GAMBUSIA AFFINIS AFFINIS

(BAIRD AND GIRARD 1854) -

SUSCEPTIBILITY TO DDT.

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1.0 INTRODUCTION

Increases in tolerance or resistance[1] to pesticides have been reported in only a few vertebrates. A DDT-tolerant strain of Mus musculus has been produced by selection in which 1.7 times more DDT was required to produce an LD_{50} (see appendix I) in the ninth selected generation than in the original stock (Ozburn and Morrison, 1962). The failure of standard dosages of diphacinone and warfarin to control Norwegian rats (Rattus norvegicus) in the field led to laboratory tests, the results of which indicated apparent resistance of the rats to these poisons (Boyle, 1960). Further evidence of warfarin resistance in this population has been shown by Cuthbert (1963). A possible example of increased tolerance to DDT in natural populations of the southern cricket frog, Acris gryllus, and the northern cricket frog, A. crepitans, has also been shown (Boyd et

Resistance to DDT was first reported in the mosquitofish, Gambusia affinis affinis (Baird and Girard) by Vinson et al. (1963). Subsequently, Ferguson et al. (1964)

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(1) The terms "resistance" and "tolerance" have been arbitrarily defined by Boyd and Ferguson (1964) in the following manner: "If 10 times as much insecticide is required to cause equivalent mortalities in one of two populations, that population is termed resistant; differences less than 10-fold are termed a tolerance."

have shown populations of mosquitofish in waters near cotton fields with a history of DDT exposure to be resistant to normally lethal doses. The observed resistance is thought to be genetically based, resulting from the selective action of the insecticide. Resistant mosquitofish (F_1 generation) reared in an insecticide-free environment may have as much as 300 times the natural resistance (ibid.). Toxicity values for fish as many as three generations removed from exposure to insecticides remain essentially unchanged from those of the original selected parental population.

The adaptive physiological mechanisms that bring about resistance in fishes have not been identified. Factors which may confer resistance include mechanical exclusion of the pesticide at exposed surfaces, detoxication and conversion of the toxicant to a less toxic state (normally involving an enzyme system), and storage wherein the animal is able to tolerate higher levels in its tissues. DDT resistance in mosquitofish is thought to be accomplished by detoxication (Ferguson 1965).

The aim of this study was to compare the susceptibilities to DDT of DDT-exposed and unexposed populations of mosquitofish which have been introduced locally to control

mosquitoes, and to elucidate the factor or factors responsible for any differences which might have occurred.

2.0 SELECTION OF STUDY POPULATIONS

It was necessary for the populations of mosquitofish chosen for comparison of susceptibilities to have the following characteristics :

a) gene flow between populations to be zero;

b) the DDT-exposed population to have been continuously exposed to DDT for a number of years;

c) the unexposed population not to have had a history of exposure to DDT or to related compounds such as aldrin, dieldrin, or heptachlor, since exposure to one of these insecticides may produce crossresistance to DDT (Boyd and Ferguson, 1964);

d) that their habitats be permanent, not ephemeral, since ephemeral water bodies in the Metropolitan area are restocked each year with mosquitofish from other areas.

The water bodies containing populations most likely to meet these criteria were visited. Those examined were :

Camel Paddock (swamp near Perry Lakes)

Lake Leschenaultia

Garden Island - stream formed by water-bore overflow

Lake Coollelal (Wanneroo)

Drainage channel at north end of Lake Monger

Ellam St. Victoria Park drainage channel

Craig St. Victoria Park drainage channel

Herdsman's Lake

Perry Lakes

Lake Monger

Lake Joondalup

Lake Coogee

Three unnamed swamps in Spearwood

Only the first six of these habitats contained mosquitofish in numbers and densities such that suitable-sized samples could be taken in a few hours.

Camel Paddock, although suitable by other criteria, was rejected as it was an ephemeral swamp. Following the dry winter of 1970 it had dried up for the first time in eight years. Ithad therefore been restocked in May of 1971.

The mosquitofish populations of Lake Leschenaultia and Garden Island appeared to be unexposed to DDT. There was no evidence of pesticide having been used on or around

the lake; its catchment area was uncultivated land. Similarly, there was no evidence of DDT having been used near the stream at Garden Island.

The Lake Coollelal population has been exposed to DDT and many other pesticides for at least six years. Run-off from market gardens drains into the Lake from both sides. Interviews with local market gardeners revealed that the following pesticides have been or are being used each year on vegetable crops grown around the Lake :

Calcine	Dipterex
Cuprose	Maneb
DDD	Terra Thimet
DDT	Thiodan
Difolotan	Zineb

DDT is used in large amounts and at frequent intervals. One gardener freely admitted to applying DDT to his cauliflower crops at five times the approved application rate, once a fortnight (and also on the night before harvesting).

According to Mr. N. Silich of the Perth City Council, the mosquitofish population of the Lake Monger drainage channel had also been exposed to DDT for a number of years.

It was not known whether the Ellam Street Victoria Park population had been exposed. Subsequent analysis of mud samples from this locality showed it to be unexposed.

In summary, five populations were found to meet the necessary criteria. Of these, three (Lake Leschenaultia, Garden Island, and Ellam Street) had not been exposed to DDT, and two (Lake Coollelal and Lake Monger) had been exposed.

3.0 EXPERIMENT I

INTRODUCTION

The aim of this experiment was to compare the susceptibilities to DDT of fish from two localities - Lake Coollelal (DDT-exposed population) and Garden Island (unexposed population). Percentage mortalities of the two populations were to be compared at each of four different concentrations of DDT.

3.2 MATERIALS and METHODS

3.21 Test Fish

Test fish were collected from Garden Island and Lake Coollelal with a fine-mesh scoop net and tipped into "eskies" containing habitat water. Scoops were made along the water's edge, and through shoals of fish in deeper water. Approximately 200 fish were sampled at each locality. They were held initially at the Zoology Department in conditioned water (see appendix II) in concrete tanks, and were fed small amounts of ground "sheep nuts" twice a week.

3.22 Test Containers

DDT is readily absorbed from solution by plastics (Burke and Ferguson, 1969). For this reason five battery jars (all-glass construction) were used as test containers.

The jars measured 23 cm x 31 cm x 50 cm deep and had a capacity of 18 litres. The test containers were made chemically clean by washing with chromic acid, then with tap water, and finally with distilled water. All glassware used for the preparation and holding of stock solutions was cleaned in the same manner.

Each of the five jars was divided into two approximately equal compartments by a screen of plastic mesh. This ensured that the two groups of test fish were exposed to the same test concentration. Although the use of this type of mesh to some extent defeated the purpose of using all-glass containers, it was the only suitable mesh readily available. There were not enough battery jars to use one for each group of fish at each of five concentrations.

3.23 Test Solutions

DDT was dissolved in acetone (reagent grade) to obtain a 0.05% stock solution. This was further diluted in acetone to give 0.0001%, 0.0004%, 0.0016%, and 0.0064% solutions. 20 litres of tap water was poured into each of five glass battery jars. 20 mls. of the appropriate dilution was then added to each of four jars to give test concentrations of 0.001, 0.004, 0.016, and 0.064 ppm DDT. 20 mls. of acetone was added to the fifth jar to give a control solution (0.00 ppm DDT). It was anticipated that the LC_{50}'s (48 hours) for

both populations would lie within this range of concentrations, since published LC_{50} (72 hour) values for mosquitofish range from 0.01 to 0.05 ppm (Johnson, 1969; Vinson, Boyd, and Ferguson, 1963). The concentrations were chosen to increase geometrically, since toxic effect is related to the logarithm of the dose, rather than to the dose itself.

3.24 Experimental Procedure

Fifty fish from each of the two population samples were introduced into the five partitioned battery jars, so that each jar contained twenty fish (ten from each locality, separated by the mesh dividing screen). Fish less than 2 cm or more than 3 cm in length (measured from tip of the mouth closed to the tip of the caudal fin) were not used for testing (cf. Doudoroff, "...the length of the longest fish used in an individual bio assay should not be more than 1.5 times the length of the smallest specimen used."). No account was taken of sex, since it has previously been established that the sexes do not differ in regard to susceptibility to DDT (Vinson, Boyd, and Ferguson, 1963).

20 mls. of the appropriate solution was then added to each of the five jars to give test concentrations of 0.00 (control), 0.001, 0.004, 0.016, and 0.064 ppm DDT. Dead fish were removed and the percentage mortality was recorded at 6-hourly intervals for the following 42 hours.

The temperature of the test water affects the toxicity of pesticides to poikilotherms. Bridges et al. (1963) found toxicity of DDT increased with temperature decrease, with the effects levelling below 9.0°C and above 29.5°C. Cope (1965) reported DDT more toxic to rainbow trout and bluegill at a low temperature (13°C) than at high temperatures (18.5°C and 23°C). The temperature of the test water was therefore kept as constant as possible by using an air conditioner in the laboratory. Water temperature was 20 ± 2°C throughout the experiment.

Artificial lighting was used only while mortality was otherwise natural lighting prevailed throughout the experiment.

The fish were not fed during testing.

Early symptoms of DDT poisoning of Gambusia affinis were short bursts of exaggerated swimming movements. While poisoned animals showed these movements, control animals rested quietly on the bottom of the test container. Fish which died from DDT poisoning lay on the bottom with their bodies contorted sideways and their opercula extended at right angles. Thus there was little difficulty in deciding whether a fish was dead or not when mortality was being scored.

