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F. F. Hooper
A. R. Grzenda

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STUDIES ON THE USE OF TOXAPHENE AS A FISH POISON[1]

Frank F. Hooper and Alfred R. Grzenda

Michigan Department of Conservation
Ann Arbor, Michigan

Abstract

Tests on the insecticide toxaphene performed in the laboratory using standard bio-assay procedures indicated that this chemical is more toxic to fish in hard water than in soft water. Its toxicity to the bluntnose minnow is increased approximately three-fold by raising the temperature from 50° F. to 75° F. This insecticide was found to be much more toxic to the bluntnose minnow than to any of four aquatic invertebrates tested. This laboratory finding supports field observations made on two Michigan lakes which indicate that many groups of invertebrates killed by toxaphene treatment reappear while the lake water is still toxic to fish.

Detoxification of water stored in the laboratory proceeds most rapidly when it is exposed to light, when abundant oxygen is present, and when the temperature is high. Hard water (alkalinity, 212 p.p.m .) detoxifies more rapidly than soft water (alkalinity, 6 p.p.m.). In the laboratory, the detoxification process is greatly accelerated by water movements which bring the toxicant in contact with the walls and bottom of the aquarium. The detoxification rate is increased by the addition

[1] Contribution from the Institute for Fisheries Research

of a substrate of glass marbles or gravel. Substrates of sand and mud assist detoxification to a lesser extent. Sterilization of the substrate with dilute formalin and carbolic acid inhibits detoxification; this indicates that microorganisms are responsible for reducing toxicity.

Introduction

Recently there has been considerable interest in the use of the insecticide toxaphene (chlorinated camphene) as a fish poison. The present study of the influence of environmental conditions upon the toxicity of this chemical to fish and fish-food organisms was undertaken with the hope of acquiring a better understanding of its proper use in fish eradication.

The effectiveness of toxaphene as a fish toxicant was recognized when dead fish appeared in waterways adjacent to fields which had been sprayed or dusted with the chemical (Lawrence, 1950; Tarzwell, 1950). Extensive laboratory tests have since been made of its toxicity to fish. The lower limits of toxicity as reported in the literature range from 0.2 p.p.m. to less than 0.005 p.p.m. depending upon species of fish, place of test (aquaria or earthen pond), and undoubtedly upon other experimental conditions which in many papers have not been clearly defined. Duodoroff, Katz, and Tarzwell (1953) estimated that the median tolerance limit of goldfish exposed for 24 hours to toxaphene dust was about 0.025 p.p.m. They believed the 10-day median tolerance limit of goldfish to be somewhat below 0.05 p.p.m. Lawrence (1950) found that a concentration of 0.05 p.p.m. killed goldfish, largemouth bass, and bluegills in aquaria but noted that this concentration did not kill the same species when

tests were made in an earthen pond. Surber (1948) found that a concentration of 0.02 p.p.m. did not kill goldfish but was lethal to 10 other species of freshwater fish. Trout fingerlings were killed at a concentration as low as 0.005 p.p.m. Tanner and Hayes (1955) reported that a variety of concentrations ranging from 0.05 to 0.2 p.p.m. all killed carp in about the same length of time (180 minutes). Both the laboratory test of Duodoroff, Katz, and Tarzwell (1953) and the field trials of Tanner and Hayes (1955) have indicated that long exposure periods are required to kill fish in the lower ranges of concentration. Many of the discrepancies in reported values for toxicity doubtlessly can be traced to the fact that laboratory tests have been of short duration.

Confusion concerning lethal concentrations of toxaphene has perhaps been in part responsible for the difficulties encountered in using it for fish eradication. On the basis of experiments showing that 0.1 p.p.m. of toxaphene killed carp at 65° F. in 72 hours, Hemphill (1954) used this concentration in treating two shallow Arizona lakes. These lakes apparently detoxified promptly. The concentration used by Hemphill (0.1 p.p.m.) is 20 times greater than the estimated 10-day median tolerance limit to goldfish given by Duodoroff, Katz, and Tarzwell (1953). This suggests that Hemphill's recommended dosage is high and that it might be reduced considerably if the influence of various field conditions upon toxicity is known.

