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James Mayhew
Iowa State Conservation Commission

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Introduction

Today, with the development and use of an increasing number of more potent insecticides there exists a real and potential danger to many forms of wildlife. DDT was the first of these insecticides to be used and it is still very popular and widely applied. Its widespread use on agricultural lands, forests, marshes, and water quickly aroused the interest of persons and agencies who were concerned with the ways in which it might directly or indirectly affect animals other than insects.

Most of the studies have been concerned with the toxicity of DDT, but within recent years a number of new insecticides have been developed which are more toxic to animals than DDT. Since little information is available concerning the toxicity of these insecticides to fish it seemed expedient to experimentally test the relative toxicity of several of these newer insecticides in comparison to DDT.

This study was made to determine the relative toxicity of dieldrin, toxaphene, heptachlor, aldrin, methoxychlor, chlordane, and DDT to fingerling rainbow trout, *Salmo gairdnerii* (Richardson). Small fingerling trout were chosen for experimental purposes because they are most easily maintained under laboratory conditions and because smaller fishes are affected to a greater extent by insecticides than are larger fishes (Hoffman and Surber, 1945; Stock, 1950; Surber and Hoffman, 1949).

The problem not only involved a study of the relative toxicity of these seven insecticides, but also a study of the tolerance of trout to different formulations and concentrations of each insecticide. Since these insecticides are generally applied in the form of emulsifiable concentrates, wettable powders, and dusts, these were the formulations tested. Although all three types of formulations were not available for each insecticide, at least two formulations were tested for each.

Materials

Experimental quantities of 75 per cent chlordane emulsifiable concentrate, 50 per cent wettable powder, and 25 per cent dust, together with 25 per cent heptachlor emulsifiable concentrate, 50

per cent wettable powder were furnished with experimental quantities of inert materials by the Velsicol Corporation of Chicago, Illinois.

Julius Hyman and Company of Denver, Colorado, furnished experimental quantities of 23 per cent aldrin emulsifiable concentrate, 23.8 per cent wettable powder, 2.5 per cent dust, 24 per cent dieldrin emulsifiable concentrate, 25 per cent wettable powder, and 1.5 per cent dust together with inert materials used in the formulations of the insecticides.

The 25 per cent toxaphene emulsifiable concentrate and 50 per cent toxaphene wettable powder, together with the inert materials which were used in these toxaphene formulations, were furnished by the Hercules Powder Company, Wilmington, Delaware.

E. I. du Pont de Nemours and Company of Wilmington, Delaware, furnished the 25 per cent methoxychlor emulsifiable concentrate, 50 per cent methoxychlor wettable powder, 25 per cent DDT emulsifiable concentrate, 50 per cent DDT wettable powder, and 75 per cent DDT dust.

Methods

Eighty-one experiments were conducted in an effort to test the relative toxicity of the various insecticides. Stock solutions of one part in a thousand by weight were prepared for the emulsifiable concentrates and wettable powders. The desired concentrations for experimental purposes were then prepared by adding a measured quantity of the stock solution to a known quantity of water in the experimental equaria. The dusts were weighed and applied directly to the surface in amounts necessary to obtain the desired concentrations.

The concentrations of active insecticidal ingredients tested were 0.05, 0.1, 0.25, 0.5, and 1.0 p.p.m., except in those cases when no mortality occurred within 24 hours. Whenever no mortality occurred within 24 hours smaller concentrations were not tested.

Dissolved oxygen, free carbon dioxide, and hydrogen ion concentration determinations were made at the start and finish of each experiment. A constant record of the temperature during the 24 hour experimental period was made by means of a Bristol (model-500T) recording thermometer.

A total of 810 fingerling rainbow trout were used in the experimental work. Ten fish were used in the experimental aquaria and nine or more in each control aquaria. The fish in the experimental aquaria were exposed to the insecticide for a period of 24 hours or until a 100 per cent mortality occurred. All experimental fish remaining alive after the 24-hour period of exposure were transferred

to fresh water and observed until they died or for an additional 72 hours. Thus, the maximum period of time any fish was under observation was 96 hours.

In control aquaria the equivalent amount of inert materials were added that had been used in the experimental aquaria.