All fish scored as being dead were removed from the test containers and placed in a container of tap water. None recovered.

Experimental results were as follows :

CONTAINER	DDT CONCN.	POPULATION SAMPLE	PERIOD OF EXPOSURE (HRS) 0	6	12	18	24	30	36	42
1	0.00 ppm CONTROL	Lake Coollelal	0	1	4	8	8	9	9	9
		Garden Island	0	1	1	1	7	10	11	11
2	0.001 ppm	Lake Coollelal	0	1	6	9	12	18	18	18
		Garden Island	0	0	0	0	0	0	0	0
3	0.004 ppm	Lake Coollelal	0	0	3	9	9	9	10	10
		Garden Island	0	0	0	1	5	8	9	9
4	0.016 ppm	Lake Coollelal	0	1	5	7	9	10	10	10
		Garden Island	0	0	0	0	4	7	9	10
5	0.064 ppm	Lake Coollelal	0	1	7	9	10	10	10	10
		Garden Island	0	0	1	1	5	8	10	10

Figures indicate number of fish dead at given times.

It can be seen from the results that the mesh dividers in containers 1 and 2 were not satisfactory.

All control fish died within 42 hours of commencement of the experiment. At this time three of the fish exposed to DDT were still alive.

Initially, a higher mortality occurred amongst the Lake Coollelal fish than amongst the Garden Island fish in all concentrations of DDT and also in the control tank.

3.4 DISCUSSION

The results gave no information on the toxicity of DDT to the fish in the two samples because of the high control mortality - 75% in 24 hours. This was well above the acceptable 10% maximum level (Doudoroff et al., 1951).

There were numerous possible explanations for the high control mortality. Since the test water was not aerated during the experiment it was possible that the fish died from lack of oxygen. The fish might have also been particularly susceptible to the acetone introduced into their water. The tap water may also have contained toxic chemicals in lethal concentrations, chlorine and copper ion being the most likely.

Whatever the cause of the high mortality, the Lake Coollelal fish were more susceptible than those from Garden Island. The reason for this difference in susceptibility was not evident.

4.0 EXPERIMENT II

4.1 INTRODUCTION

Prior to their death, the fish of Experiment I swam about slowly, visibly gasping. Lack of oxygen was thus thought to be the most likely explanation for the high control mortality of that experiment. Experiment I was therefore repeated with alterations which, it was hoped, would reduce control mortality to less than 10% and would also prevent mingling of the two groups of fish in each container.

4.2 MATERIALS and METHODS

The materials and methods of Experiment I were used with the following alterations :

Airstones fed by compressed air were used to oxygenate the test water during the experiment.

The simple mesh dividers were replaced by five-walled plastic mesh enclosures (each with four sides and a bottom) so that the two groups of fish were unable to mingle.

A different range of DDT concentrations was also used, as the two lowest concentrations of the previous experiment were thought to be too low to cause significant mortality during tests of 48 hours duration or less. The new range of concentrations was 0.00 (control), 0.02, 0.04, 0.08, and 0.16 ppm DDT.

4.3 RESULTS

Experimental results were as follows :

CONTAINER	DDT CONCN.	POPULATION SAMPLE	PERIOD OF EXPOSURE (HRS) 0	6	12	18	24	30	36	42	48	54
1	0.00 ppm (control)	Lake Coollelal	0	0	1	4	7	8	8	8	10	10
		Garden Island	0	0	0	1	1	5	7	9	9	9
2	0.02 ppm	Lake Coollelal	0	1	2	5	9	10	10	10	10	10
		Garden Island	0	0	2	3	7	9	10	10	10	10
3	0.04 ppm	Lake Coollelal	0	0	1	2	3	5	8	10	10	10
		Garden Island	0	0	0	3	6	7	10	10	10	10
4	0.08 ppm	Lake Coollelal	0	2	4	5	9	10	10	10	10	10
		Garden Island	0	1	3	5	6	7	8	10	10	10
5	0.16 ppm	Lake Coollelal	0	0	1	1	3	7	9	10	10	10
		Garden Island	0	0	0	0	3	5	6	7	7	7

Figures indicate number of fish dead at given t imes.

The mesh dividers used in this experiment prevented any mingling of the two groups of fish.

Control mortality was again high; within 42 hours of the commencement of the experiment, 17 of the 20 control fish had died.

Aeration of the test water did not reduce the control mortality to the acceptable 10% level. It therefore appeared likely that the water used for testing (tap water) contained some toxic chemical, and that mortality was not due to lack of oxygen. Experiment III was designed to examine this possibility.

5.0 EXPERIMENT III

5.1 INTRODUCTION

The high control mortality of Experiments I and II was now thought to be due to some toxic chemical in the tap water. In Experiment III the mortality of fish in tap water was compared to the mortality in "conditioned" water.

It was also obvious that the very large surface area of the plastic mesh compartments of Experiment II defeated the purpose of using glass battery jars as test containers. Use of these non-disposable containers also meant that they had to be acid-washed after each experiment. Having only five such containers limited the number of fish that could be tested at each concentration of DDT, and precluded the use of replicates at each concentration. For these reasons the battery jars were discarded as test containers, being replaced by half-gallon ice-cream buckets.

Experiment III was also designed to establish a suitable number of fish to use per container in future toxicity tests. The numbers of fish per container tried were : 3, 5, 7, and 10.

5.2 MATERIALS and METHODS

Twenty-four clear-plastic ice-cream containers ($\frac{1}{2}$ gallon

or 2.27 litre capacity) were used as test containers. One litre of tap water was poured into each of the first twelve, and one litre of "conditioned" water into each of the remainder. Gambusia from the Lake Coollelal stock were placed in the test containers as follows :

WATER TYPE	NUMBER OF CONTAINERS	NUMBER OF FISH PER CONTAINER	TOTAL NUMBER OF FISH
TAP	3	3	9
	3	5	15
	3	7	21
	3	10	30
CONDITIONED	3	3	9
	3	5	15
	3	7	21
	3	10	30

There was no acclimatization period for this experiment. Water temperature was maintained between 17°C and 21°C. The fish were not fed during the experiment. Mortality was scored at irregular intervals for 96 hours.

5.3 RESULTS

The experimental results were as follows :

NUMBER OF FISH PER CONTAINER	NUMBER OF CONTAINERS	TOTAL NUMBER OF FISH	WATER TYPE	PERIOD OF EXPOSURE (HRS 0	1	3	6	16	24	96
3	3	9	TAP	0	22	56	67	100	100	100
	3	9	CONDIT.	0	0	0	0	0	22	33
5	3	15	TAP	0	0	27	53	93	93	100
	3	15	CONDIT.	0	0	0	0	13	20	20
7	3	21	TAP	0	5	14	24	62	81	93
	3	21	CONDIT.	0	0	5	10	19	24	29
10	3	30	TAP	0	0	20	37	73	87	100
	3	30	CONDIT.	0	0	0	3	10	17	23

The figures indicate percentage mortality at given times.

5.4 DISCUSSION

Exposure to tap water under the test conditions produced

between 80% and 100% mortality in 24 hours. Exposure to "conditioned" water produced between 17% and 24% mortality in the same period. Obviously the tap water contained some toxin or toxins which are lethal to Gambusia affinis affinis, and which are not present, or are present in lower concentrations, in the "conditioned" water. For this reason tap water was no longer used as a solvent for DDT test solutions. Instead, "conditioned" water was used for Experiment IV.

Mortality at 24 hours was in all cases more than the 10% acceptable limit for control fish. It will be noticed, however, that for the groups with 5, 7, and 10 fish per container, the mortality in the following 72 hours was less than 10% (0%, and 8% respectively). Because of this, in the following experiment fish were maintained in the laboratory for 24 hours prior to testing, to enable them to acclimatize to test conditions. At the end of 24 hours dead fish were replaced by other acclimatized fish.

The percentage mortality in containers of 10 fish in the interval 24-96 hours was less than 10%. Since it was preferable to use as many fish as possible at each concentration, 10 fish were used per container in experiment IV.

6.0 EXPERIMENT IV

6.1 INTRODUCTION

By using conditioned water as a solvent of DDT test solutions, and by maintaining the animals under test conditions for 24 hours before testing, it was believed that control mortality could be kept below 10% for experiments of at least 72 hours duration.

The aim of this experiment was to test the comparative susceptibilities of populations of mosquitofish from Lake Monger, Lake Leschenaultia, and the Ellam Street Victoria Park drainage channel.

It was expected that the selective pressure exerted by low habitat levels of DDT would have resulted in the Lake Monger population being able to tolerate higher test concentrations of DDT than the unexposed Lake Leschenaultia and Ellam Street populations.

6.2 MATERIALS and METHODS

Approximately 200 fish were collected from each of the three study populations by means of a scoop net. The fish were transported to the laboratory in "eskies", each containing water from the appropriate habitat.

The sample from Ellam Street, as well as containing Gambusia affinis affinis, contained another subspecies of mosquitofish, G. affinis holbrooki (Girard 1859). Both these subspecies occur together in the drainage channel. This is the only locality in Western Australia to which G. a. holbrooki is known to have been introduced. The two subspecies are easy to distinguish; G. a. holbrooki are heavily spotted, whereas G. a. affinis are not. They also differ in body shape.

