THE EFFECT OF SOME ENVIRONMENTAL FACTORS ON THE TOXICITY OF DDT TO FISH

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This is to certify that the

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Peter J. Tait
Major professor

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THE EFFECT OF SOME ENVIRONMENTAL FACTORS ON THE TOXICITY OF DDT TO FISH

By

Robert Nels Thompson

A THESIS

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Robert N. Thompson

May, 1953

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INTRODUCTION

In the science of insect control probably the most significant development of the past decade has been the recognition and use of DDT. The insecticidal potency of this chlorinated hydrocarbon, when combined with the extended action of its residue, has made DDT the most widely known and most widely used of all the modern insect-killing compounds.

In practice, DDT is effective in extremely minute concentrations and therefore it is greatly diluted with petroleum fractions, dusts, or water before application is made. However, since DDT is one of the least water soluble organic compounds, some form of processing becomes necessary when its dispersion through water is desirable. Various formulations of wettable powders and emulsions are used for this purpose.

Because of its extensive use on or near water areas, particularly as a mosquito larvicide, DDT has been brought in contact with many other forms of aquatic life. When used on such a large scale, the effect of this chemical on even one group of organisms becomes important. For this reason various state, federal, and other agencies have done many experiments in an effort to obtain a more complete understanding of the relationship between different doses and formulations of DDT and their effect upon aquatic life in general.

The most intensive research directed toward any one group of aquatic organisms has been done with fish. Laboratory and field tests have resulted in a great accumulation of data, some of which are contradictory, and much of which are so variable that they lend little support to any conclusion except that DDT is generally toxic to fish.

In 1945, Eide et al reported that goldfish used in laboratory tests were killed by concentrations of DDT in excess of .2 ppm when applied as water suspensions. Odum and Sumerford (1946) found that Gambusia affinis, the mosquitofish, was much less resistant to DDT in acetone than were goldfish; the average lethal dosage being .1 ppm for goldfish and .01 ppm for mosquitofish. In South Africa, Pielou (1946) observed that .03 ppm DDT in paraffin produced 100 percent mortality in young bream (Tilapia kafuensis) after 72 hours exposure, and that a .05 ppm concentration killed all the test fish within 24 hours.

In field tests, it was observed by Tarzwell (1948) that an application of .2 pound DDT per acre in the form of an emulsion gave significant fish-kills in small ponds after individual treatments. (1 pound per acre is equal to approximately .2 ppm on an acre of water two feet deep) In Pennsylvania, DDT applied to small hardwater ponds at the rate of 1 pound per acre as an oil solution, an emulsion, and as a suspension, had varying effects on the fish population of small bluegill sunfish and largemouth bass. The suspension killed very few fish; the solution

killed 50 to 60 percent of the bluegills, but very few bass; the emulsion killed all of both species. In one other shallow pond treated with a DDT oil solution at the rate of one pound DDT per acre, there was an 80 percent loss of bluegills, a 93 percent loss of red belted sunfish, and a 78 percent kill of yellow perch (Cottam and Higgins, 1946). Surber (1946) reports that using DDT in oil, sprayed on a section of river at the rate of two pounds DDT per acre, live boxes stocked with bluegills and perch showed a 79 to 97 percent bluegill mortality while the perch kill was only 10 percent. Gjullin et al (1949) observed the effect of various DDT formulations on rainbow trout in Alaskan streams and report that the maximum tolerance for this species, when exposed to an emulsion, is three ppm and, when exposed to an acetone solution formulation, is 30 ppm. Tiller and Cory, in 1947, sprayed a two acre pond with DDT in oil at a concentration of three pounds DDT per acre. After one week, the fish population of the pond, which consisted entirely of mosquitofish, (Gambusia sp.) showed no mortality. Six days later the pond received another treatment, this time using three pounds DDT per acre in a water dispersible emulsion. Observations 24 hours later showed no mortality among the fish or to frogs which the authors report were the only other animals in the pond. Quite to the contrary, in a pond which was described as being very weedy, possessing a mud bottom, and containing much litter, it was re-

ported by Pielou (1946) that a three ounce per acre application of DDT in paraffin caused a 70 percent kill of young bream.

Such wide differences in the toxicity of DDT become more understandable when one realizes the degree of influence which different conditions exert on its toxic action. Hoffman and Linduska (1949) report that, besides formulation variation and species susceptibility, there are indications that other factors affect the toxicity of DDT to fish. Young fish have less resistance than adults, and well-fed fish are not affected as much as those that are undernourished. Physical and chemical characteristics of the water, such as warm temperatures, reduced oxygen tension, and soft water all increase DDT toxicity. Field and laboratory tests indicate that clarity of the water has an appreciable bearing on toxicity. DDT at .25 pounds per acre, when applied to dirt-free aquaria, killed 84 to 100 percent of the contained rainbow, brook, and brown trout. The same dosage caused up to 39 percent mortality in these same species when applied to aquaria containing a layer of mud.(the extent of turbidity was not mentioned, nor were the numbers of fish involved, although the mortality percentages indicate that the numbers used were adequate to insure differences greater than would be expected by chance of sampling) This effect has also been noted in the field by Linduska and Surber (1948). On a small trout stream in Wyoming, DDT in oil, sprayed by air-

plane at the rate of 2.5 pounds per acre, resulted in a kill of only 11 cutthroat trout. This unusually light mortality was partly attributed to an almost continuous chain of beaver dams which kept the DDT formulation floating on the surface film by slowing the flow and minimizing the mixing action. Also, it is believed that many fish were saved by the activity of beavers which stirred up much organic debris and mud.

According to many workers, the presence of aquatic plants in treated waters has an influence on the toxicity of DDT. In one case, it was reported that goldfish survived when placed in a fish bowl containing a lethal dose of DDT and 10 grams wet weight of Elodea and Cabomba (Odum and Sumerford, 1946). Tarzwell (1948) also noted the modifying effect of plants on DDT. He observed that one heavily vegetated pond, when subjected to 14 DDT treatments at the rate of .1 pound of DDT per acre, showed no fish-kill whereas another deeper pond, which contained no rooted vegetation, had a significant fish-kill after receiving the same number of treatments at a DDT concentration of .05 pound per acre.

These reports of variable mortalities under different conditions point to the need of investigations directed toward the determination and evaluation of the modifying factors. The work presented here is concerned with the influence of various environmental components on the toxicity of DDT to fish. It endeavors to measure the change

in the toxicity in different environments by observing the effect of the DDT on fish placed in these environments. This form of experimentation is biological assay, which D. J. Finney, the noted British statistician, defines in its broadest sense as "the measurement of the potency of any stimulus by means of its reactions on living material."

The experimental plan consisted of conducting tests on the toxicity of two formulations of DDT in the presence of separate or isolated environmental factors. One series of tests in the most simple type of environment was used as a point of reference to which the results of tests with other environmental factors were compared.

At the outset, it was planned to thoroughly investigate the effects of several environmental components but, after the experiment was well underway, it became evident that the restrictions of time and equipment would limit the extended testing to only those environmental factors which produced the most pronounced toxicity modifications in the initial tests. This resulted in the abandonment of one formulation of DDT as a test toxicant and in the running of only three tests on the influence of aquatic plants, soft water, and washed sand. Extended testing, consisting of six or more replications of the environment, was done with warm water, cold water, moving water, and water containing organic muck.

EXPERIMENTAL MATERIALS

The northern red belly dace, Chrosomus eos, was used as the test organism in this work. All fish were taken from the Michigan State College Experiment Station at Lake City during the period of July through October of 1952. Only adults were selected, and, following the recommendations of Douderroff et al (1951), the length of the largest test individual was not more than 1.5 times the length of the smallest specimen. The modal fish was approximately 4.5 centimeters in length and weighed 1.2 grams.