Hemphill believed that the pH of the water influenced the breakdown of toxaphene. He found that alkaline waters lost their toxicity within four weeks. Other workers, however, have noted that toxicity may be persistent even in alkaline waters. A reservoir treated by Tanner and Hayes (1955) remained toxic to fish for more than seven months although it constantly maintained a pH greater than 8.0. A Michigan lake

(Horseshoe Lake, Alcona County) treated with a concentration of 0.1 p.p.m. remained toxic for 10 months even though the water was consistently alkaline. Water hardness may be a better indicator of chemical conditions influencing the breakdown of toxaphene than pH, but little reliable information exists regarding the effect of hardness upon toxicity and upon the rate of detoxification of toxaphene and related compounds. Temperature is known to have a profound influence upon the effectiveness of rotenone (Leonard, 1939). Temperature and light probably also influence the rate of dissipation of rotenone-bearing fish poisons (Clemens and Martin, 1953). Data concerning the influence of these conditions upon toxaphene are needed.

Records dealing with the influence of toxaphene upon fish-food organisms are fragmentary. Hemphill (1954) stated that insects are severely affected but not eliminated by it. He suggested that the reappearance of zooplankton may be used as an indication that a lake has detoxified. Tanner and Hayes (1955), however, noted that zooplankters were present before water lost its toxicity to fish.

Methods

Methods used in the toxicity studies followed closely the procedures recommended by Doudoroff et al (1951). The test fish used were the bluntnose minnow (Pimephales promelas) and the creek chub (Semotilus atromaculatus). Fish were carefully selected for uniformity of size and condition. Nearly all fish were between 2.0 and 2.5 inches in length. Prior to trials, fish were conditioned for 48 hours at the test water temperature. Two gallons of test water per minnow were used in all determinations of the median tolerance limit. To determine whether or not this ratio of water to fish was sufficiently high to avoid detoxification of the water by the fish themselves, after tests were completed at

the lowest concentrations tested (0.003 and 0.005 p.p.m.) the same water was retested with a second group of fish. The average survival time of the second group of fish in the water did not differ significantly from the average survival time of the first group of fish. This indicated that the first group had not removed enough poison to affect the solution's potency.

Although test aquaria were not aerated during bio-assays, the concentration of dissolved oxygen remained above 5 p.p.m. Controls were maintained for all experiments. Results were disregarded when more than 10 percent of the control animals died during tests in which the median tolerance limit was being determined. Only natural waters were used. The water hereafter referred to as "hard" water was taken from Flemming Creek, Washtenaw County, Michigan. This water had a methyl orange alkalinity ranging from 200 to 212 p.p.m. The "soft" water used was from Weber Lake, Cheboygan County, Michigan, and had an alkalinity of 6 p.p.m. An analysis of the mineral composition of Weber Lake water has been published (Hooper, 1953). Temperature was maintained at a constant level by immersing aquaria in a water bath whose temperature was thermostatically controlled. The temperature fluctuation did not exceed 5° F.

The index used to compare absolute toxicities in this study was the 24-hour median tolerance limit (TLm). Procedures used in deriving this datum are those given by Litchfield and Wilcoxon (1949). Percentages of animals surviving 24-hour test periods at various concentrations were plotted on logarithmic probability paper (Codex 3128). Ten experimental animals were used at each concentration tested. In cases of either 100 percent or 0 percent survival, a corrected percentage was plotted from a table of maximal and minimal corrected probits. A straight line was then fitted by inspection to the plotted points. Particular attention

was given to points in the 30 percent to 70 percent range of survival. A chi square test was then made of the fit of the line to the plotted data. If the fit was poor, a second line was fitted and tested. The median tolerance limit was determined by reading the concentration at which the fitted line intercepted the 50 percent survival ordinate. Confidence limits of the median lethal dose were calculated from the plotted data (c.f. Litchfield and Wilcoxon, p. 102).

Invertebrates used in toxicity tests were conditioned for 48 hours at the experimental temperature. The Daphnia magna tested were secured from the laboratory culture maintained by the Department of Zoology, University of Michigan. All other invertebrates were collected in the field. Mayfly nymphs (Ephemera simulans) were taken from Cedar Lake, Washtenaw County, Michigan. Asellus intermedius and Gammarus fasciatus were obtained from Houghton Creek, Ogemaw County, Michigan. The toxaphene used in all experiments was an emulsified concentrate marketed under the trade name Cooper-Tox, by William Cooper and Nephews, Chicago, Illinois. It contains six pounds of technical toxaphene per gallon.