Forty-five half-gallon, clear-plastic ice-cream buckets were arranged in a block (15 x 3) to be used as test containers. One litre of conditioned water was poured into each of the containers. The containers were randomly assorted within the block, which was then divided into three equal blocks (each 5 x 3). 150 fish from Lake Monger were then randomly assorted into the first of these blocks, 10 fish per container. 150 fish were taken from each of the other two samples and assorted randomly into the remaining two blocks of containers.

Fish of less than 2 cm or more than 3 cm were not used in the experiment. No attempt was made to separate the two subspecies of mosquitofish in the Ellam Street sample.

The 450 fish were acclimatized to test conditions for 24 hours prior to commencement of the experiment. Dead fish

were then removed and replaced by spare fish which had also been acclimatized.

The only fish of the Ellam Street sample to die were G. a. holbrooki individuals, all of which died. The reason for this is not known. These were replaced with G. a. affinis individuals from the same sample.

Unfortunately there were not enough spare fish to replace all of those from the Lake Leschenaultia sample which had died during acclimatization. Two of the three 0.16 ppm DDT Lake Leschenaultia replicates (B and C, see below) therefore contained no fish.

One ml. of the appropriate stock DDT solution was then added to each of the 45 containers so that each block of 15 containers consisted of 3 replicates of each of 5 DDT concentrations, these being 0.00 (control), 0.02, 0.04, 0.08, and 0.16 ppm DDT. The three blocks were then regrouped and the 45 containers randomized a second time.

Note that all randomizations were done by using a table of random numbers.

The resultant arrangement of fish, test concentrations, and replicates is shown below :

LM 0.08 A	LL 0.00 A	LM 0.16 B	LM 0.02 A	LL 0.08 A	LL 0.16 B	LM 0.08 B	LM 0.04 A	VP 0.16 B	VP 0.04 C	LL 0.04 B	VP 0.04 B	LL 0.02 C	LM 0.00 C	LL 0.04 C
LM 0.04 C	VP 0.04 A	VP 0.16 C	VP 0.00 C	LM 0.02 B	LM 0.02 C	VP 0.02 B	LL 0.00 C	LM 0.16 A	LM 0.04 B	LM 0.16 C	VP 0.00 A	LM 0.08 C	LL 0.02 B	LL 0.08 C
LL 0.16 A	VP 0.16 A	VP 0.08 B	LM 0.00 A	VP 0.02 A	LM 0.00 B	LL 0.02 A	LL 0.16 C	LL 0.00 B	LL 0.04 A	VP 0.00 B	VP 0.02 C	LL 0.08 B	VP 0.08 C	VP 0.08 A

KEY: LM – Lake Monger Drainage Channel

VP – Victoria Park Drainage Channel

LL – Lake Leschenaultia

A, B, C – refer to replicates

Figures refer to concentrations of DDT in ppm.

Containers LL 0.16 B and LL 0.16 C, although containing no fish, were retained in the experiment. This was because, at this stage in the experiment there was insufficient time to make the necessary calculations for the randomization of 43 containers, the calculations for 45 containers having been made in advance.

The 1 ml aliquots were added at 1-minute intervals, and mortality was subsequently recorded in each container at 1-minute intervals, in the order in which the DDT had been added. Thus the scoring of mortality required 45 minutes every 6 hours for four days.

Water temperature in the test containers was held at 17 ± 1°C for the duration of the experiment. Mortality was scored each day for four days at 1604, 2204, 0404, and 1004 hours. Artificial lighting was used at these times only; at other times normal daily light cycles prevailed.

Experimental results are shown below in condensed form and are graphically illustrated in Figures 1-6. Raw data are given in Tables 1-16.

DDT CONCN.	POPULATION SAMPLE	PERIOD OF EXPOSURE (HRS)				
		24	36	48	72	96
0.00	LM	0	0	0	0	3
	LL	0	0	0	0	0
	VP	0	10	0	13	13
0.02	LM	3	3	3	10	13
	LL	3	3	7	10	13
	VP	3	3	3	6	10
0.04	LM	6	22	26	29	36
	LL	3	10	13	17	23
	VP	7	7	7	23	30
0.08	LM	43	80	87	90	90
	LL	20	37	47	53	63
	VP	7	27	33	47	63
0.16	LM	67	80	97	100	100
	LL	20	40	70	70	80
	VP	17	37	73	90	97

Figures indicate percentage mortality at given times and concentrations.

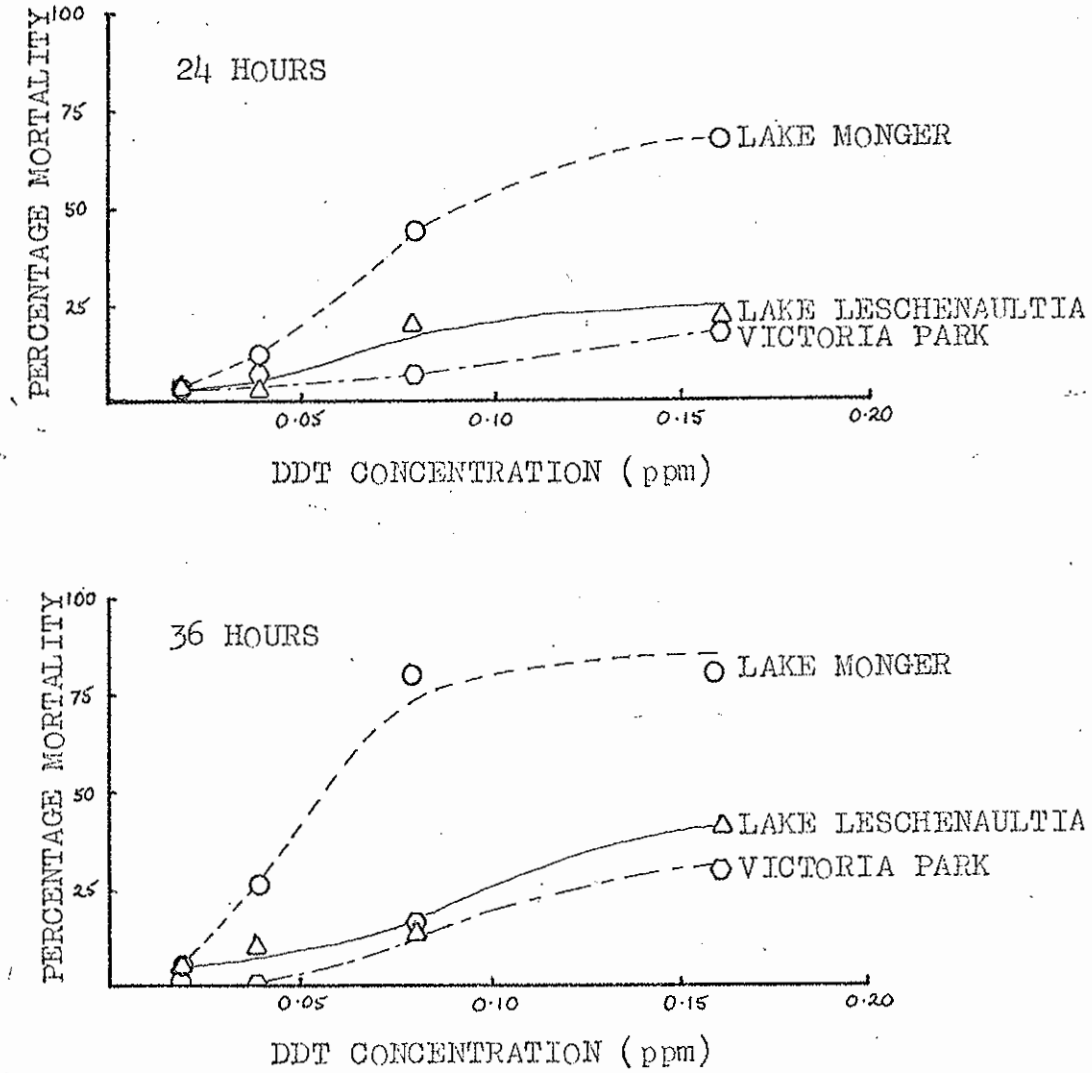


FIGURE I : Dosage/mortality curves for 24 and 36 hour exposures of three populations of Gambusia affinis affinis to a range of DDT concentrations.

