17	.13	.22	.13	.10	.17
Vapona (DDVP), Tech. 93%	Acetone	S	Fatheads	NF			7.9	6.2	10.	4.0	3.2	5.0
	Acetone	S	Bluegills	NF			.32	.24	.40	.27	.22	.35
	Acetone	H	Bluegills	.87	.67	1.8	.46	.36	.58	.35	.27	.44
Bayer 29493 (Baytex),	Acetone	S	Fatheads	3.4	2.9	4.0	3.3	2.8	3.9	3.3	2.8	3.9
Tech. 93%	Water blend	S	Fatheads	3.3	2.8	3.9	3.2	2.7	3.8	3.2	2.7	3.8
	Acetone	H	Fatheads	3.5	3.0	4.1	3.5	3.0	4.1	3.5	3.0	4.1
	Acetone	S	Bluegills	3.7	3.3	4.3	3.7	3.3	4.3	3.1	2.5	4.0
	Acetone	S	Guppies	5.0	3.9	8.8	3.6	3.0	4.5	3.1	2.4	4.1
Bayer 25141, Tech.	Acetone-blend	S	Bluegills	.20	.17	.25	.11	.08	.15	.056	.009	.10
	Acetone-blend	H	Bluegills	.26	.18	4.0	.11	.09	.15	.070	.02	.16
American Cyanamid 12009, Tech.	Acetone	S	Fatheads	.32	.17	.45	.32	.17	.45	.32	.17	.45
	Acetone	S	Bluegills	.0081	.0064	.010	.0075	.0059	.0095	.0075	.0059	.0095
	Acetone	H	Bluegills	.0081	.0064	.01	.0075	.0059	.0095	.0075	.0059	.0095
	Acetone	S	Guppies	.011	.0078	.0132	.010	.0078	.0132	.010	.0073	.013
Chlorinated Hydrocarbons												
Thiodan, Tech. 96.6%	Acetone	S	Bluegills	.0036	.0028	.0046	.0033	.0025	.0041	.0033	.0025	.0041
	Acetone	H	Bluegills	.0046	.0037	.0060	.0044	.0035	.0056	.0044	.0035	.0056
	Acetone	S	Guppies	.0046	.0034	.0066	.0039	.0030	.0049	.0037	.0037	.0048
Ovex, Tech.	Acetone	S	Bluegills	3.2	2.7	3.8	3.1	2.5	4.2	2.5	2.0	3.2
	Acetone	H	Bluegills	3.4	2.8	4.3	2.3	1.7	3.0	2.2	1.7	2.7

NF = Not Found S = Soft H = Hard

and 95 per cent confidence limits are reported for the different species of fish and the two dilution waters. All values are reported from calculations as milligrams per liter of compound or formulation as initially added to the test solution.

There was a great variation in the acute toxicity of these pesticides with 96-hour TL_m values, ranging from 0.0033 to 4.0 mg/l. Thiodan, a chlorinated hydrocarbon, and Thimet, an organic phosphorus pesticide, were the most toxic (TL_m value the smallest) to bluegills; and Bayer 29493, an organic phosphorus compound, and Fermate, a carbamate, were the least toxic.

In about half of these tests, toxicity increased significantly with an increase of exposure time from 24 to 96 hours. Table 2, a summary of the results of the

TABLE 2

Significance of difference between estimated 96- and 24-hour TL_m values[a]

Compound	Fatheads		Bluegills		Guppies	
	Soft	Hard	Soft	Hard	Soft	Hard
Vancide 51Z	−0.13508		−0.08856			
Manganese salt of Vancide 51	−0.5708	−0.1588				
Methyl Zimate	−0.11298	0.12183				
Cumate	1.50875		0.71438			
Fermate	0.23123	0.22413	0.11551			
Thimet	−0.27091		−0.02472	0.11238		
Vapona	−0.11482		0.87963	0.24784		
Ethion			−0.04002	1.30939	0.02586	
Bayer 29493	−0.07898	0.08464	2.48739		0.10744	
	−0.08385					
Bayer 25141			0.40668	0.35678		
American Cyanamid 12009	−0.25564		−0.12277	−0.12277	−0.13783	
Thiodan			−0.10668	−0.13032	−0.02283	
Ovex			0.01404	0.07205		

[a]The two TL_m estimates will be judged significantly different if number is ≥ 0. See Harris (1959).

analysis, compares the difference between estimated 24-hour and the 96-hour TL_m values in both soft and hard water. A pair of the TL_m estimates was judged to be significantly different at a 95 per cent confidence limit if the number was zero or greater.

The toxicity of some pesticides is known to be influenced by water characteristics such as dissolved oxygen, hardness, turbidity, and temperature. Bioassays were conducted with two waters widely different in pH, alkalinity, and hardness, but similar in dissolved oxygen and temperature, to determine whether these qualities would influence the toxicity of these pesticides. The average characteristics of the two dilution waters are given in the section on Methods.

The toxicity of the pesticides to different species in soft water and hard water is compared in Table 3. The acute toxicity of the organic phosphorus and chlorinated hydrocarbon pesticides does not appear to be influenced by the water quality characteristics examined. The TL_m values of these phosphorus and chlorinated hydrocarbon pesticides are not significantly different in these two experimental waters. Among the carbamates, the 96-hour TL_m value of Fermate is significantly lower in hard water and Cumate, the copper salt of zimate, is more toxic in soft water. The 96-hour TL_m values for the manganese salt of Vancide 512 and methyl zimate are not significantly different.