The 28 gallon rectangular test aquaria were 16 inches wide and about 25 inches long. The sides were made of glass and the ends and bottom were composed of a talc stone called "Alberene".

The water for the experiment came from the Michigan State College water supply reservoir which is maintained by several deep wells located on the campus. This source was chosen over that of a surface water because the composition of the well water was known and was less likely to vary during the course of the experiment. A chemical analysis of this water was made in the spring of 1952 by the Michigan State College Department of Sanitary Engineering and showed the following approximate composition:

dissolved oxygen....	4 ppm	total hardness.....	310 ppm
carbon dioxide.....	40 ppm	calcium hardness...	240 ppm
alkalinity.....	320 ppm	magnesium hardness..	70 ppm
pH.....	7.1	sulfates.....	14 ppm
total solids.....	310 ppm	chlorides.....	16 ppm
silicon dioxide.....	8 ppm	iron.....	0.2 ppm

Two formulations of the toxicant were used in the experiment. A commercial brand of 50 percent DDT wettable powder ("Deenate", E. I. DuPont, Wilmington, Del.) was used at the beginning of the experiment, but the major part of the testing was done with a DDT emulsion formulation consisting of 20 percent technical grade DDT, 60 percent xylene, and 20 percent emulsifier ("Triton B-1956", Rohm and Haas Chemical Co., Philadelphia, Pa.).

LABORATORY PREPARATIONS

The test fish were transported from Lake City to the college in milk cans and were placed in a large concrete holding tank for at least 10 days to permit the sick and weak fish to die off before the survivors were readied for experimental purposes. During the holding period, the fish were fed finely ground dry dog food which they readily took. Although the composition of the water in the holding tank was generally the same as that of the water supply, all fish were acclimatized for a period of five days prior to the starting of a test by placing them in water which was almost identical in composition to that used in the experiments. During the last two days of this acclimatizing period, the fish were not fed. This practice served to bring about more uniformity of the test animals by emptying their digestive tracts before exposure to DDT.

In the preparation of the wettable powder formulation for introduction into the test aquarium, the procedure was merely to place the dose in a small beaker of test water and stir until the particles separated and became evenly distributed in the beaker. The emulsion formulation was made with a high percentage of emulsifier to give small particle size. This formulation, when used with water in the ratio of one part emulsion to 10,000 parts water, produced a moderately stable emulsion of extremely fine particles after a short period of vigorous shaking. As a precaution against

any great variation in particle size, all test emulsions were made with a one part emulsion to 10,000 parts water dilution in half-filled liter bottles which were given a rough form of standardized agitation consisting of 30 vigorous shakes. Preliminary tests showed that a variable procedure for making emulsions with this formulation directly influenced toxicity and that some type of standardization was necessary for valid experimentation.

The water on the campus is chlorinated, and for this reason it was necessary to agitate, by aeration, 100 liter volumes of test water for 24 hours to obtain satisfactory chlorine removal. This agitation dispelled much carbon dioxide from the water causing an appreciable precipitation of soluble magnesium and calcium bicarbonates as insoluble normal carbonates. In this way, the high hardness of the well water (310 ppm) was reduced to a hardness of 200 ppm which more closely approximates the hardness condition of natural surface waters. Also, as a direct result of the loss of carbon dioxide, the reserve pH of the water was brought out so that, in all tests, the pH was above 8.0 and was usually 8.5.

The tests were carried out in 80-liter volumes of water 11 inches deep. All test water was transferred from the aeration tanks, used for chlorine removal, to the test aquaria in order to avoid the precipitation of carbonates in the experimental environments.

The degree to which each environmental influence was

established in the test aquaria was more or less arbitrarily determined by the author's judgement of what constituted an average natural condition, and also by the available supply of the aquatic pondweed, Naias flexilis. Once the weight of this plant material (40 grams per test) was determined, other environmental factors which could be weighed were used in the same amounts so that the influence of equal weights could be compared. This weight of pondweed was judged to represent a moderate to sparsely vegetated aquatic situation.

The warm water tests were carried out at a temperature of 26 degrees centigrade with not more than a one-degree plus or minus variation. All tests, with the exception of cold water, were conducted at this temperature. The cold water tests were run in a constant temperature room at a temperature of 16 plus or minus one degree centigrade.

A moving water environment was produced by introducing air through two ¼-inch glass tubes that extended to the bottom of the test aquarium at its center and which were connected to a compressed air supply.

Organic muck was taken from the basin of a woodlot pond, dried, ground to a powder, sifted through several layers of cheese cloth, and presoaked before being added to the aquaria. The turbidity of the test water was measured with a colorimeter one minute after the organic material had been stirred into the aquaria, and also after 14 hours of standing. Forty grams of the dried bottom material were used in each test of this environmental factor.

The effect of white sand, which was collected at Lake Lansing in Ingham County, was tested by adding 40 grams of this bottom material to each aquarium after the sand had been thoroughly washed in soft water and dried.

Equal parts of conditioned tap water and distilled water were used to make a soft water environment. Since distilled water by itself was found to be lethal to the test fish, it was necessary to experiment with different proportions of the tap water-distilled water mixtures to determine a satisfactory ratio. It was observed that a mixture containing 25 percent or less of conditioned tap water produced some mortality. The probability of testing with a toxic environment was then greatly reduced by increasing to 50 percent the amount of tap water present in the mixture. This resulted in a water with a total hardness content of 90 ppm.

EXPERIMENTAL PROCEDURE

In each test, the desired dosage of DDT was evenly distributed in the water after the specific environmental factor had been established and before the fish were added. In the case of the wettable powder, which tended to settle out, the dosage was kept partly in suspension by the constant agitation of the test water with large air bubbles.

With the exception of two tests in moving water, one of which was carried out with eight fish and the other with nine, between 10 and 13 fish were used in each test of the emulsion. These numbers, in 80 liters of test water, provided at least six liters of water for each fish tested. In preliminary tests, using only one liter of water per fish, the corresponding decrease in the total amount of DDT available to the fish caused a reduction in mortality. Prevost et al (1948) found that at least two liters of water per test fish (Chrosomus eos) were necessary before the amount of DDT absorbed by the fish did not reduce the concentration to a sub-lethal level. They observed that a concentration of DDT which produced 100 percent mortality with two liters of water per fish caused no kill when .2 liter per fish was used.

The duration of the tests was determined by the speed with which the DDT formulation acted upon the test fish. The wettable powder was slow in producing its toxic effects and, after running the first two tests using a 72-hour

period, the remainder of the wettable powder experiments were conducted with a 96-hour exposure period. A 48-hour period was used for testing the emulsion which affected the fish more quickly. It was generally observed that most of the fish which were destined to die in a given DDT concentration could not tolerate the full length of the exposure period. In other words, the fish which were not affected by the concentration during the exposure period, were usually not affected by that same dosage during a much longer period. In the event that any fish were in distress at the end of an exposure period, they were immediately transferred to 4-liter "recovery jars" which contained only conditioned tap water at the same temperature as the water of the test. These fish were kept in this water until they either died or no longer showed DDT toxicity symptoms which consisted of excitability, tremors, and loss of equilibrium. Any mortality in the recovery jars was considered as evidence that the fish had passed beyond its recovery point during the exposure period and should be entered in the data as dying in the test.