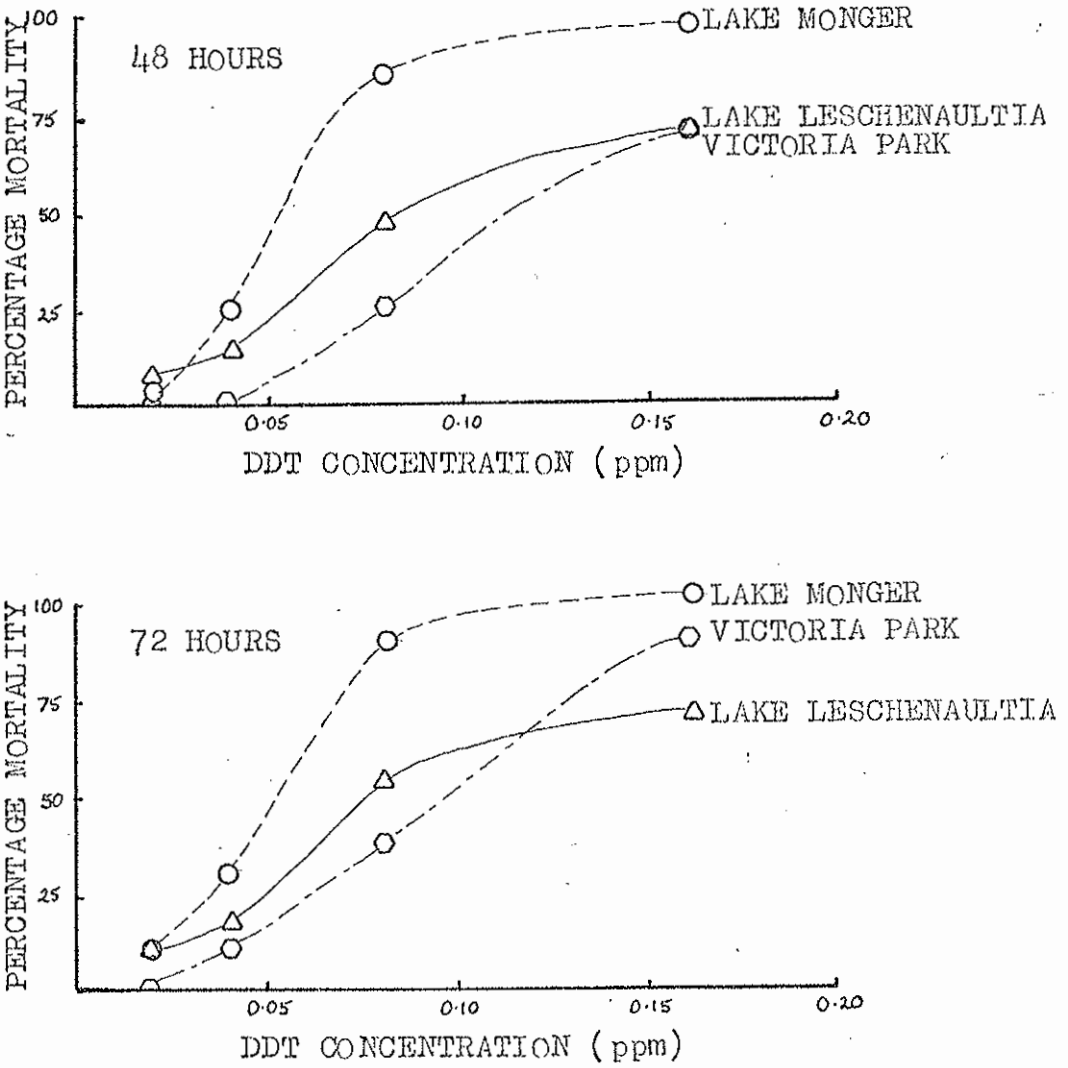


FIGURE 2 : Dosage/mortality curves for 48 and 72 hour exposures of three populations of Gambusia affinis affinis to a range of DDT concentrations.

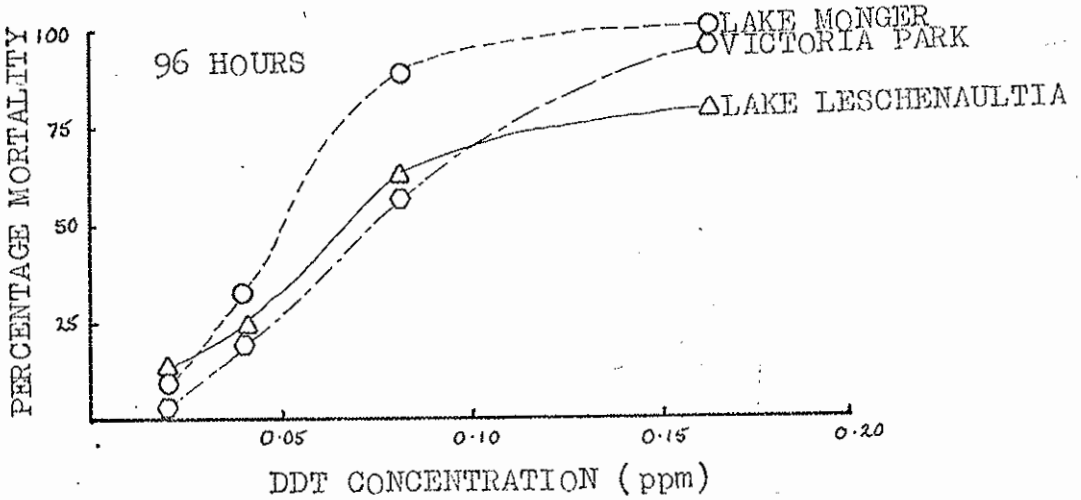


FIGURE 3 : Dosage/mortality curve for 96 hour exposure of three populations of Gambusia affinis affinis to a range of DDT concentrations.

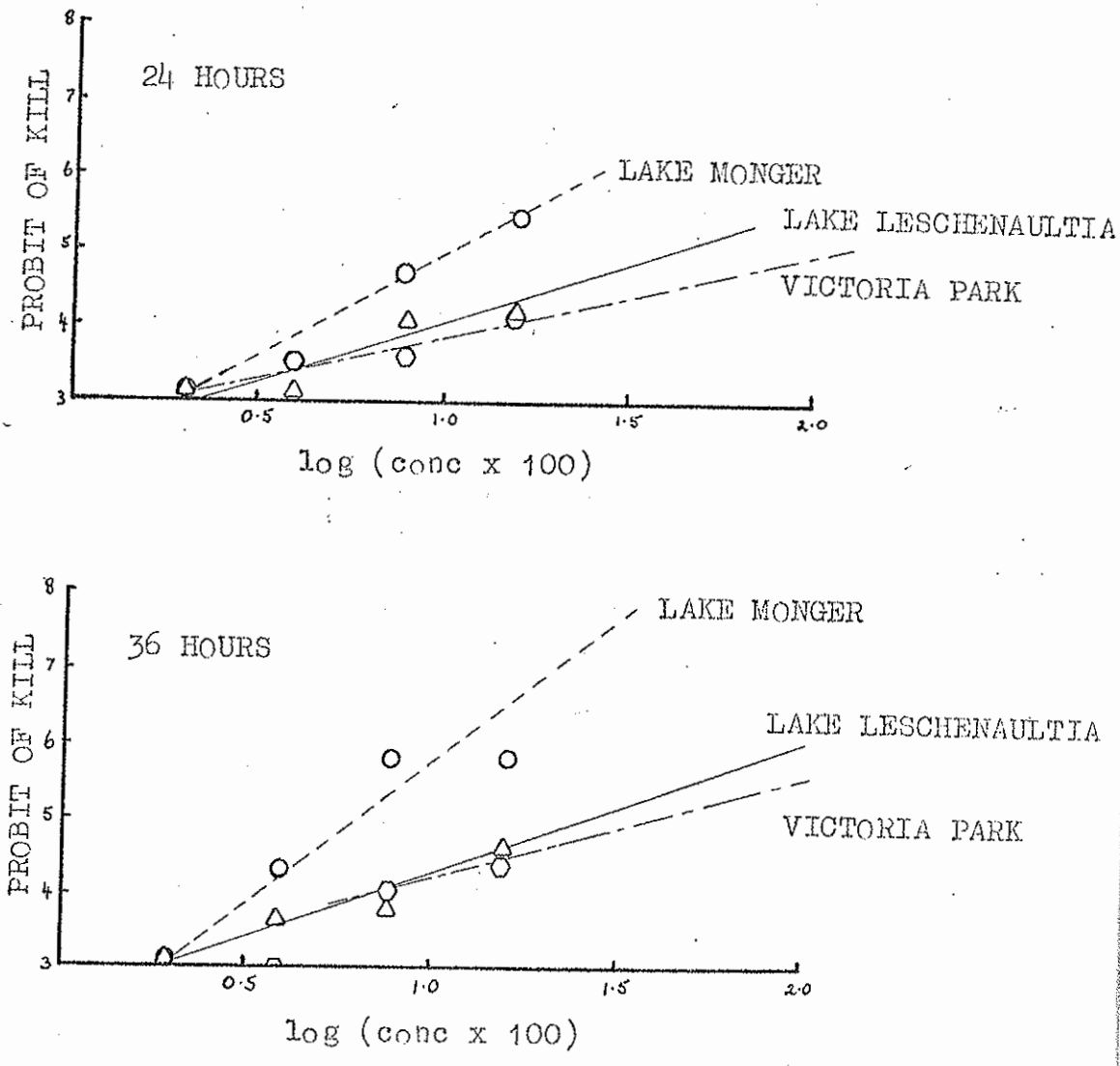


FIGURE 4 : Relationship between dosage and probit of kill for 24 and 36 hour exposures of three populations of Gambusia affinis affinis to a range of DDT concentrations.