Relative Toxicity

General

All other insecticides used in the experiments, with the exception of heptachlor wettable powder, proved to be more toxic to rainbow trout than DDT. Dieldrin was consistently the most toxic of any of the seven insecticides with toxaphene second. Chlordane was slightly more toxic than DDT in most concentrations and formulations. A rather wide variation occurred in the relative toxicity of aldrin, heptachlor, and methoxychlor formulations at different concentrations

The emulsifiable concentrates were consistently the most toxic of the various formulations tested, whereas the wettable powder and dust varied considerably in their relative toxicity. Differences in the toxicity of the three formulations is thought to be due to the difference in availability of the active principles of the insecticides to the fish.

Emulsifiable Concentrates

As indicated in Table I, dieldrin and toxaphene were the most toxic of the emulsifiable concentrates. Both these insecticides killed all the fish at a concentration of 1.0 p.p.m. within four hours, but dieldrin killed all the fish within 12 hours at a concentration of 0.5 p.p.m., whereas toxaphene killed 40 per cent of the fish within 12 hours at this concentration. Aldrin emulsifiable concentrate, with few exceptions, appeared to be the third most toxic of the insecticides. The toxicity of chlordane and DDT emulsifiable concentrates was quite similar, with chlordane tending to be slightly more toxic at a concentration of 1.0 p.p.m. while DDT was the most toxic at lower concentrations. When Stock (*Op. Cit.*) experimentally tested the relative toxicity of four emulsifiable concentrates to brown trout he found dieldrin most toxic, toxaphene second, aldrin third, and chlordane fourth. This is exactly the same order of toxicity for the emulsifiable concentrates of these four insecticides as determined by this study.

Wettable Powders

The wettable powders were the least toxic of the three formulations as shown in Table 2. Toxaphene and dieldrin were the most potent of the wettable powders. Dieldrin killed all the fish within

Table 1.

Comparative Toxicity of Emulsifiable Concentrate Formulations

Insecticide	Concentration (p.p.m.)	Average Length (mm.)	Average Weight (grams)	Per Cent Mortality (hours) 4	12	16	24
Dieldrin	1.0	46.8	1.24	100			
Toxaphene		55.8	2.08	100			
Heptachlor		50.0	1.28	100			
Aldrin		49.0	1.76	80	100		
Methoxychlor		52.6	1.44	50	100		
Chlordane		48.3	1.63	0	100		
DDT		50.8	1.63	0	70	100	
Dieldrin	0.5	50.2	1.65	100			
Toxaphene		55.3	2.08	40	100		
Aldrin		50.6	1.77	30	100		
Methoxychlor		51.9	1.00	20	100		
Heptachlor		45.8	1.20	0	100		
DDT		52.3	1.42	0	40	90	100
Chlordane		37.0	1.20	0	20	70	100
Dieldrin	0.25	47.4	1.31	100			
Toxaphene		50.9	1.56	10	100		
Aldrin		48.3	1.34	10	100		
Methoxychlor		54.9	1.56	0	100		
Heptachlor		44.9	1.05	0	100		
Chlordane		43.7	1.36	0	0	20	60
DDT		55.7	1.77	0	0	10	90
Dieldrin	0.1	49.3	1.52	90	100		
Toxaphene		46.8	1.34	0	100		
Methoxychlor		50.1	1.42	0	100		
Aldrin		53.6	1.94	0	90	100	
Heptachlor		47.3	1.23	0	20	30	80
DDT		55.3	1.83	0	0	0	40
Chlordane		47.0	1.33	0	0	0	0
Dieldrin	0.05	50.8	1.71	40	100		
Toxaphene		47.6	1.39	0	40	100	
Aldrin		49.3	1.45	0	80	90	100
Methoxychlor		53.9	1.61	0	40	80	100
Heptachlor		44.5	1.19	0	0	0	10
DDT		52.6	1.39	0	0	0	0

four hours at 1.0 p.p.m., whereas toxaphene killed 80 per cent of the fish within four hours at this concentration. Aldrin was the third most toxic of the wettable powders followed by methoxychlor, chlordane, and DDT in that order. Heptachlor wettable powder had no ill effects upon the fish at either concentration of 1.0 or 0.5 p.p.m. This was thought to be due to the near insolubility of the formulation since it would settle rapidly to the bottom of the aquarium unless constantly agitated.