TABLE 3
A comparison of the acute toxicity of pesticides to fish in different dilution waters[a]

	Soft Water			Hard Water			Significance of difference	Test fish
	96-hour TL_m	95 percent Conf. limits		96-hour TL_m	95 percent Conf. limits			
Vancide 51Z[b]	0.59	0.51	0.70	0.51	0.41	0.67	−0.00206	Guppies
Manganese salt Vancide 51	0.83	0.66	1.1	0.71	0.55	0.90	−0.04506	Fatheads
Methyl Zimate	0.25	0.19	0.33	0.24	0.19	0.31	−0.17269	Fatheads
Cumate	0.073	0.054	0.089	0.23	0.16	0.31	1.50875	Fatheads
Fermate	3.1	2.4	4.0	1.3	0.97	1.6	0.28260	Fatheads
Thimet	0.0047	0.0037	0.0060	0.0049	0.0038	0.0067	−0.1668	Bluegills
Vapona	0.27	0.22	0.35	0.35	0.27	0.44	−0.05039	Bluegills
Bayer 29493	3.3	2.8	3.9	3.2	2.7	3.8	−0.05244	Fatheads
	3.5	3.0	4.1	3.2	2.7	3.8	−0.06757	Fatheads
Bayer 25141	0.056	0.0090	0.10	0.070	0.02	0.16	−0.30639	Bluegills
American Cyanamid 12009	0.0075	0.0059	0.0095	0.0075	0.0059	0.0095	−0.15742	Bluegills
Thiodan	0.0033	0.0025	0.0041	0.0044	0.0035	0.0056	−0.2283	Bluegills
Ovex	2.5	2.0	3.2	2.2	1.7	2.7	−0.08562	Bluegills

[a]The two TL_m estimates will be judged significantly different if number is ≥ 0. See Harris (1959).
[b]Values are for 24 hours instead of 96.

DISCUSSION

The carbamates are important fungicides. Their emergence as useful broad-spectrum pesticides is a major development of recent years (Johnson, et al., 1963). Cumate, the copper salt of zimate, was the most toxic of the carbamates, as determined by the 96-hour TL_m value. This pesticide was significantly more toxic in soft water, and the 96-hour TL_m value was significantly lower than the 24-hour TL_m value. The manganese and iron salts were less toxic than methyl zimate, the non-metallic carbamate. On the basis of active ingredient concentration, the zinc salt is highly toxic, but only slightly more so than methyl zimate. The 96-hour TL_m value for Fermate in hard water was significantly lower than the value in soft water.

The acute toxicity of the organic phosphorus insecticides is characterized by great variation in toxicity to different species of fish. Among those tested, the bluegills were generally the most sensitive (Pickering, et al., 1962). Because the bluegill appears to be sensitive to the organic phosphorus insecticides, all of these compounds were bioassayed with the bluegill. In addition, five of these pesticides were bioassayed with both the bluegill and fathead minnow. Tests of the significance of the difference of 96-hour TL_m values in soft water indicated that Thimet, Vapona, and Ethion are more toxic to the bluegill than to the fathead minnow. The 96-hour TL_m value of Thimet for fathead minnows was about 50 times greater than that for the bluegill. Of these compounds, Thimet was the most toxic and Bayer 29493 the least toxic. As indicated in Table 3, a comparison of the 96-hour TL_m values of the organic phosphorus pesticides did not give a difference to a species tested in soft or hard dilution water.

As indicated in Table 2, there is much variation in tests comparing the significance of the difference between 24- and 96-hour TL_m values. The change in the TL_m value for American Cyanamid 12009 was not significantly different for any species or type of water tested. Bayer 25141 was significantly more toxic to bluegills after 96 hours in both soft and hard water. Vapona was significantly more toxic in 96 hours for the bluegill, but not for the fathead minnow.

Only two chlorinated hydrocarbon pesticides were bioassayed. Thiodan was the most toxic of the compounds examined, and it had a 96-hour TL_m value of 0.0033 mg/l to bluegills in soft water. Ovex was one of the least toxic. For both of these compounds, the 96-hour TL_m value was not significantly different in soft or hard dilution water. The 96-hour TL_m values for Thiodan were not significantly different from the 24-hour values, while the 96-hour TL_m values for ovex were significantly lower than the 24-hour values.

According to Lehman et al. (1955), a substance proposed for use in food should show no chronic toxicity to animals in an amount equivalent to 100 times the amount proposed for use in the human diet; that is, a safety factor of at least 100 should be present. In the necessary use of a chemical, such as a drug or pesticide, some believe this margin of safety may be reduced, and the recognized risk of its use may be weighed against its value to the human economy. Such safety factors have not been developed for aquatic life.

At the present time, there are no magic numbers to plug into a formula for the estimation of a so-called "safe concentration" from static, acute toxicity bioassays. Subacute and chronic studies utilizing both fish and fish food organisms are necessary to determine sublethal effects. Then significant physiological effects may be used for the development of water quality criteria for aquatic life.

The hazard of a pesticide to aquatic life depends upon both its toxicity and its use. The acute toxicity values reported here indicate that several of these pesticides are extremely toxic and could represent a hazard to aquatic life.

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