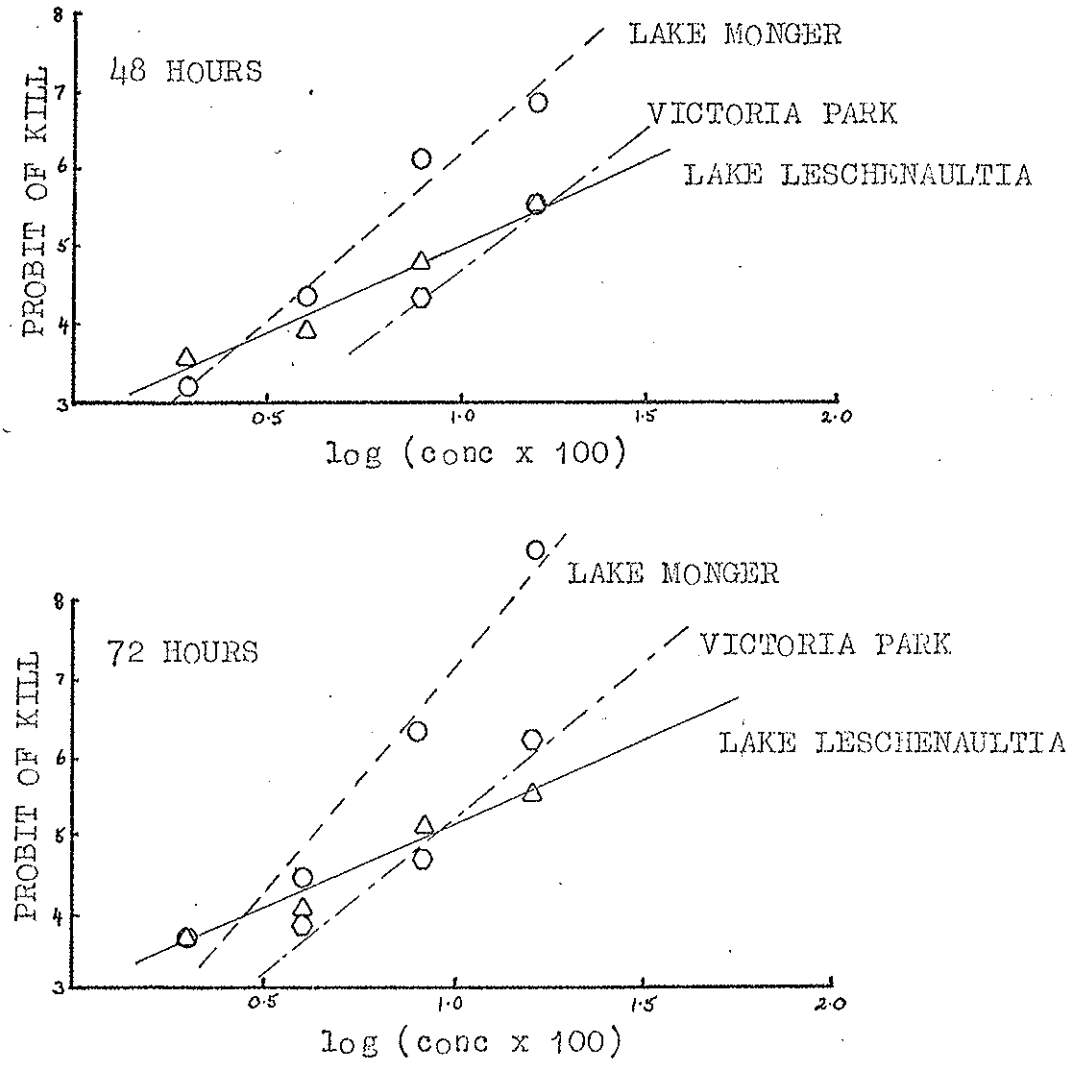
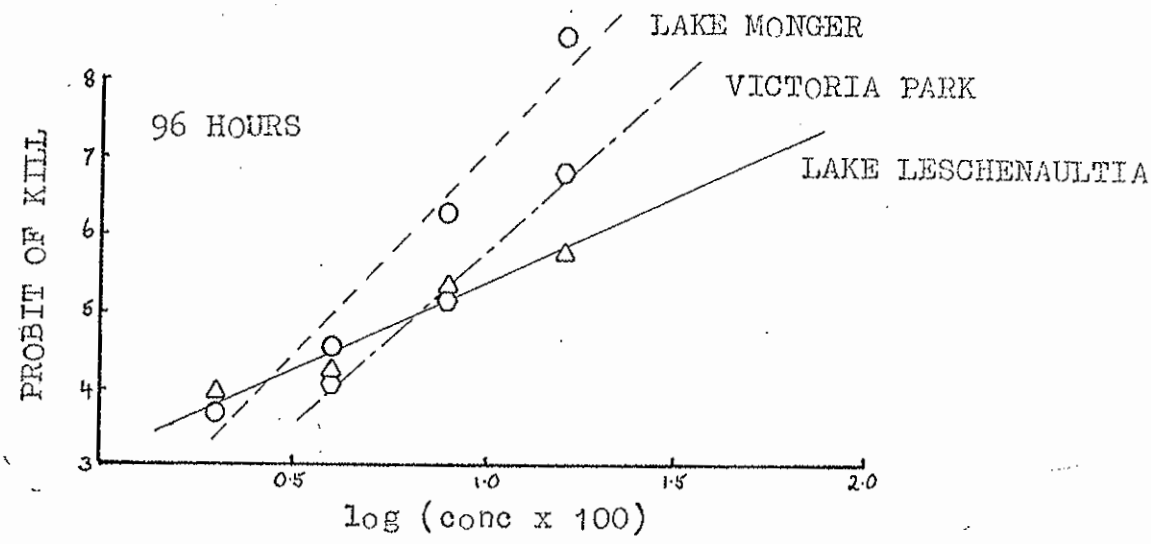


FIGURE 5 : Relationship between dosage and probit of kill for 48 and 72 hour exposures of three populations of Gambusia affinis affinis to a range of DDT concentrations.



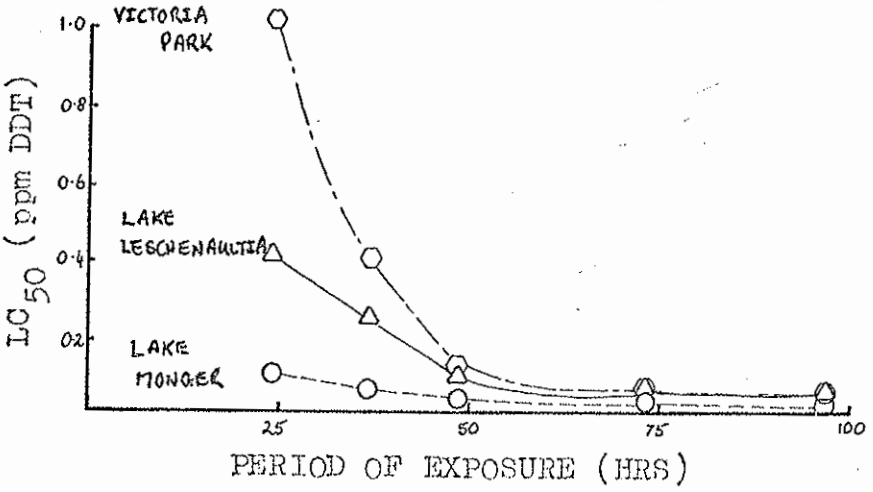


FIGURE 6

TOP : Relationship between dosage and probit of kill for 96 hour exposure of three populations of Gambusia affinis affinis to a range of DDT concentrations.

BOTTOM : Relationship between duration of test (period of exposure) and resultant LC_{50} values of the three populations.

Plotting the percentages killed against the concentrations (Figures 1-3) results in a skewed sigmoid curve. This curve, however, is ill-suited for either interpolation or extrapolation. A preferred procedure is to express dosage in logarithms, which normalizes the distribution of mortality with respect to dosage, and to express mortality in terms of standard deviations from the mean. When increased by a factor of 5, these standard deviations become probits. A plot of log dosage versus probits gives a line which, for a homogeneous group, is approximately straight over a mortality range of 20% to 80% (Hoskins, 1960).

Log dosage-probit lines have been constructed for the exposure times most commonly found in the literature, that is, 24, 36, 48, 72, and 96 hours (Figures 4-6). The most important features of these graphs are their slope, which measures the variance in response of the majority of the individuals, and the LC_{50} point, the concentration of toxicant at which 50% of the test animals are killed for specified periods of exposure. The transformed data are given in Tables 17-21. LC_{50} values are tabled below.

POPULATION SAMPLE	PERIOD OF EXPOSURE (HRS)				
	24	36	48	72	96
	ppm DDT	ppm DDT	ppm DDT	ppm DDT	ppm DDT
LAKE MONGER	0.10	0.06	0.05	0.04	0.04
LAKE LESCHENAULTIA	0.40	0.25	0.10	0.08	0.07
ELLAM STREET, VICTORIA PARK	1.00	0.40	0.12	0.08	0.07

Where necessary, observed mortalities have been corrected for control mortalities in the following manner :

$$P = \frac{P^1 - C}{1 - C}$$

where P = the corrected mortality, P^1 = the observed mortality, and C = the control mortality. This is commonly known as Abbott's formula.

Note that, by accident, 29 Lake Leschenaultia and 31 Ellam Street fish were exposed to 0.02 ppm DDT and 31 Lake Monger fish were exposed to 0.04 ppm DDT. This has been taken into account when calculating percentage mortalities.

DISCUSSION

As can be seen from the results, the new stratagem of using conditioned water and not tap water as the test solution, and allowing a 24 hour acclimatization period before commencing testing, reduced control mortality considerably. There was no control mortality at 24 hours exposure. Control mortality did not exceed the acceptable 10% level until 66 hours, and then only in the Ellam Street sample. Control mortality was still well below the 10% level for both the Lake Monger and the Lake Leschenaultia populations 96 hours after testing commenced.

In comparing the relative susceptibilities of the three populations, I will firstly consider the populations of Lake Monger and Lake Leschenaultia.