Influence of Alkalinity and Temperature upon Toxicity

Of the water properties easily measured and generally available from lake survey records, temperature and alkalinity are perhaps the ones most likely to influence toxicity. In Michigan lakes, alkalinity appears to be a more discriminating indicator of chemical conditions than pH. Low alkalinity ordinarily indicates that acid water is present at some time of the year, but that the pH fluctuates considerably. High alkalinity usually indicates that pH fluctuations are small and that the pH is maintained near that of a calcium bicarbonate solution (pH 8.3).

The 24-hour median tolerance limit (TLm) of the bluntnose minnow to toxaphene increased from 0.02 in hard water (212 p.p.m.) to 0.036 in

soft water (6 p.p.m.) when tests were made at a temperature of 50°F. (Table 1). The 95 percent confidence limit of these TLm values overlap slightly, however the 90 percent limits are separate. Both of these test waters were alkaline during the trials. The hard water had a pH of 8.6; the soft water a pH of 8.1. The pH of the hard water remained practically constant but the soft water fluctuated somewhat, falling as low as 7.4 during some of the 24-hour tests.

The effect of temperature upon toxicity was more pronounced. The 24-hour TLm increased from 0.0057 p.p.m. at 75° F. to 0.020 p.p.m. at 50° F. (Table 1). Similar increases in potency with increasing water temperatures have been noted in the case of rotenone (Leonard, 1938; Burdick, et al., 1955).

Comparative Toxicity to Fish-food Organisms

All fish-food organisms were tested in hard water at 55° F. At this temperature, stocks of invertebrates could be maintained in the laboratory and there was little or no mortality among control animals during experiments. At a lower temperature (45° F.) Daphnia became quiescent and so insensitive to the chemical that a 24-hour TLm could not be determined. All invertebrates were selected for uniformity of size. Only the early instars of Daphnia were used.

The 24-hour TLm of all invertebrates tested was much higher than that of the bluntnose minnow. Gammarus was most sensitive to toxaphene but its TLm at 55° F. (0.06) was three times that of Pimephales at 50° F. (0.02). Nymphs of the mayfly Ephemera simulans were most tolerant of the chemical. Nearly 10 p.p.m. were required to kill 50 percent of the nymphs in 24 hours. The observation of Tanner and Hayes (1955) that lakes treated with toxaphene may support large populations of Daphnia while they are toxic to fish is well substantiated. The 24-hour TLm of

Table 1.--The 24-hour median tolerance limit (TLm) of certain invertebrates and fish to toxaphene

(6 concentrations tested in determining each TLm)

SPECIES	Test Water		TLm (p.p.m.)	Confidence interval (0.95)
	Methyl orange alkalinity (p.p.m.)	Temperature (° F.)		
Pimephales promelas	6	50	0.036	0.026 - 0.050
Pimephales promelas	212	50	0.020	0.014 - 0.028
Pimephales promelas	212	75	0.0057	0.0041 - 0.0080
Gammarus fasciatus	212	55	0.060	0.014 - 0.22
Asellus intermedius	212	55	0.10	0.029 - 0.34
Daphnia magna	212	55	1.5	0.79 - 2.8
Ephemera simulans	212	55	9.5	5.6 - 16.0

Daphnia at 55° F. was 75 times greater than that of Pimephales at a temperature of 50° F. These results indicate that Daphnia can survive in solutions of toxaphene strong enough for fish eradication.

Studies made on the benthos of two Michigan lakes treated with 0.1 p.p.m. toxaphene indicate that, at this concentration, a large part of the invertebrate fauna is killed within a month (Table 2). In Horseshoe Lake, mollusks (Unionidae, Sphaeriidae, gastropods) and certain aquatic obligochaetes (Tubificidae) were the only groups which did not appear to be harmed. A few midges and mayflies survived for more than three weeks (September 21-October 15) but these species were not collected the following spring.