During the running of each environmental test, certain measurements of test conditions were made. The temperature was checked as often as possible and was kept within one degree centigrade of the set standard. The pH and the total hardness of the test water were determined at the beginning and end of each test. The turbidity of the organic muck environment was measured in one test of this factor both at

the beginning and after 14 hours of the test. The oxygen content of the water was also measured at the end of this test as a check on the oxygen depleting powers of the organic material.

A control was run on every environmental factor except that of aquatic plants. In the case of two of the components which in themselves might prove toxic, organic muck and warm water, the influence of the factor was intensified. The amount of organic material added to the control water was twice that used in the tests and the temperature of the warm water was raised two degrees centigrade over test conditions.

A control was also run on the xylene emulsifier formulation at a concentration of one ppm which was over twice the strength of the formulation in any test. Everhart and Hassler (1945) found that emulsified exlene was not toxic to fingerling brook trout in concentrations up to 7 ppm, which indicates that the control concentration in this experiment was considerably below the tolerance limit of the test fish.

Occasionally during the course of the experiment a simultaneous control was run on the conditioned tap water and on the condition of the fish in the holding tank. After observing the effect of the conditioned water on the fish for a two-or four-day period, depending on the duration of the tests in which the fish were to be used, the condition of the fish was roughly checked by keeping them in the control water for three weeks without food. Any sick or distressed fish were noted after this starvation period and these obser-

vations were compared with those of other periods for an indication of any change in the condition of the stock fish. It might be mentioned here that, during the entire experiment, no fish died in the holding tank after the 10-day holding period given all new stocks of fish before use in the experiment.

The analysis of experimental results of tests in which six or more replications were made was aimed at establishing the median lethal dose of the emulsion and its 95 percent confidence limits in each environment.

Since mortality and dosage have a sigmoid rather than a linear relationship, the median lethal dosage was determined by the probit method outlined by Finney (1947). In this procedure, which is standard statistical practice for toxicity work, percent mortality is converted to probits which are plotted against log dose to transform the normal sigmoid curve to a straight line.

For data with the value of chi-square clearly significant, it was necessary to spread the dosage limits for 95 percent confidence. The degree of spread was obtained by first inserting into the computation of the standard error, a heterogeneity factor equal to the chi-square value of the data divided by one less than the number of degrees of freedom; then this value of the standard error was multiplied by an abnormal deviate (t value) under the 5 percent probability column in a t table corresponding to one degree of freedom less than exists in the data. These modifications

follow the recommendations of Finney (1947).

Experimentation on certain environmental factors, namely washed sand, soft water, and aquatic plants, was not carried to the extent that the computation of the median lethal dose was justifiable, yet some information is available from these data. Tests concerning the statistical significance of the proportional mortality difference between each of these specific environmental components and the warm water reference environment reveal whether the factor concerned influenced the toxicity of the DDT. The criterion used in examining such significance is the chi-square test (Snedecor, 1946).

In making this comparison of the summarized proportional mortalities for several dosage levels in two different environments, the data have a clear biological interpretation only when the distribution of test animals over the several dosage levels is proportional in the two series of environmental tests. For example, in the following set of hypothetical data, half of the animals died in each environmental series, but it is evident that environment "A" caused a heavier mortality for each dosage tested.

TABLE I. ILLUSTRATIVE DATA FOR STATISTICAL MODIFICATIONS

	Dose	Dead	Alive	Totals
Environment "A"	10	30	60	90
	20	30	0	30
Totals.....		60	60	120
Environment "B"	10	0	30	30
	20	60	30	90
Totals.....		60	60	120

To demonstrate the true relationship, the same proportion of the total number must be assigned to each dosage in each series. This is accomplished by inserting positive or negative values into classes with excess or deficient numbers so as to obtain an equality of proportions. Environment "B" in table I might be altered to give proportions matching those of environment "A" by adding 240 to the number surviving at dose 10. This would result in 270 animals being assigned to this dose with still 90 individuals tested at the dosage level of 20 and would make the proportion of the total numbers tested at each dose the same for both environments; i.e., 270 and 90 are in the same ratio as 90 and 30. Inspection of the preceding table will show that reducing the data of environment"B"and also reducing or inflating the data from "A" could be employed to obtain proper proportions.

This procedure of inflating and deflating data has been used by Mainland and Murray (1952) for the application of disproportionate samples to tables used in contingency tests. The technique is justifiable on the basis that these modifications, when analyzed properly, do not alter the measurement of significance. That is, differences demonstrated to be significant after the data have been reduced imply a greater significance in the original data which involve greater numbers. When testing two ratios, increasing the numbers involved, always keeping the ratios constant, increases the chi-square value proportionally. By

the same reasoning, when increasing the numbers fails to produce an apparently significant value of chi-square, the differences are not significant in the original values.

In other circumstances, if, after reducing the data, differences are not significant, then this result merely gives by its proximity to the significant level, an indication of the original ratio differences. If an inflation of the data yeilds a significant difference, this information also is only approximate and has little reliability since the additions to the data, and not true ratio differences, may have caused the resulting significant level.

In considering the final alternative, where the chi-square test does not indicate a significant difference when the data are deflated, but does reveal significance between ratios when the data are enlarged, no determination of a significant difference can be made and it is only possible to say that the probability of the ratio differences occurring by chance is somewhere near the significant level.

In the tests of the environmental factors of washed sand, soft water, and aquatic plants, the distribution of the experimental animals at the various dosage levels did not agree exactly with those proportions found among the test fish in the warm water reference environment. Therefore equal proportions at each dosage level were attained by adding dummy values to the warm water data. The following table shows the data modifications for the comparison between the washed sand and warm water results. The con-

clusions drawn from these data are given elsewhere in this report.

TABLE II

ILLUSTRATIVE DATA FOR STATISTICAL MODIFICATIONS

	Washed Sand				Warm Water			
	Dose	Dead	Alive	Total	Dose	Dead	Alive	Total
Before Addition	50	9	4	13	50	29	15	44
	40	7	5	12	40	26	16	42
	30	1	10	11	30	5	15	20
After Addition	50	9	4	13	50	30.5	15.5	46
	40	7	5	12	40	26	16	42
	30	1	10	11	30	9.5	29.5	39

It should be emphasized that when the data are inflated in this method of comparison, no conclusions are drawn from the numbers added, since these are not actual experimental data, but only imaginary values added to allow information to be extracted from the original data.

RESULTS AND DISCUSSION OF WETTABLE POWDER TESTS

TABLE III

RESULTS OF TESTS WITH
50 PERCENT DDT WETTABLE POWDER

Wettable Powder Dosage	Fish Per Test	Percent Kill	Exposure Period	Centigrade Temperature	Other Conditions*
4 ppm	9	22%	72 hrs	26 deg.	
6 ppm	7	43%	72 hrs	26 deg.	
5 ppm	10	12½%	96 hrs	18 deg.	
7 ppm	10	20%	96 hrs	18 deg.	
5 ppm	10	20%	96 hrs	26 deg.	
7 ppm	10	60%	96 hrs	26 deg.	
5 ppm	10	9%	96 hrs	26 deg.	plants
7 ppm	10	20%	96 hrs	26 deg.	plants
6 ppm	10	20%	96 hrs	26 deg.	
6 ppm	10	10%	96 hrs	26 deg.	
8 ppm	10	0%	96 hrs	26 deg.	
8 ppm	10	0%	96 hrs	26 deg.	