It was expected that since the Lake Monger population had been exposed to DDT for some time, it might be more tolerant to DDT than the Lake Leschenaultia population. However, the LC_{50} values for 24, 36, 48, 72, and 96 hours exposure of the Lake Monger population were in all cases less than the corresponding values of the Lake Leschenaultia population (see Results). This would indicate that the Lake Monger population was in fact more susceptible to DDT.

The slope of the log dosage-probit lines for the Lake Monger population are steeper than those of the Lake

Leschenaultia population, indicating that in the former population there is a smaller variance of response to DDT poisoning. This is also atypical of tolerant populations; their log dosage-probit line usually has a lesser slope - cf. Hoskins (1960) : "A clear decrease in slope is a sure sign that resistance has appeared."

An explanation for these unexpected results is offered. It is believed that the fish from Lake Monger which were used in the experiment, unlike those from Lake Leschenaultia, were carrying a sublethal burden of DDT. Because of this burden, the amount of additional toxicant required to reach the lethal level was reduced. This reduction resulted in an LC_{50} value lower than would be obtained for the same fish free from DDT contamination, and lower than the LC_{50} value of the Lake Leschenaultia fish.

The level of exposure to DDT which produced sublethal contamination in the Lake Monger fish would have killed the more susceptible members of that population, thus reducing the variance of response of the population, and steepening the slope of the log dosage-probit line, as was observed.

The results obtained from the unexposed Ellam Street population are more difficult to interpret. At 72 and 96 hours exposure the LC_{50} values obtained from this

population are the same as those of the Lake Leschenaultia population. This is to be expected, since both populations have not been exposed to DDT and therefore would presumably have the same tolerance. At 24, 36, and 48 hours exposure, however, the Ellam Street population has higher LC_{50} values, indicating greater tolerance. Also, at 96, 72, and 48 hours exposure the variance of response of the Ellam Street fish is less than that of the Lake Leschenaultia fish, whereas at 36 and 24 hours exposure it is more (Figures 4-6). No explanation is offered for these ambiguities.

Whether or not the presence of sublethal levels of DDT in the Lake Monger fish is the reason for the apparently greater susceptibility of this population, the possibility of this factor affecting the results obtained in similar studies is raised.

To the author's knowledge, no account has been taken of this possibility in any previous studies involving susceptibility comparisons. Obviously, if tolerant or resistant populations of previous studies involving persistent environmental contaminants have been carrying sublethal levels of the toxicants under study, which is highly likely, the true tolerance of these populations will have been underestimated to varying degrees. It would appear mandatory that in future studies in this field, test animals

be "flushed out" or consideration at least be given to the influence which undetermined sublethal levels may exert on results.

In this study no conclusions can be drawn about the true susceptibility of the Lake Monger population and its relationship with that of the unexposed Lake Leschenaultia population.

7.0 SUGGESTIONS FOR FUTURE WORK

The next step in this investigation, the testing of the hypothesized explanation for the greater susceptibility of the Lake Monger population, would best be tested in the following manner :

Large samples (400 or more fish) of the Lake Monger and Lake Leschenaultia populations should be held in conditioned water in suitable tanks, the holding water being changed once per week. Fish should be sampled in these tanks weekly, commencing on the day of initial sampling of the population, and these weekly samples should be analysed for whole-fish DDT concentrations. This would :

a) show the level of DDT contamination of the Lake Monger population, and the absence of DDT in the Lake Leschenaultia population; and

b) establish the half-life of DDT in the Lake Monger sample which is now free from further contamination. (The half-life of DDT in the goldfish Carassius auratus has been established as 27 days by Grzenda (1970), using radioactively-labelled DDT. This more popular technique is unnecessary here.)

When most of the body burden has been metabolised and eliminated by the Lake Monger fish (as judged by DDT estimation

of weekly samples), the LC_{50}'s of the two population samples should be re-determined for comparison.

Samples were taken from each population on three occasions for this purpose; however all fish in each sample died inexplicably after two to four weeks maintenance in tanks in the Zoology Department animal yards. On one of these occasions frogs which were in adjacent tanks also died. Testing of the hypothesis in the intended manner was thus prevented.

8.0 SUMMARY AND CONCLUSIONS

The relative susceptibilities of a number of populations of mosquitofish (Gambusia affinis affinis) to DDT were tested. Some of these populations had been exposed to DDT for many years, others had no history of exposure to DDT.

Early attempts to compare susceptibilities were hampered by high control mortality; experiments showed this mortality to be due to some toxicant or toxicants contained in tap water but not in conditioned water. In later experiments, therefore, conditioned water was used.

It was anticipated that the exposed populations, due to selective pressure imposed upon them by low habitat levels of DDT, would be less susceptible to DDT than the unexposed population. The opposite was found to be the case; fish sampled from a drainage channel at Lake Monger (exposed to DDT) were more susceptible to DDT than unexposed fish from Lake Leschenaultia. It is hypothesized that the fish from Lake Monger drainage channel were carrying sub-lethal amounts of DDT before exposure to test concentrations, thus reducing the amount of DDT required to reach the lethal level, and increasing their apparent susceptibility.

Suggestions for future work include the "flushing out"

of DDT from test animals of exposed populations before comparison of susceptibilities between exposed and unexposed populations are made.

It is also suggested that other workers should take into account the possibility of sublethal levels of toxicants affecting their results, before arriving at any conclusion regarding the relative susceptibilities of populations which they may be studying.

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11.0 APPENDICES

APPENDIX I

LD_{50} is an abbreviation of "lethal dose for 50% mortality" and is the dose required to kill 50% of a population sample; it is specific for a particular period of exposure, e.g. LD_{50} (24 hours) = 0.1 ppm.

LC_{50} is the lethal concentration required to kill 50% of a population sample.

The LD_{50} measure of susceptibility is used in feeding-trials where the actual amount of pesticide consumed by the test animal is known. LC_{50} is used for aquatic and marine animals, since only the concentration to which they are exposed, and not the dosage of toxicant which they receive, is known.

"Conditioned" water.

"Conditioned" water is scheme water which has passed through three open concrete tanks (interconnected) in the Zoology Department animal yards. The water which was drawn from these tanks and used in experiments had been standing for at least one week.

APPENDIX III

POPULATION SAMPLE	REPLICATE	DDT CONCENTRATION (ppm) 0·00	0·02	0·04	0·08	0·16
LAKE MONGER	A	0	0	0	0	0
	B	0	0	0	0	0
	C	0	0	0	0	0
	TOTAL	0	0	0	0	0
	PERCENTAGE MORTALITY	0	0	0	0	0
LAKE LESCHENAULTIA	A	0	0	0	0	
	B	0	0	0	1	
	C	0	0	0	0	0
	TOTAL	0	0	0	1	0
	PERCENTAGE MORTALITY	0	0	0	3	0
VICTORIA PARK	A	0	0	0	0	0
	B	0	1	0	0	0
	C	0	0	0	0	0
	TOTAL	0	1	0	0	0
	PERCENTAGE MORTALITY	0	3	0	0	0

TABLE 1

: Mortality of Gambusia affinis affinis after 6 hours exposure to a range of DDT concentrations.

POPULATION SAMPLE	REPLICATE	DDT CONCENTRATION (ppm) 0·00	0·02	0·04	0·08	0·16
LAKE MONGER	A	0	0	0	1	0
	B	0	0	0	0	0
	C	0	0	0	0	1
	TOTAL	0	0	0	1	1
	PERCENTAGE MORTALITY	0	0	0	3	3
LAKE LESCHENAULTIA	A	0	0	0	0	
	B	0	0	0	1	
	C	0	0	0	0	0
	TOTAL	0	0	0	1	0
	PERCENTAGE MORTALITY	0	0	0	3	0
VICTORIA PARK	A	0	0	0	0	0
	B	0	1	0	0	0
	C	0	0	0	0	0
	TOTAL	0	1	0	0	0
	PERCENTAGE MORTALITY	0	3	0	0	0

TABLE 2 : Mortality of Gambusia affinis affinis after 12 hours exposure to a range of DDT concentrations.

POPULATION SAMPLE	REPLICATE	DDT CONCENTRATION (ppm) 0·00	0·02	0·04	0·08	0·16
LAKE MONGER	A	0	0	0	2	6
	B	0	0	0	0	4
	C	0	0	0	2	3
	TOTAL	0	0	0	4	13
	PERCENTAGE MORTALITY	0	0	0	13	43
LAKE LESCHENAULTIA	A	0	0	0	0	
	B	0	0	0	1	
	C	0	0	0	2	0
	TOTAL	0	0	0	3	0
	PERCENTAGE MORTALITY	0	0	0	10	0
VICTORIA PARK	A	0	0	0	0	0
	B	0	1	0	1	1
	C	0	0	0	0	0
	TOTAL	0	1	0	1	1
	PERCENTAGE MORTALITY	0	3	0	3	3

TABLE 3 : Mortality of Gambusia affinis affinis after 18 hours exposure to a range of DDT concentrations.