By mid-summer of the year following treatment, repopulation by most groups of invertebrates was well underway. In Horseshoe Lake, dragonfly nymphs reappeared in abundance by July 29 although many of the aquatic diptera, e.g. Chaoborus, were not present. A highly successful hatch of the midge Tendipes plumosus produced a standing crop of benthos in McCarthy Lake on June 23, 1955 (11 months after treatment) that appeared to be somewhat larger than the crop present prior to treatment. This spectacular repopulation of the lake bottom was perhaps made possible by the absence of fish and other predators.

Duration of Toxicity

Waters treated with toxaphene may retain their toxicity for long periods. Although Hemphill (1954) indicated that "alkaline waters" can be replanted with fish four weeks after treatment, Tanner and Hayes (1955) found that toxicity persisted for more than seven months in a Colorado reservoir. Of the ten Michigan lakes treated in 1949 and 1950 with Fish-Tox (a fish poison now known to have contained toxaphene), six

Table 2.—Invertebrates collected before and after treating two Michigan lakes with toxaphene (0.1 p.p.m.)

Lake and dates of samples	Number of samples	Average number of animals per square foot						
		Diptera	Ondonata	Ephemeroptera	Oligochaeta	Mollusca	Miscel-laneous	All organisms (total)
Horseshoe Lake [1] Sept. 20, 1954 (Before treatment)	12	92	6.4	48	0.5	1.78	3.0	156
Oct. 15, 1954	12	15.6	...	4.0	4.8	4.8	0.40	29.2
April 29, 1955	6	0.4	...	...	1.2	0.8	...	4.0
July 29, 1955	6	3.4	10.8	...	10.8	6.4	...	31.2
McCarthy Lake [2] Aug. 3, 1954 (Before treatment)	15	428	...	1.2	...	...	0.4	43.2
Aug. 24, 1954	6	0.64	...	...	0.64	0.6	...	1.9
May 25, 1955	6	...	...	...	4.4	...	...	4.4
June 23, 1955	6	916	...	...	...	16	...	932

[1] Treated on Sept. 21, 1954

[2] Treated on Aug. 4, 1954

remained toxic for more than eight months. One of these lakes was toxic for 33 months. The amount of toxaphene in the Fish-Tox used in the Michigan lakes is not known, hence it is impossible to say what concentration brought about such persistent toxicity. The detoxification period of four bodies of water in Michigan treated in 1954 and 1955 using a concentration of 0.1 p.p.m. toxaphene ranged from 1 to 10 months. A shallow pond freshened by a flow of spring water detoxified most rapidly (two months). Shallow lake basins also detoxify rapidly. In the case of Horseshoe Lake, with a shallow and a deep basin joined by a narrow connection, the shallow basin lost its toxicity three months before the deep basin. Although persistent toxicity may be undesirable from the standpoint of preventing fishing for an extended period, it has the advantage of assuring a complete kill, and thus may be especially useful in treating those shallow ponds and lakes which detoxify too rapidly when treated with rotenone (Clemens and Martin, 1954). A toxicant which breaks down rapidly may not have an opportunity to mix throughout the lake basin before losing its potency. A toxicant that can be used in the fall after the close of the fishing season and that will retain its toxicity through the fall overturn period but will detoxify in time for spring or early summer planting, would be highly desirable for many Michigan lakes.

In terrestrial soils, microorganisms utilize toxaphene and render it non-toxic (Smith and Wenzel, 1947). It is not known whether or not microorganisms of hydrosols break down this compound. The slow detoxification of lake waters is perhaps circumstantial evidence that detoxification of the water mass takes place only at interface boundaries. The more rapid detoxification of shallow basins as compared to deep basins suggests that a high ratio of interface surfaces (bottom soil-water and air-water) to water volume favors detoxification.

Detoxification during Laboratory Storage

Water samples were treated with 0.1 p.p.m. toxaphene and then stored in the laboratory in glass containers under a variety of conditions. With one exception, all water samples were 30 liters in volume. Storage conditions for various waters are listed in Table 3.