* pH 8.5; total hardness 200 ppm; plant concentration= 40 grams per test; control-no mortality

The failure to obtain results from which a median lethal dosage could be calculated led to the abandonment of this formulation as a test toxicant. No definite answer was found for the variable results. Chemical analyses of the test water showed that approximately five percent of the DDT added at the start of the tests remained in suspension at the end of a 96 hour exposure period. The first tests, which produced at the same dosage, a greater mortality than the later tests, were conducted during a hot weather period in August. At all times, however, the temperature did not vary more than one degree from the standard temperature condition of 26 degrees centigrade. When the results were graphed, it was noted that the general tendency was toward reduced mortality as time progressed, in-

dicating perhaps, that the formulation was decreasing in toxicity. However, chemical analysis of a supposedly random sample from the wettable powder stock supply, taken three months after the experiment was started, revealed a DDT content of approximately 47 percent. On the theory that agitation of the water by aeration might have varied sufficiently between tests to cause a change in the suspended concentration, or in the direct toxicity of DDT, small scale experiments were performed comparing strong agitation with still water at 8 ppm wettable powder concentrations. In both cases, no deaths occurred during the 96-hour exposure period.

One factor which was recognized, but not investigated, was the possibility of variation in DDT particle size when different portions of the stock powder were withdrawn for test use. A portion of powder containing a majority of relatively large DDT particles would differ markedly in its toxic properties when compared to the same weight of powder made up of smaller sized particles. If a stratification of different sized particles occurred in the stock powder, various portions of equal weight could well differ in poisonous properties. No information regarding particle size could be obtained from the manufacturer.

EMULSION TEST RESULTS

Results of More Extended Testing With 20 Percent DDT Emulsion

TABLE IV. WARM WATER REFERENCE ENVIRONMENT
TEMPERATURE 26 DEGREES CENTIGRADE

Micrograms DDT Per Liter	Fish Per Test	Percent Mortality	Exposure Period	Other Conditions*
60	11	91%	48 hrs	pH 8.5
50	10	60%	48 hrs	pH 8.5
50	10	90%	48 hrs	pH 8.5
50	12	83%	48 hrs	pH 8.5
50	12	33%	48 hrs	pH 8.5
40	10	60%	48 hrs	pH 8.5
40	10	80%	48 hrs	pH 8.5
40	10	60%	48 hrs	pH 8.5
40	12	33%	48 hrs	pH 8.5
30	10	30%	48 hrs	pH 8.5
30	10	20%	48 hrs	pH 8.5

*test volume=80 liters; total hardness=200 ppm; control-no mortality

TABLE V. ORGANIC MUCK ENVIRONMENT
TEMPERATURE 26 DEGREES CENTIGRADE

Micrograms DDT Per Liter	Fish Per Test	Percent Mortality	Exposure Period	Other Conditions*
150	10	100%	48 hrs	pH 8.1
140	10	90%	48 hrs	pH 8.1
120	10	100%	48 hrs	pH 8.1
110	10	80%	48 hrs	pH 8.1
100	10	60%	48 hrs	pH 8.1
100	10	30%	48 hrs	pH 8.1
90	10	10%	48 hrs	pH 8.1
90	10	10%	48 hrs	pH 8.1

*test volume=80 liters; total hardness=200 ppm; dried muck concentration=40 grams per test; turbidity at start of tests=34 ppm; turbidity after 14 hours=8 ppm; control-no mortality

Results of More Extended Testing With
20 Percent DDT Emulsion

TABLE VI. COLD WATER ENVIRONMENT
TEMPERATURE 16 DEGREES CENTIGRADE

Micrograms DDT Per Liter	Fish Per Test	Percent Mortality	Exposure Period	Other Conditions*
50	11	100%	48 hrs	pH 8.5
40	10	80%	48 hrs	pH 8.5
30	10	90%	48 hrs	pH 8.5
20	10	60%	48 hrs	pH 8.5
20	10	60%	48 hrs	pH 8.5
20	10	40%	48 hrs	pH 8.5

*test volume=80 liters; total hardness=200 ppm; control-no mortality

TABLE VII. MOVING WATER ENVIRONMENT
TEMPERATURE 26 DEGREES CENTIGRADE

Micrograms DDT Per Liter	Fish Per Test	Percent Mortality	Exposure Period	Other Conditions*
400	11	55%	48 hrs	pH 8.5
300	12	42%	48 hrs	pH 8.5
250	8	50%	48 hrs	pH 8.5
250	11	0%	48 hrs	pH 8.5
100	9	22%	48 hrs	pH 8.5
100	12	0%	48 hrs	pH 8.5

*test volume=80 liters; total hardness=200 ppm; control-no mortality

TABLE VIII. STATISTICAL MEASUREMENTS
ON PRECEDING RESULTS

Environment	Micrograms Per Liter: Median Lethal Dosage	Micrograms Per Liter: 95% Confidence Limits	Homogeneity Chi Square Value	Degrees of Freedom
Warm Water	38.2	30-48	23.0	9
Cold Water	18.8	14-26	3.2	4
Moving Water	418.5	244-717	6.7	4
Organic Muck	102.6	98-108	6.8	6

Figure 1. Results from the warm water environment after a 48 hour exposure period with a water temperature of 26 degrees centigrade. Percentages have been substituted in the probit scale of the ordinate and the log values of the abscissa have been converted to micrograms.