POPULATION SAMPLE	REPLICATE	DDT CONCENTRATION (ppm) 0·00	0·02	0·04	0·08	0·16
LAKE MONGER	A	0	1	2	4	7
	B	0	0	0	4	7
	C	0	0	0	5	6
	TOTAL	0	1	2	13	20
	PERCENTAGE MORTALITY	0	3	6	43	67
LAKE LESCHENAULTIA	A	0	1	1	2	
	B	0	0	0	1	
	C	0	0	0	3	2
	TOTAL	0	1	1	6	2
	PERCENTAGE MORTALITY	0	3	3	20	20
VICTORIA PARK	A	0	0	1	1	1
	B	0	1	1	0	3
	C	0	0	0	1	1
	TOTAL	0	1	2	2	5
	PERCENTAGE MORTALITY	0	3	7	7	17

TABLE 4 : Mortality of Gambusia affinis affinis after 24 hours exposure to a range of DDT concentrations.

POPULATION SAMPLE	REPLICATE	DDT CONCENTRATION (ppm) 0·00	0·02	0·04	0·08	0·16
LAKE MONGER	A	0	1	4	8	8
	B	0	0	2	6	8
	C	0	0	1	6	7
	TOTAL	0	1	7	20	23
	PERCENTAGE MORTALITY	0	3	22	67	87
LAKE LESCHENAULTIA	A	0	1	2	2	
	B	0	0	1	2	
	C	0	0	0	5	3
	TOTAL	0	1	3	9	3
	PERCENTAGE MORTALITY	0	3	10	30	30
VICTORIA PARK	A	1	0	1	2	2
	B	0	1	1	0	4
	C	1	0	0	3	1
	TOTAL	2	1	2	5	7
	PERCENTAGE MORTALITY	7	3	7	17	23

TABLE 5 : Mortality of Gambusia affinis affinis after 30 hours exposure to a range of DDT concentrations.

POPULATION SAMPLE	REPLICATE	DDT CONCENTRATION (ppm) 0·00	0·02	0·04	0·08	0·16
LAKE MONGER	A	0	1	4	8	8
	B	0	0	2	8	8
	C	0	0	1	8	8
	TOTAL	0	1	7	24	24
	PERCENTAGE MORTALITY	0	3	22	80	80
LAKE LESCHENAULTIA	A	0	1	2	4	
	B	0	0	1	2	
	C	0	0	0	5	4
	TOTAL	0	1	3	11	4
	PERCENTAGE MORTALITY	0	3	10	37	40
VICTORIA PARK	A	2	0	1	4	3
	B	0	1	1	0	5
	C	1	0	0	4	3
	TOTAL	3	1	2	8	11
	PERCENTAGE MORTALITY	10	3	7	27	37

TABLE 6 : Mortality of Gambusia affinis affinis after 36 hours exposure to a range of DDT concentrations.

POPULATION SAMPLE	REPLICATE	DDT CONCENTRATION (ppm) 0·00	0·02	0·04	0·08	0·16
LAKE MONGER	A	0	1	4	9	9
	B	0	0	2	9	10
	C	0	0	2	8	8
	TOTAL	0	1	8	26	27
	PERCENTAGE MORTALITY	0	3	26	87	90
LAKE LESCHENAULTIA	A	0	1	2	4	
	B	0	0	1	2	
	C	0	1	1	6	5
	TOTAL	0	2	4	12	5
	PERCENTAGE MORTALITY	0	7	13	40	50
VICTORIA PARK	A	2	0	1	4	7
	B	0	1	1	1	7
	C	1	0	0	4	5
	TOTAL	3	1	2	9	19
	PERCENTAGE MORTALITY	10	3	7	30	63

TABLE 7 : Mortality of Gambusia affinis affinis after 42 hours exposure to a range of DDT concentrations.

POPULATION SAMPLE	REPLICATE	DDT CONCENTRATION (ppm) 0·00	0·02	0·04	0·08	0·16
LAKE MONGER	A	0	1	4	9	9
	B	0	0	2	9	10
	C	0	0	2	8	10
	TOTAL	0	1	8	26	29
	PERCENTAGE MORTALITY	0	3	26	87	97
LAKE LESCHENAULTIA	A	0	1	2	4	
	B	0	0	1	3	
	C	0	1	1	7	7
	TOTAL	0	2	4	14	7
	PERCENTAGE MORTALITY	0	7	13	47	70
VICTORIA PARK	A	2	0	1	4	7
	B	0	1	1	2	8
	C	1	0	0	4	7
	TOTAL	3	1	2	10	22
	PERCENTAGE MORTALITY	10	3	7	33	73

TABLE 8 : Mortality of Gambusia affinis affinis after 48 hours exposure to a range of DDT concentrations.

POPULATION SAMPLE	REPLICATE	DDT CONCENTRATION (ppm) 0·00	0·02	0·04	0·08	0·16
LAKE MONGER	A	0	2	4	9	9
	B	0	0	2	9	10
	C	0	0	2	8	10
	TOTAL	0	2	8	26	29
	PERCENTAGE MORTALITY	0	7	27	87	97
LAKE LESCHENAULTIA	A	0	1	2	4	
	B	0	0	1	3	
	C	0	1	2	7	7
	TOTAL	0	2	5	14	7
	PERCENTAGE MORTALITY	0	7	17	47	70
VICTORIA PARK	A	2	0	2	4	7
	B	0	1	1	2	8
	C	1	0	1	4	8
	TOTAL	3	1	4	10	23
	PERCENTAGE MORTALITY	10	3	13	30	77

TABLE 9 : Mortality of Gambusia affinis affinis after 54 hours exposure to a range of DDT concentrations.

POPULATION SAMPLE	REPLICATE	DDT CONCENTRATION (ppm) 0·00	0·02	0·04	0·08	0·16
LAKE MONGER	A	0	3	4	9	9
	B	0	0	2	9	10
	C	0	0	2	8	10
	TOTAL	0	3	8	26	29
	PERCENTAGE MORTALITY	0	10	27	87	97
LAKE LESCHENAULTIA	A	0	1	2	4	
	B	0	0	1	4	
	C	0	1	2	7	7
	TOTAL	0	2	5	15	7
	PERCENTAGE MORTALITY	0	7	17	50	70
VICTORIA PARK	A	2	0	3	7	8
	B	0	1	1	2	8
	C	1	0	2	4	8
	TOTAL	3	1	6	13	24
	PERCENTAGE MORTALITY	10	3	20	43	80

TABLE 10 : Mortality of Gambusia affinis affinis after 60 hours exposure to a range of DDT concentrations.

POPULATION SAMPLE	REPLICATE	DDT CONCENTRATION (ppm) 0·00	0·02	0·04	0·08	0·16
LAKE MONGER	A	0	3	4	9	10
	B	0	0	2	9	10
	C	0	0	2	8	10
	TOTAL	0	3	8	26	30
	PERCENTAGE MORTALITY	0	10	27	87	100
LAKE LESCHENAULTIA	A	0	1	2	4	
	B	0	0	1	5	
	C	0	1	2	7	7
	TOTAL	0	2	5	16	7
	PERCENTAGE MORTALITY	0	7	17	53	70
VICTORIA PARK	A	3	0	3	7	9
	B	0	1	1	3	8
	C	1	0	3	4	8
	TOTAL	4	1	7	14	25
	PERCENTAGE MORTALITY	13	3	23	47	83

TABLE 11 : Mortality of Gambusia affinis affinis after 66 hours exposure to a range of DDT concentrations.

POPULATION SAMPLE	REPLICATE	DDT CONCENTRATION (ppm) 0.00	0.02	0.04	0.08	0.16
LAKE MONGER	A	0	3	4	9	10
	B	0	0	2	9	10
	C	0	0	3	9	10
	TOTAL	0	3	9	27	30
	PERCENTAGE MORTALITY	0	10	29	90	100
LAKE LESCHENAULTIA	A	0	2	2	4	
	B	0	0	1	5	
	C	0	1	2	7	7
	TOTAL	0	3	5	16	7
	PERCENTAGE MORTALITY	0	10	17	53	70
VICTORIA PARK	A	3	1	3	7	9
	B	0	1	1	3	10
	C	1	0	3	4	8
	TOTAL	4	2	7	14	27
	PERCENTAGE MORTALITY	13	6	23	47	90

TABLE 12 : Mortality of Gambusia affinis affinis after 72 hours exposure to a range of DDT concentrations.

POPULATION SAMPLE	REPLICATE	DDT CONCENTRATION (ppm) 0·00	0·02	0·04	0·08	0·16
LAKE MONGER	A	0	3	4	9	10
	B	0	0	2	9	10
	C	0	1	3	9	10
	TOTAL	0	3	9	27	30
	PERCENTAGE MORTALITY	0	10	29	90	100
LAKE LESCHENAULTIA	A	0	2	2	5	
	B	0	0	1	5	
	C	0	2	3	7	8
	TOTAL	0	4	6	17	8
	PERCENTAGE MORTALITY	0	14	20	57	80
VICTORIA PARK	A	3	1	3	7	10
	B	0	1	1	3	10
	C	1	0	3	4	8
	TOTAL	4	2	7	14	28
	PERCENTAGE MORTALITY	13	6	23	47	93

TABLE 13 : Mortality of Gambusia affinis affinis after 78 hours exposure to a range of DDT concentrations.