The influence of light was investigated by storing samples in an aquarium from which all light was excluded by a covering of black paper. All other samples were stored under laboratory fluorescent lamps. One water sample was stored in a water bath at a temperature of 55° F.; others were stored at laboratory temperature (75° F.). One sample was of low alkalinity (6 p.p.m.); others were of high alkalinity (212 p.p.m.). One water sample (6 liters in volume) was boiled to reduce the oxygen tension. After cooling, toxaphene was added and samples were stored in sealed 1-gallon jugs. The oxygen content of this sample was 0.69 p.p.m. as compared to the range of from 6.4 to over 8.0 p.p.m. for other samples. The low oxygen samples were stored for 23 weeks while all others were stored for only 12 weeks. To test the influence of water circulation and aeration, one sample was supplied with an air stone delivering a flow of 2 liters of air per minute. A control sample was stored for 12 weeks under laboratory conditions of temperature and light. This water was exposed to the air, but was not aerated. In the case of the control, the fish toxicant was added after the period of storage.

The potency of samples after storage was tested by recording the survival of minnows after one, two, and seven days of exposure. A chi square test was made of the hypotheses that survival of fish in water stored under various test conditions did not differ from the survival of fish in control water. This hypothesis could be rejected when the 95 percent probability level was used only in the case of the two water

Table 3.--Effect of storage for a 12-week period under various laboratory conditions upon toxicity of water treated with 0.1 p.p.m. toxaphene

Toxicity after storage measured by the survival of minnows

Methyl orange alkalinity (p.p.m.)	Storage conditions				Percentage of 10 test fish surviving					
					Semotilus atromaculatus			Pimephales promelas		
	Temperature (° F.)	Aeration (water circulation)	Dissolved oxygen (p.p.m.)	Light	24 hrs.	48 hrs.	7 days	24 hrs.	48 hrs.	7 days
212	75	none	6.4-7.0	light	100	100	90	50	10	10
212	75	air stone	8.0+	light	100	100	80	100	100	50
212	75	none	6.4-7.0	dark	50	10	0	0	0	0
212	55	none	6.4-7.0	light	30	0	0	10	0	0
212[1]	75	none (sealed)	0.69	light	30	20	0	...	...	...
6	75	none	6.4-7.0	light	30	0	0	30	10	0
212 (Control[2])	75	none	6.4-7.0	light	20	0	0	10	10	0

[1] Water stored for 23-week period.

[2] Control water stored as indicated but toxicant added immediately before testing.

samples of high alkalinity, stored under normal laboratory conditions of temperature and light, and with abundant oxygen (Table 3). When Semotilus was used as the test fish, both the sample aerated with an air stone and the sample of standing water (without aeration) gave a survival significantly higher than that of control water for 24-hour, 48-hour, and 7-day test periods. In tests with Pimephales, survival was significantly greater than that of controls for the aerated sample during all three test periods, and for samples of standing water for the 24-hour test period. Survival of fish in water stored under other combinations of conditions did not differ significantly from that of controls. Further tests showed that in the case of standing water, the toxicity did not change enough to influence the survival of minnows for the first six weeks of storage, i.e., during this period the test fish died about as fast in stored water as they did in the water before storage. After this period, however, survival in the stored water increased steadily.

Circulation of water in aquaria during storage increased survival. Water containing 0.1 p.p.m. toxaphene circulated by an air stone, using a flow rate of two liters of air per minute, detoxified in some trials after 168 hours of storage. This detoxifying influence appeared to be associated with the water movements caused by the aerator rather than with the physical or chemical changes produced by air bubbles in their passage through the solution. This was indicated by several tests in which a mechanical stirrer was substituted for the air stones. By rapid mechanical agitation, 30 liters of water could be detoxified within 48 hours.

The sharp decrease in detoxification time brought about by water circulation suggested that the toxicant was being removed by some type of absorption on the walls of the aquaria. To test this hypothesis, the

water-glass contact area in the aquarium was increased approximately three-fold by lining the bottom of the aquarium with glass marbles. This reduced the time required for detoxification using an air stone as a water circulation device from 168 hours to 24 hours. Similar results were obtained when gravel was substituted for marbles as a substrate (Table 4). Other substrates such as sand and mud also had a detoxifying effect, although they appeared to be less active as detoxifying agents than gravel or marbles.