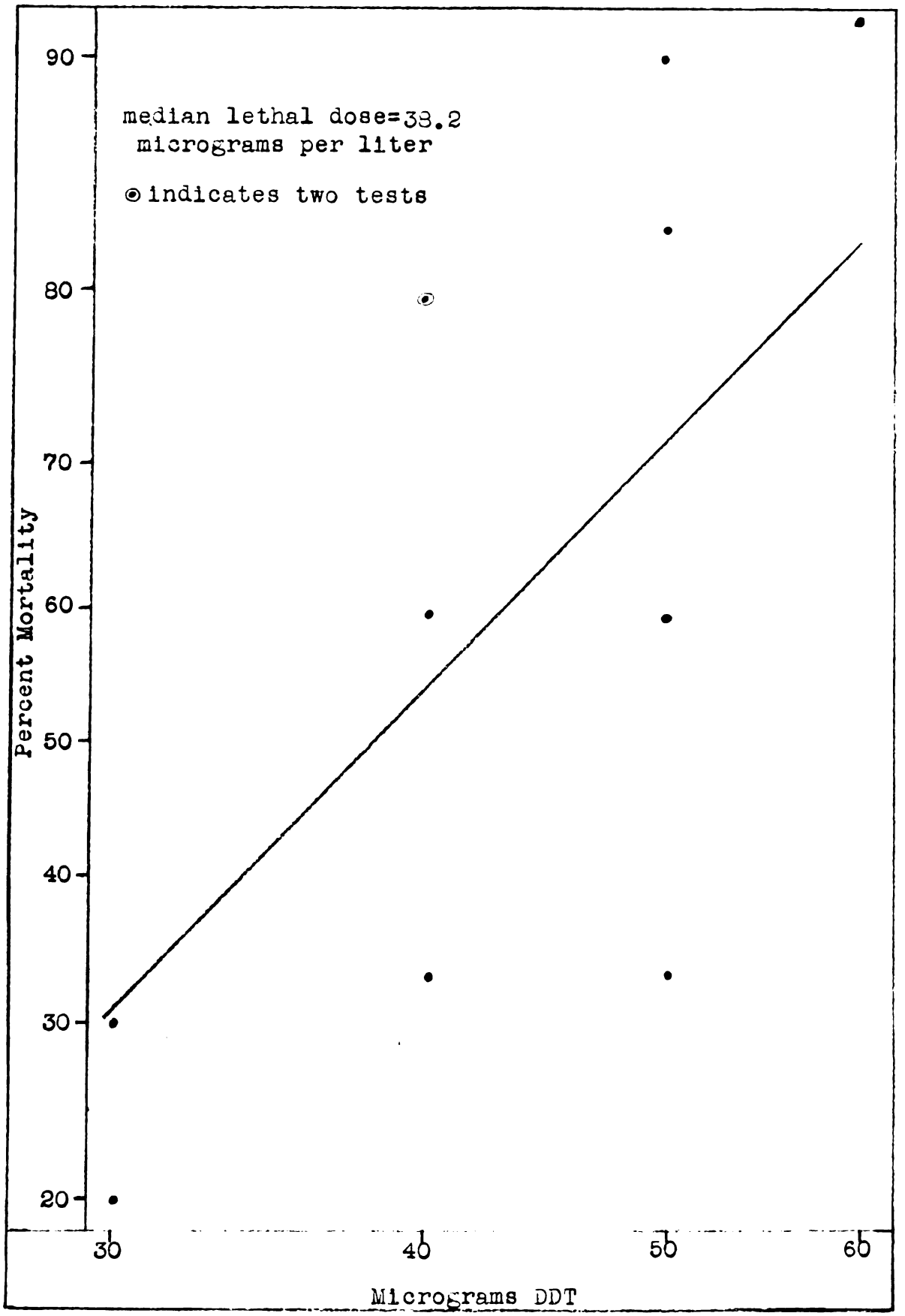

median lethal dose=38.2
micrograms per liter
⊙ indicates two tests
90
80
70
60
50
40
30
20
Percent Mortality
30
40
50
60
Micrograms DDT

Figure 2. Results from the organic muck environment after a 48 hour exposure period with a water temperature of 26 degrees centigrade. Percentages have been substituted in the probit scale of the ordinate and the log values of the abscissa have been converted to micrograms.

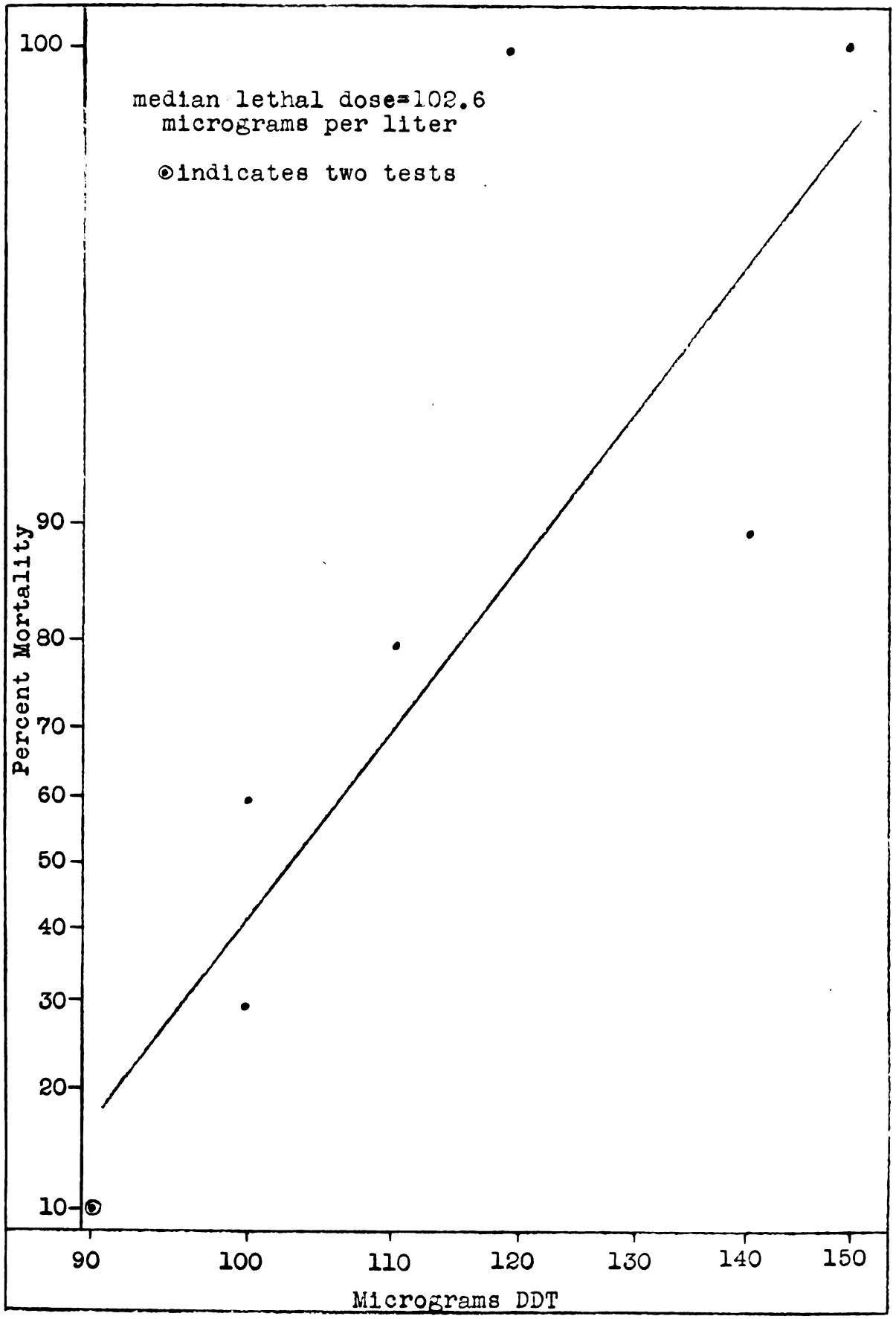

median lethal dose=102.6
micrograms per liter
⊙indicates two tests
Percent Mortality
100
90
80
70
60
50
40
30
20
10
90
100
110
120
130
140
150
Micrograms DDT

Figure 3. Results from the cold water environment after a 48 hour exposure period with a water temperature of 16 degrees centigrade. Percentages have been substituted in the probit scale of the ordinate and the log values of the abscissa have been converted to micrograms.