POPULATION SAMPLE	REPLICATE	DDT CONCENTRATION (ppm) 0·00	0·02	0·04	0·08	0·16
LAKE MONGER	A	0	3	4	9	10
	B	0	0	2	9	10
	C	0	1	3	9	10
	TOTAL	0	4	9	27	30
	PERCENTAGE MORTALITY	0	13	29	90	100
LAKE LESCHENAULTIA	A	0	2	2	5	
	B	0	0	1	5	
	C	0	2	3	7	8
	TOTAL	0	4	6	17	8
	PERCENTAGE MORTALITY	0	14	20	57	80
VICTORIA PARK	A	3	1	3	8	10
	B	0	1	1	3	10
	C	1	0	3	4	9
	TOTAL	4	2	7	15	29
	PERCENTAGE MORTALITY	13	6	23	50	97

TABLE 14 : Mortality of Gambusia affinis affinis after 84 hours exposure to a range of DDT concentrations.

POPULATION SAMPLE	REPLICATE	DDT CONCENTRATION (ppm) 0·00	0·02	0·04	0·08	0·16
LAKE MONGER	A	0	3	4	9	10
	B	0	0	2	9	10
	C	1	1	3	9	10
	TOTAL	1	4	9	27	30
	PERCENTAGE MORTALITY	3	13	29	90	100
LAKE LESCHENAULTIA	A	0	2	2	6	
	B	0	0	2	5	
	C	0	2	3	7	8
	TOTAL	0	4	7	18	8
	PERCENTAGE MORTALITY	0	14	23	60	80
VICTORIA PARK	A	3	1	3	8	10
	B	0	1	1	5	10
	C	1	0	3	5	9
	TOTAL	4	2	7	18	29
	PERCENTAGE MORTALITY	13	6	23	60	97

TABLE 15 : Mortality of Gambusia affinis affinis after 90 hours exposure to a range of DDT concentrations.

POPULATION SAMPLE	REPLICATE	DDT CONCENTRATION (ppm) 0·00	0·02	0·04	0·08	0·16
LAKE MONGER	A	0	3	5	9	10
	B	0	0	3	9	10
	C	1	1	3	9	10
	TOTAL	1	4	11	27	30
	PERCENTAGE MORTALITY	3	13	36	90	100
LAKE LESCHENAULTIA	A	0	2	2	6	
	B	0	0	2	6	
	C	0	2	3	7	8
	TOTAL	0	4	7	19	8
	PERCENTAGE MORTALITY	0	13	23	63	80
VICTORIA PARK	A	3	1	4	8	10
	B	0	2	1	6	10
	C	1	0	4	5	9
	TOTAL	4	3	9	19	29
	PERCENTAGE MORTALITY	13	10	30	63	97

TABLE 16 : Mortality of Gambusia affinis affinis after 96 hours exposure to a range of DDT concentrations.

LAKE MONGER

DDT CONCN	NUMBER OF FISH	NUMBER KILLED	PERCENTAGE KILLED	$\log_{10}$ (concn × 100)	EMPIRICAL PROBIT
0·02 ppm	30	1	3·3	0·301	3·162
0·04 ppm	31	2	6·5	0·602	3·486
0·08 ppm	30	13	43·3	0·903	4·831
0·16 ppm	30	20	66·7	1·204	5·432
0·10 ppm				1·02	5·000

LC_{50} = 0·10 ppm DDT

LAKE LESCHENAULTIA

0·02 ppm	29	1	3·4	0·301	3·175
0·04 ppm	30	1	3·3	0·602	3·162
0·08 ppm	30	6	20	0·903	4·158
0·16 ppm	10	2	20	1·204	4·158
0·40 ppm				1·60	5·000

LC_{50} = 0·40 ppm DDT

VICTORIA PARK

0·02 ppm	31	1	3·2	0·301	3·148
0·04 ppm	30	2	6·7	0·602	3·575
0·08 ppm	30	2	6·7	0·903	3·575
0·16 ppm	30	5	16·7	1·204	4·034
1·00 ppm				2·00	5·000

LC_{50} = 1·00 ppm DDT

TABLE 17 : Determination of Empirical Probits and LC_{50}'s for 24 hours exposure to DDT. Where necessary, percentage kills have been adjusted (using Abbot's formula) to account for control mortality.

LAKE MONGER

DDT CONCN	NUMBER OF FISH	NUMBER KILLED	PERCENTAGE KILLED	$\log_{10}$ (concn x 100)	EMPIRICAL PROBIT
0·02 ppm	30	1	3·3	0·301	3·162
0·04 ppm	31	7	25·8	0·602	4·350
0·08 ppm	30	24	80	0·903	5·842
0·16 ppm	30	24	80	1·204	5·842
0·06 ppm				0·815	5·000

LC_{50} = 0·06 ppm DDT

LAKE LESCHENAULTIA

0·02 ppm	29	1	3·4	0·301	3·175
0·04 ppm	30	3	10	0·602	3·718
0·08 ppm	30	11	13·3	0·903	3·888
0·16 ppm	10	4	40	1·204	4·747
0·25 ppm				1·40	5·000

LC_{50} = 0·25 ppm DDT

VICTORIA PARK

0·02 ppm	31	1	0	0·301	...
0·04 ppm	30	2	0	0·602	...
0·08 ppm	30	8	18·6	0·903	4·107
0·16 ppm	30	11	29·7	1·204	4·467
0·40 ppm				1·60	5·000

LC_{50} = 0·40 ppm DDT

TABLE 18 : Determination of Empirical Probits and LC_{50}'s for 36 hours exposure to DDT. Where necessary, percentage kills have been adjusted (using Abbot's formula) to account for control mortality.

DDT CONCN	NUMBER OF FISH	NUMBER KILLED	PERCENTAGE KILLED	$\log_{10}$ (concn x 100)	EMPIRICAL PROBIT
0·02 ppm	30	1	3·3	0·301	3·162
0·04 ppm	31	8	25·8	0·602	4·350
0·08 ppm	30	26	86·7	0·903	6·112
0·16 ppm	30	29	96·7	1·204	6·838
0·05 ppm				0·72	5·000

LC_{50} = 0·05 ppm DDT

LAKE LESCHENAULTIA

0·02 ppm	29	2	6·9	0·301	3·517
0·04 ppm	30	4	13·3	0·602	3·888
0·08 ppm	30	14	46·7	0·903	4·817
0·16 ppm	10	7	70·0	1·204	5·524
0·10 ppm				1·00	5·000

LC_{50} = 0·10 ppm DDT

VICTORIA PARK

0·02 ppm	31	1	0	0·301	...
0·04 ppm	30	2	0	0·602	...
0·08 ppm	30	10	25·9	0·903	4·354
0·16 ppm	30	22	70·3	1·204	5·533
0·12 ppm				1·08	5·000

LC_{50} = 0·12 ppm

TABLE 19 : Determination of Empirical Probits and LC_{50}'s for 48 hours exposure to DDT. Where necessary, percentage kills have been adjusted (using Abbot's formula) to account for control mortality.

LAKE MONGER

DDT CONCN	NUMBER OF FISH	NUMBER KILLED	PERCENTAGE KILLED	$\log_{10}$ (concn x 100)	EMPIRICAL PROBIT
0·02 ppm	30	3	10	0·301	3·718
0·04 ppm	31	9	29	0·602	4·447
0·08 ppm	30	27	90	0·903	6·282
0·16 ppm	30	30	100	1·204	8·719
0·04[illegible] ppm				0·62	5·000

LC_{50} = 0·04[illegible] ppm DDT

LAKE LESCHENAULTIA

0·02 ppm	29	3	10	0·301	3·718
0·04 ppm	30	5	16·7	0·602	4·034
0·08 ppm	30	16	53·3	0·903	5·083
0·16 ppm	10	7	70	1·204	5·524
0·08 ppm				0·92	5·000

LC_{50} = 0·08 ppm DDT

VICTORIA PARK

0·02 ppm	31	2	0	0·301	...
0·04 ppm	30	7	11·5	0·602	3·800
0·08 ppm	30	14	38·4	0·903	4·705
0·16 ppm	30	27	88·5	1·204	6·200
0·08 ppm				0·92	5·000

LC_{50} = 0·08 ppm DDT

TABLE 20 : Determination of Empirical Probits and LC_{50}'s for 72 hours exposure to DDT. Where necessary, percentage kills have been adjusted (using Abbot's formula) to account for control mortality.

LAKE MONGER

DDT CONCN	NUMBER OF FISH	NUMBER KILLED	PERCENTAGE KILLED	$\log_{10}$ (concn x 100)	EMPIRICAL PROBIT
0·02 ppm	30	4	10·3	0·301	3·735
0·04 ppm	31	11	33·3	0·602	4·568
0·08 ppm	30	27	89·7	0·903	6·625
0·16 ppm	30	30	100	1·204	8·719
0·04 ppm				0·62	5·00

LC_{50} = 0·04 ppm DDT

LAKE LESCHENAULTIA

0·02 ppm	29	4	13·8	0·301	3·911
0·04 ppm	30	7	23·3	0·602	4·271
0·08 ppm	30	19	63·3	0·903	5·340
0·16 ppm	10	8	80·0	1·204	5·842
0·07 ppm				0·83	5·000

LC_{50} = 0·07 ppm DDT

VICTORIA PARK

0·02 ppm	31	3	0	0·301	...
0·04 ppm	30	9	19·3	0·602	4·133
0·08 ppm	30	19	57·7	0·903	5·194
0·16 ppm	30	29	96·1	1·204	6·762
0·07 ppm				0·83	5·000

LC_{50} = 0·07 ppm DDT

TABLE 21 : Determination of Empirical Probits and LC_{50}'s for 96 hours exposure to DDT. Where necessary, percentage kills have been adjusted (using Abbot's formula) to account for control mortality.