Table 2.

Comparative Toxicity of Wettable Powder Formulations

Insecticide	Concentration (p.p.m.)	Average Length (mm.)	Average Weight (grams)	Per Cent Mortality (hours) 4	12	16	24
Dieldrin	1.0	54.7	2.02	100			
Toxaphene		49.6	1.43	80	100		
Aldrin		56.7	1.99	40	100		
Methoxychlor		54.1	1.71	20	100		
Chlordane		43.6	1.15	0	30	90	100
DDT		51.9	1.35	0	0	40	90
Heptachlor		51.4	2.09	0	0	0	0
Toxaphene	0.5	47.2	1.26	50	100		
Dieldrin		52.5	1.91	40	100		
Aldrin		47.6	1.58	0	100		
Methoxychlor		58.2	2.27	0	90	100	
Chlordane		44.9	1.17	0	0	50	90
DDT		53.5	1.42	0	0	0	20
Heptachlor		51.0	1.99	0	0	0	0
Toxaphene	0.25	53.0	1.80	0	100		
Dieldrin		50.4	1.64	0	100		
Aldrin		57.0	2.52	0	40	100	
Methoxychlor		54.1	1.67	0	20	70	100
Chlordane		46.9	1.37	0	0	0	30
DDT		51.4	1.38	0	0	0	0
Toxaphene	0.1	49.8	1.51	0	70	100	
Dieldrin		49.2	1.55	0	0	40	80
Aldrin		54.0	2.28	0	0	20	70
Methoxychlor		54.5	1.79	0	0	20	70
Chlordane		46.0	1.15	0	0	0	0
Toxaphene	0.05	50.0	1.59	0	30	100	
Dieldrin		52.6	1.87	0	0	30	100
Aldrin		51.3	1.73	0	0	0	60
Methoxychlor		55.8	1.78	0	0	0	10

Dusts

Although dust formulations of only four insecticides were tested they were more toxic in general than wettable powders of the same insecticide, except in the case of chlordane. Chlordane wettable powder tended to be slightly more potent than dusts at lower concentrations. As show by Table 3, dieldrin and aldrin were the most toxic of the four dusts and their degree of toxicity was very similar. Chlordane and DDT were very similar in their toxicity; however, chlordane was slightly more toxic at all concentrations. Neither of these insecticides killed any fish within 24 hours at a concentration of 0.1 p.p.m.

Relationship of Certain Factors to Toxicity

The results of this study, as well as those of other investigators, indicate that a wide variation occur in the response of fish and

Table 3.

Comparative Toxicity of Dust Formulations.

Insecticide	Concentration (p.p.m.)	Average Length (mm.)	Average Weight (grams)	Per Cent Mortality (hours) 4	12	16	24
Dieldrin	1.0	52.7	1.89	100			
Aldrin		48.7	1.57	60	100		
Chlordane		43.1	1.13	0	20	90	100
DDT		56.0	2.08	0	20	70	100
Dieldrin	0.5	51.5	1.87	60	100		
Aldrin		48.3	1.43	40	100		
Chlordane		43.9	1.05	0	0	40	100
DDT		51.7	1.80	0	0	0	50
Aldrin	0.25	47.5	1.52	20	100		
Dieldrin		53.4	1.94	10	100		
Chlordane		43.7	1.11	0	0	20	60
DDT		49.6	1.09	0	0	0	40
Dieldrin	0.1	53.1	1.86	0	90	100	
Aldrin		49.9	2.09	0	70	100	
Chlordane		47.7	1.23	0	0	0	0
DDT		54.4	1.46	0	0	0	0
Aldrin	0.05	47.4	1.50	0	20	90	100
Dieldrin		51.8	1.85	0	20	80	100

other vertebrates to the toxic effects of insecticides. As previously pointed out, the emulsifiable concentrates were the most toxic type of formulation. However, the toxic qualities of the individual insecticides are also extremely important because regardless of the type of formulation applied dieldrin and toxaphene were always the most toxic, while chlordane and DDT were generally least toxic.