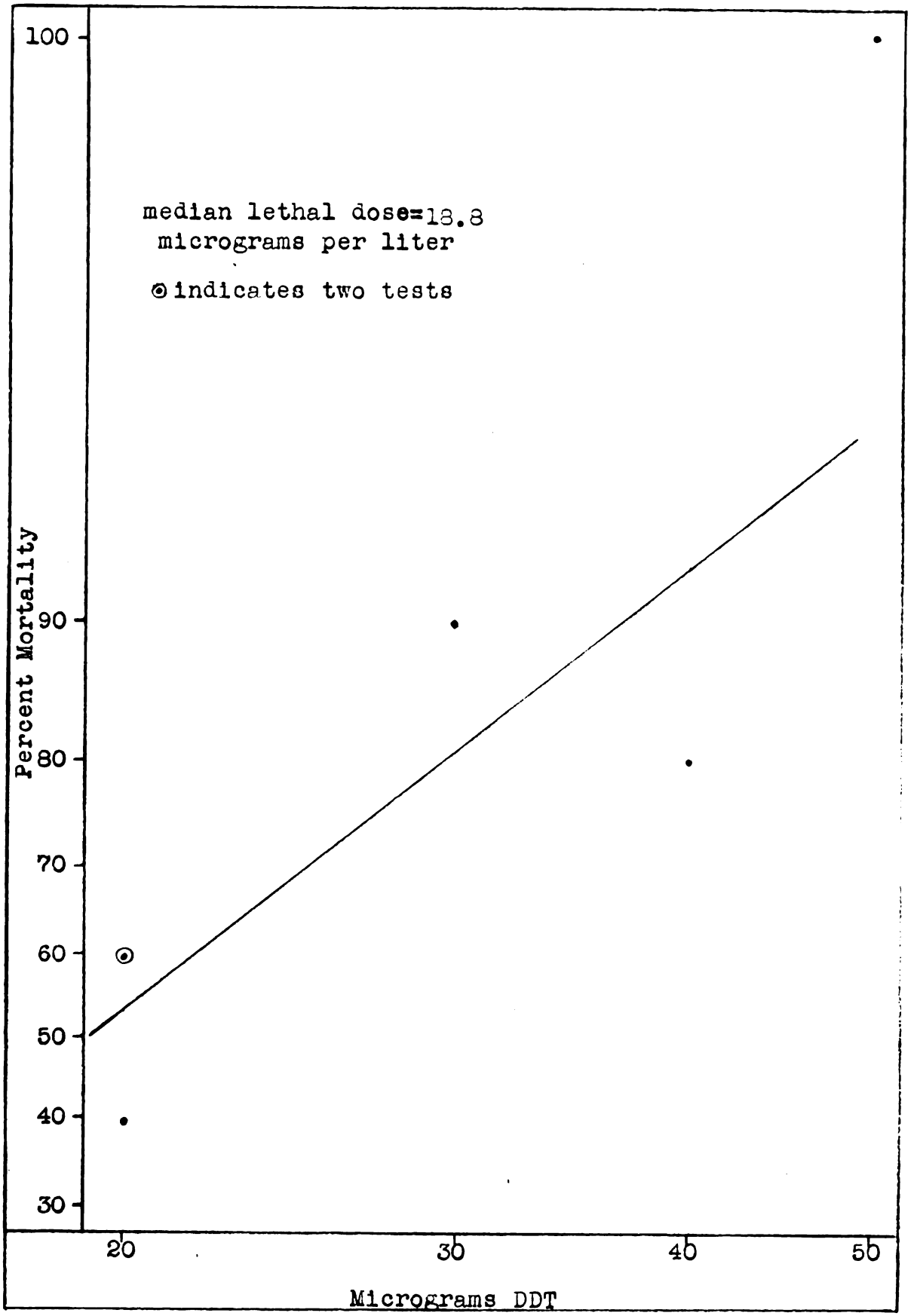

100
90
80
70
60
50
40
30
Percent Mortality
median lethal dose=18.8
micrograms per liter
⊙ indicates two tests
20
30
40
50
Micrograms DDT

Figure 4. Results from the moving water environment after a 48 hour exposure period with a water temperature of 26 degrees centigrade. Percentages have been substituted in the probit scale of the ordinate and the log values of the abscissa have been converted to micrograms.

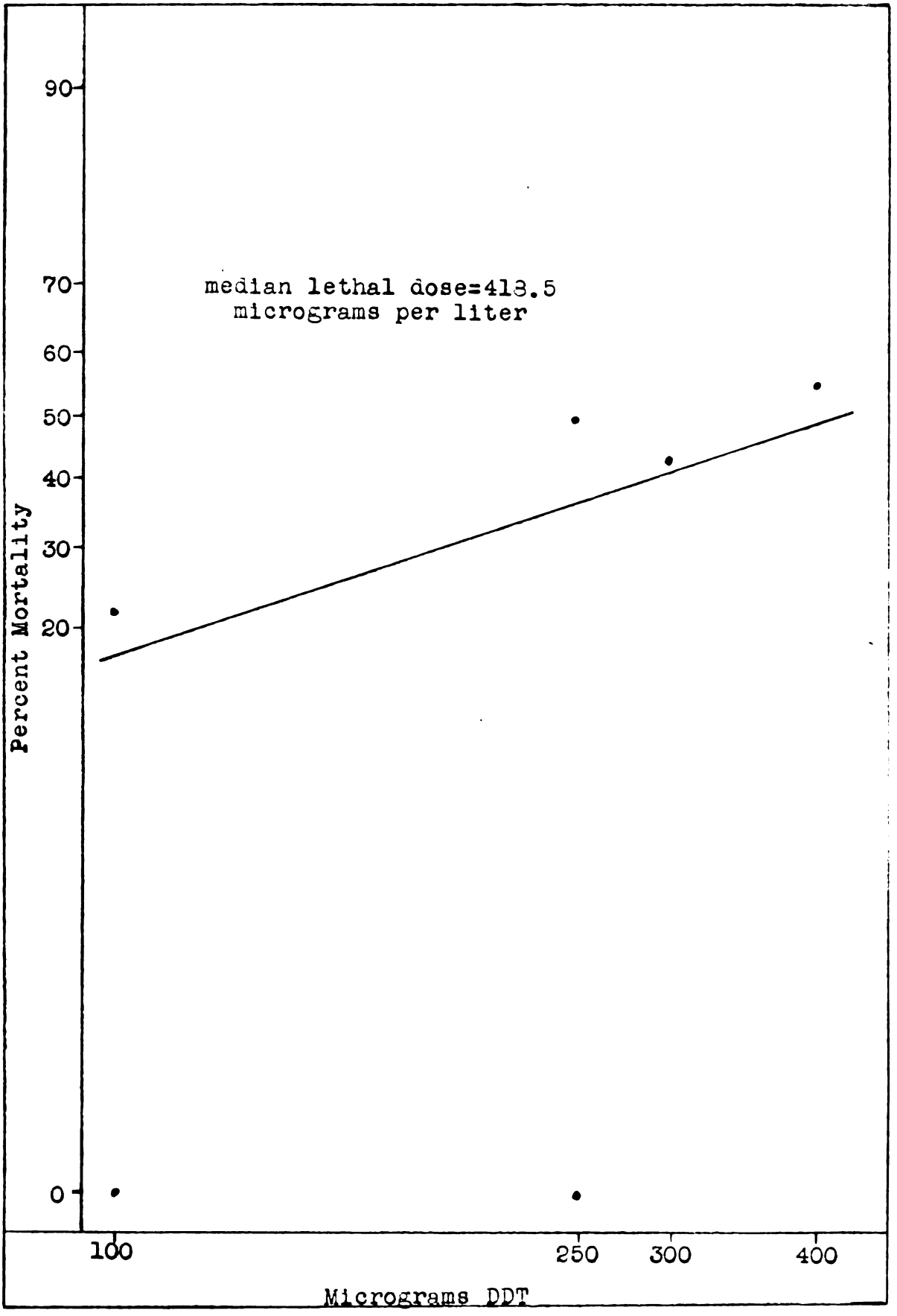
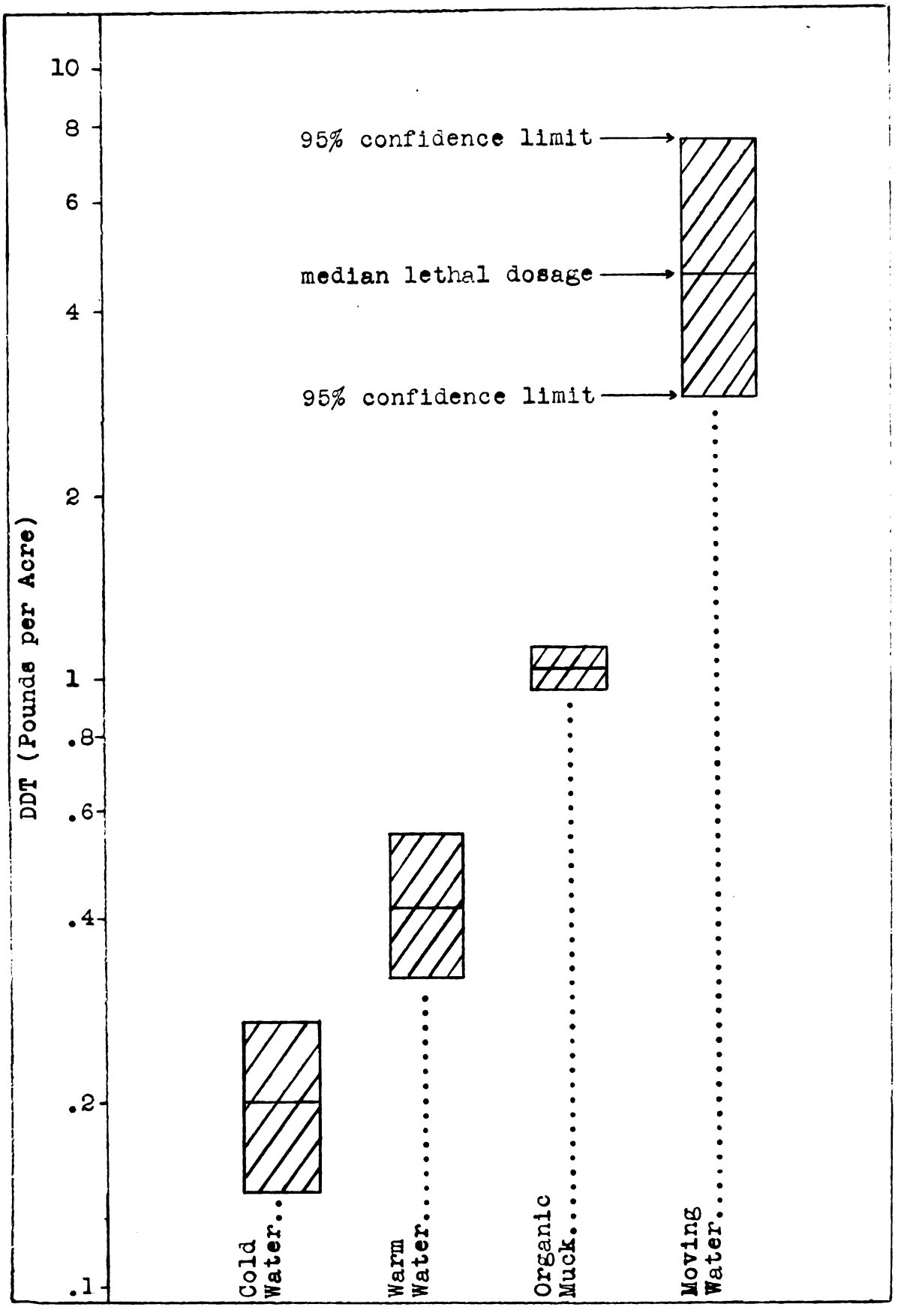