After a water sample had completely detoxified, the water was siphoned off, and the substrate and the inside surfaces of the aquarium were washed thoroughly with two liters of fresh water. The wash water gave no indication of toxicity when tested with minnows. However, a toxic material believed to be toxaphene was recovered from the inside of the aquaria and from the marble and gravel substrates by the following procedure: the aquarium and substrate were washed with 50 milliliters of benzene. The benzene was evapored to dryness in an oven at 60° C. The residue was treated with three milliliters of isopropyl alcohol. This extract was added to three liters of water and this mixture was then tested with minnows. Extracts prepared in this way killed Semotilus in four hours. These fish showed distress reactions characteristic of fish dying from toxaphene poisoning. A second 3-liter water sample treated with three milliliters of isopropyl alcohol served as a control.

If the substrate of gravel or marbles was removed from the aquarium at 6-hour intervals, sterilized with a dilute solution of either formalin or carbolic acid, washed with distilled water, and returned to the aquarium, the detoxifying influence of the substrate is inhibited (Table 4). If this sterilization procedure is stopped, the substrate within 48 hours again becomes a detoxifying agent. This experiment

Table 4.—Influence of substrate upon rate of detoxification during storage of water containing toxaphene

Storage conditions			Biossay of stored water with Semotilus atromaculatus				
Substrate[1] in aquarium	Duration of storage (hours)	Concentration of toxaphene (p.p.m.)	Number of test fish	Percentage survival 24 hours	48 hours	7 days	Mean survival (days)
None	0	0.1	20	20	5	0	0.85
None	24	0.1	10	20	0	0	0.70
None	48	0.1	10	10	10	0	0.76
Marbles	24	0.1	20	100	90	90	[2]
Marbles (sterilized[3])	24	0.1	10	70	30	10	[2]
Gravel	24	0.1	10	70	70	70	[2]
Gravel (sterilized[4])	24	0.1	10	30	0	0	0.87
Gravel	48	0.1	10	90	80	80	[2]
Gravel	48	0.2	10	90	70	30	[2]
Sand	24	0.1	10	40	10	0	1.1
Sand	48	0.1	10	80	70	70	[2]
Mud	24	0.1	10	60	20	10	[2]
Mud	48	0.1	10	80	80	80	[2]

[1] Water circulation maintained in all aquaria by air stones delivering a flow of 2 liters per minute.
[2] Not determined, experiment terminated after 7 days.
[3] Aquarium and substrate sterilized with dilute formalin.
[4] Aquarium and substrate sterilized with dilute carbolic acid.

strongly suggests that detoxification is due to removal of the chemical by microorganisms attached to the substrate. Glass slides suspended in water samples being detoxified rapidly developed a fauna and flora of microorganisms. Within 48 hours, a variety of protozoa, rotifers, diatoms, and bacteria were present. Which of these organisms are concerned in the removal of toxaphene from solution was not determined.

Use of Toxaphene in Lake Management

Final judgement as to whether or not this insecticide can be used as a fish toxicant without risking prolonged periods of toxicity must await results of experimental treatments now underway on several lakes. Treatments of Michigan lakes to date and the above laboratory experiments indicate that a concentration of 0.05 p.p.m. of emulsified toxaphene is sufficient for fish eradication. Laboratory tests suggest that somewhat lower concentrations can be used successfully in shallow hard-water lakes having a high range of temperature (70°-80° F.). The detoxifying influence of the substrate that was demonstrated in the laboratory suggests that lakes in which the entire water mass can circulate freely over the bottom sediments are apt to detoxify most rapidly. Conversely, detoxification is likely to proceed slowly or not at all in the hypolimnia of deeper lakes, during summer stagnation. Since the hypolimnion may retain its toxicity longer than the epilimnion, both strata should be tested with live fish before restocking. Survival of test fish in only the epilimnion should not be considered reliable evidence that a lake has detoxified, since upwelling of toxic water from the hypolimnion may make the shallower regions toxic again.

The laboratory results suggest that for prompt detoxification, treatments should be timed to take advantage of natural water movements.

Thus late spring or summer treatments should be avoided. Early fall treatments would make use of the detoxifying influence of the fall overturn.

This chemical appears to be more toxic to fish than to many, if not all, aquatic invertebrates. Since bottom invertebrates killed by toxaphene reappear in the lake before it loses its toxicity to fish, food organisms are likely to be present by the time the lake is ready for restocking.

INSTITUTE FOR FISHERIES RESEARCH

By Frank F. Hooper and Alfred R. Grzenda

Approved by G. P. Cooper

Typed by A. J. Hamilton

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