The observations of Surber and Hoffman (*Op. Cit.*) and of Stock (*Op. Cit.*) indicated there was some relationship between size of fish and the toxicity of the insecticide. Although the size variation of the fish used in this study was small, in general it was noted that the larger fish were last to die.

Temperature of the water is also important in altering the rate of kill by insecticides. Everhart and Hassler (1945) and Stock (*Op. Cit.*) show that there was a definite increase in mortality of brown trout as the temperature of the water increased. This study was not designed to check this particular type of relationship and inasmuch as the temperature ranges were so narrow throughout the study no conclusions can be drawn concerning this factor.

Individual differences in fish are probably important since each fish differs physiologically and physically. These differences will be reflected in their tolerance to the insecticides. The general physiological condition and/or physical condition of the fish can not be measured or kept constant for comparative purposes, and consequently certain wide variations in the killing time of particular

formulations and concentrations of insecticides may be due to these factors. One example of an unaccountable variation in the killing time of an insecticide occurred with the experiments using heptachlor emulsifiable concentrate at a concentration of 0.25 and 0.1 p.p.m. The first fish died within 10 hours and 30 minutes with a concentration of 0.25 p.p.m., where as the first fish died within 10 hours and 15 minutes with a concentration of 0.1 p.p.m. These fish weighed exactly the same, 0.75 grams, and yet this difference occurred.

Symptoms

The symptoms of insecticidal poisoning followed a rather set pattern and were practically the same for all insecticides. The first noticeable symptom was the development of a definite irritability or excitability of the fish which gradually increased in intensity. This increase in irritability caused the fish to become exceedingly sensitive to vibrations and during part of the pattern would swim wildly about when the side of the aquarium was tapped or jarred. Eventually the fish would swim to the surface of the water, often in a vertical position, and upon tiring would slowly sink to the bottom of the aquarium. This stage was followed by a loss of equilibrium accompanied by muscular spasams and convulsions. The final stages of death were characterized by an ataxia. Just before death the fish would lie on the bottom of the aquarium with their opercula and pectoral fins exhibiting the only perceptable movement. The only variation in the pattern which occurred with the use of different insecticides was the length of time involved in the sequence of events.

A characteristic feature of the fish which were killed by the insecticides was a definite blanching of the skin. A microscopic examination of the skin revealed that the effect was caused by a contraction of the chromatophores of the skin. It is assumed this is part of the overall pattern which is due to the effects of the insecticides on the nervous system.

Summary

A total of 81 experiments were conducted in which 810 fish were used to determine the toxicity of seven different insecticides in various forms to rainbow trout.

The insecticides tested were chlordane, heptachlor, aldrin, dieldrin, toxaphene, methoxychlor and DDT. The formulations tested were emulsifiable concentrates, wettable powders, and dusts. Dusts were not available for heptachlor, toxaphene, and methoxychlor.

The desired quantities of emulsifiable concentrates and wettable powders in the form of a 1:1,000 stock solution were applied to a known quantity of water in the aquaria, whereas the desired quantities of dust were weighed and sifted on to the surface of the water.

The fish were exposed to the insecticides until a 100 per cent

mortality occurred or for a period of 24 hours. Those fish which were still alive at the end of 24 hours were placed in fresh water and observed until they died or for an additional 72 hours. Concentrations tested were 0.05, 0.1, 0.25, 0.5, and 1.0 p.p.m.

Ten fingerling, hatchery-raised rainbow trout were used in each experimental aquarium and nine or more in each control aquarium. Equivalent amounts of inert materials which were applied in the experimental aquaria were also applied in the control aquaria.

DDT was the least toxic of all the insecticides tested except heptachlor wettable powder; however, chlordane was only slightly more toxic. Dieldrin and toxaphene were the most toxic. The relative toxicity of heptachlor, aldrin, and methoxychlor varied considerably with the different formulations and concentrations used.

The emulsifiable concentrates proved to be the most toxic. A variations occurred in the toxicity of the wettable powder and dust. Chlordane wettable powder was the most toxic, whereas aldrin, dieldrin, and DDT dusts were the more toxic.

None of the inert materials which were applied to the control aquarium had any ill effects upon the trout.

Some factors, both biological and chemical, which may have affected the results are discussed.

The symptoms of insecticidal poisoning and general behavior pattern of the fish are described.

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