90
70
60
50
40
30
20
0
Percent Mortality
median lethal dose=418.5
micrograms per liter
100
250
300
400
Micrograms DDT

Figure 5. Median lethal dosage and 95 percent confidence limits for the DDT test emulsion applied in pounds per acre to a uniform water depth of four feet. The logarithmic ordinate scale is used only to give a better representation of the confidence limits.


10
8
6
4
2
1
.8
.6
.4
.2
.1
DDT (Pounds per Acre)
95% confidence limit
median lethal dosage
95% confidence limit
Cold Water
Warm Water
Organic Muck
Moving Water

Results of Less Extended Testing With
20 Percent DDT Emulsion

TABLE IX. AQUATIC PLANT ENVIRONMENT
TEMPERATURE 26 DEGREES CENTIGRADE

Micrograms DDT Per Liter	Fish Per Test	Percent Mortality	Exposure Period	Other Conditions*
60	10	70%	48 hrs	pH 8.5
50	10	80%	48 hrs	pH 8.5
50	12	17%	48 hrs	pH 8.5

*test volume=80 liters; total hardness=200 ppm; plant concentration=40 grams per test; no control

TABLE X. WASHED SAND ENVIRONMENT
TEMPERATURE 26 DEGREES CENTIGRADE

Micrograms DDT Per Liter	Fish Per Test	Percent Mortality	Exposure Period	Other Conditions*
50	13	70%	48 hrs	pH 8.5
40	12	80%	48 hrs	pH 8.5
30	11	17%	48 hrs	pH 8.5

*test volume=80 liters; total hardness=200 ppm; sand concentration=40 grams per test; control-no mortality

TABLE XI. SOFT WATER ENVIRONMENT
TEMPERATURE 26 DEGREES CENTIGRADE

Micrograms DDT Per Liter	Fish Per Test	Percent Mortality	Exposure Period	Other Conditions*
50	10	90%	48 hrs	pH 8.5
40	10	80%	48 hrs	pH 8.5
30	10	0%	48 hrs	pH 8.5

*test volume=80 liters; total hardness=90 ppm; control-no mortality

DISCUSSION OF RESULTS FROM EMULSION TESTS

The warm water environment, being the reference point for comparisons, received the most extended investigation during the experiment with 11 tests of the emulsion being conducted at four different levels of DDT dosage ranging from 30 to 60 micrograms per liter of test water. The median lethal dosage of this emulsion was computed to be 38.2 micrograms per liter with the 95 percent confidence limits at 30.1 and 48.3 micrograms per liter. Since a significant degree of heterogeneity is present in these data, the confidence limits were widened by a method previously explained.

The addition of organic material to the warm water environment resulted in a pronounced modification of the emulsion toxicity with the median lethal dosage being increased by 74 micrograms per liter. The initial turbidity produced by the organic material was measured at 34 ppm when the DDT was added to the test water whereas, after 14 hours, the turbidity remained constant at around eight ppm. As a general summary of this environmental condition, it may be said that during the test, between 38 and 39 grams of this bottom type were on the floor of the aquarium and about two grams remained in suspension with practically all of the settling out occurring during the first quarter of the exposure period. This settling out of the organic muck undoubtedly carried some DDT particles to the bottom, but it cannot definitely be said that this condition would decrease

the toxicity more than would a condition in which there was no precipitation and the turbidity remained at 34 ppm. In fact, it might be that a heavy suspension of organic material in the water would decrease the influence of the dose by the adsorption or absorption of more DDT particles than would be taken out of the water by the precipitation. In effect, then, this test is a measurement of the influence on DDT toxicity of a water that is lowering its turbidity through a settling out process.

The effect of moving water on DDT toxicity has been noted by Roegner-Aust (1949), a German worker, who found that the toxicity of "Gesarol"(a DDT preparation) was reduced in running water.

In the case of the specific emulsion formulation used in this experiment, the effect of agitation with air seems to have brought about a clumping of particles by virtue of the additional contact opportunities afforded in moving water between the particles themselves, and between particles and aquarium surfacrs. In a test of the physical effect of moving water on the dispersion of DDT particles, conducted in one-liter glass containers, a relatively high DDT concentration of 16 ppm was used to give observable results. In standing water, the emulsion was equally dispersed and maintained a uniform turbidity through the contained volume after vigorous agitation and a 24 hour settling period. After 24 hours, in water which was first strongly agitated by shaking and then gently agitated by aeration, the emulsion

produced less turbidity and formed small groups of particles on the sides and bottom of the glass container.

This property of adherence, or clumping, exhibited by these particles may be peculiar to the specific emulsion used, or it may extend to other formulations as well, since the emulsifier is one of the quick breaking types and would leave the common DDT and xylene combination as the particle components. Most noteworthy, however, is the fact that this characteristic of clumping did occur in moving water in the laboratory and may occur in moving water in the field with the subsequent reduction in the concentration of suspended DDT particles and in the toxicity of the dose.

The cold water environment enhanced the toxic effect of the DDT emulsion on the fish to an appreciable degree. The chi-square test gave a highly significant value when the cold water data were compared to results from warm water.

To postulate that the toxic activity of the DDT was increased by the cold water hardly seems reasonable. More likely, this is a case in which the environment has modified the test organism rather than the toxicant.

Generally speaking, within the ordinary temperature range of any given cold blooded aquatic animal, a rise in temperature of one degree centigrade increases the rate of the animal's metabolism about 10 percent; or, it could be said that a 10 degree centigrade change in temperature doubles or halves the metabolic rate depending upon the direction of the change (Welch, 1935). Here, where the

temperature drop is from 26 to 16 degrees centigrade, the metabolism would be reduced to about half of the warm water rate.

Since the biological effects of toxic compounds are dependent upon the rate of absorption, the amounts reaching the site of toxic action, and the toxicity at cell level (Blackman, 1952), the metabolic rate, which has an appreciable influence on these conditions, will be an important criterion in determining the effect of any poison. For this experimental species, the aggregate effect of the lowered metabolism seems to have resulted in a reduced rate of toxin absorption which was more than offset by the inability of the body to counteract the toxic action of DDT. In this respect, the choice of the test animal was a major factor influencing the results of the experiment. If brook trout were used instead of this warm water species, the drop to 16 degrees centigrade would not produce the same effect on metabolism because, at this temperature, the brook trout is more nearly in the center of its normal temperature range than is the dace. However, if the drop were from 20 to 10 degrees centigrade, the influence on the trout would be much the same as the effect of the actual test temperature on the dace, since this change is more equally related to the test condition. Therefore, it can be seen that the choice of the test organism and the test temperature are closely linked to the true influence of the toxicant as it would effect the test species in its normal habitat.

Of the environmental factors tested with but three replications, only the aquatic plant, Naias, presented any evidence of an influence on the toxicity of the DDT emulsion. The chi-square comparison of the basic warm water results with those results from the soft water and washed sand environments showed no indication of differences (respective probability levels of 42 percent and 63 percent). The aquatic plants may have influenced the toxicity of the DDT, but a definite decision is impossible on the basis of these data. In testing by chi-square for differences between these two disproportionate sets of data, reduction of the data indicated a probability level of about 7 percent while adding to the data resulted in a probability of four percent, leaving the issue in doubt.

Since only three replications of each environment were tested and the environmental factors were moderated, rather than exaggerated in comparison with natural conditions, it should be remembered that this work offers only a limited indication of the extent of influence that these factors exert on DDT the natural environment.

In some respects, the results of this experiment show a general agreement with past reports of field and laboratory observations. The aquatic plant tests gave results which bordered on being significant at the five percent level when compared to the data of warm water without plants. Since this environmental factor was only moderately established in the test aquaria, it seems likely that the pre-

sence of plants does affect the toxicity of this DDT emulsion.

Such a conclusion agrees with the reports of others including Odum and Sumerford (1946) who found that when 10 grams wet weight of Elodea and Cabomba were placed in a fish bowl, goldfish were able to survive an otherwise lethal dose of DDT. As previously mentioned, Tarzwell (1948) noted in field tests that a heavy fish kill occurred in a sparsely vegetated pond which was exposed to DDT while in another pond having a profuse growth of rooted plants, only a light fish-kill resulted when the pond was subjected to twice the dosage used in the first pond.

The influence of organic material on DDT toxicity has been observed in the field by Linduska and Surber (1948). They report that one factor which kept fish mortality low on a small trout stream exposed to DDT spraying was the presence of organic material stirred up in the water by the activity of beavers. Laboratory tests on trout, reported by Hoffman and Linduska (1949), showed that aquaria containing a layer of mud had a mortality of between zero and 39 percent while the same dose in aquaria lacking this factor produced a kill of 84 to 100 percent. Since their experiments were apparently carried out at a single dosage level, it is impossible to demonstrate more than qualitative agreement with the present study, where toxicity was much reduced in the presence of mud. A dose which would produce 100 percent kill in warm water was practically non-toxic in water

containing organic material under these test conditions.

Certain test results, namely from the cold water and soft water experiments, do not agree with literature reports. Hoffman and Linduska (l.c.) report that warm temperatures and soft water increased the toxic action of DDT. In this experiment, the effect of temperature was found to be just the reverse, with a significant increase in mortality occurring in the colder water. Also, water of 90 ppm total hardness failed to reveal any significant difference in mortality when compared to a harder water of 200 ppm total hardness. These results suggest that a number of variables must be considered before satisfactory generalizations may be issued. The formulation used, the type, age, species, and condition of the test organism, the degree of environmental establishment in the test water, and many other factors all may have a bearing upon toxicity of DDT to fish.

For determining the ability of natural environments to modify DDT toxicity to fish, there is need for more detailed work in which each component of the environment is critically evaluated by itself and in combination with other habitat factors as to their relationship with the test animals and the test formulations. When this is done, the knowledge gained could be extremely beneficial to the conservation of fish life. It is quite possible that the results may lead to the adjustment of the insecticidal application, with heavier doses being applied when the environment is such that toxicity is reduced and lighter doses being used when

the environment is not likely to protect the fish. If a relationship between formulations and environments becomes established, certain formulations which were found to be less toxic to fish under certain conditions could be applied when those conditions existed. Even the temporary establishment of a certain modifying factor such as an algal bloom or an artifically produced turbidity might be employed as a detoxifying agent.

These implications of the practical use of future test results are prompted by the wide differences in the results of the tests in warm, cold, moving, and mud containing waters. Then these results are converted to the application of this form of DDT in pounds per acre for a water mass of a uniform depth of four feet, the computed median lethal dose for warm water becomes .42 pounds per acre; for cold water, .20 pounds per acre; for moving water, 4.54 pounds per acre; and for mud containing water it is 1.12 pounds per acre. If the median lethal dose of the emulsion in the warm water environment is regarded as a base or reference point, a change to one of the other three conditions alters the median lethal dose to such an extent that the 95 percent confidence limits do not overlap. The existance of differences as great as these is strong evidence for the critical consideration of the type of aquatic habitat exposed to a DDT application and the role that it will play in preserving fish life.

SUMMARY

Aquarium tests dealing with the toxicity of DDT to the red bellied dace (Chrosomus eos) were made to study the modifying effect of certain environmental factors on this chemical. Several test replications of each environmental factor were carried out using ten fish in 80-liter volumes of water containing a hardness content of 200 ppm. An emulsion of DDT was used for most of the tests after a wettable powder had given inconsistant results.

More extended experiments allowing computation of the L.D.50 by probit analysis gave the following results:

Environmental Factor	L.D.50 (micrograms/liter)
Warm water (26 degrees centigrade).....	38.2
Cold water (16 degrees centigrade).....	18.8
Moving warm water.....	418.5
Warm water containing organic muck.....	102.6

Confidence limits (95 percent) computed for these median lethal doses did not overlap in any comparison.

In other less extended tests, the results suggested that aquatic plants may reduce DDT toxicity, but no significant effect was demonstrable for the influence of soft water (90 ppm) or washed sand.

The results of this experiment suggest that the environment has an appreciable effect on DDT toxicity, but as yet, broad generalizations are not justifiable. More work is needed, not only to support these findings, but to evaluate more critically each component of the environment by itself and in combination with other habitat factors as to their influence on the test fish and the test formulations.

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