EFFECTS OF PESTICIDES ON ECOSYSTEMS

I. PRELIMINARY REPORT ON SOME PROPERTIES OF DURSBAN

BY

MADELYN F. WEBB

REPORT TO PESTICIDE SECTION, CANADIAN WILDLIFE SERVICE,

OCTOBER, 1970

Acknowledgements

Several people and agencies have contributed to the organization and support of this programme whom I wish to acknowledge.

Financial support for the project was provided by a Province of Ontario Grant to the Biology Department of York University for the study of ecological effects of pesticides, and by the Canadian Wildlife Service.

I would like to thank Mr. R. Boelens of the Ontario Water Resources Commission for suggesting possible sites for study, and providing information on recommended pesticides.

Many thanks to Mr. D. Williams for technical assistance throughout the summer, and to Miss J. Jacks for help with the field work.

I am particularly indebted to Dr. C.D. Fowle for his guidance and participation in the project.

Samples of pesticides were provided free of charge by Dow Chemical Co. and American Cyanamid Co.

INTRODUCTION

This report describes some of the preliminary work carried out during the summer of 1970 in preparation for a field project in 1971 to study the effects of a pesticide on an ecosystem.

The plan called for the selection of a study area in a diversified habitat characterized by a fairly complex food web and a substantial floral and faunal list. After consideration of various possible locations near the headquarters at York University in Toronto, it was decided to concentrate on a swamp habitat on the northeastern shore of Lake Simcoe near Brechin, Ontario.

The site was selected with the advice of officers of the Ontario Water Resources Commission who reported that permission for aerial spraying of the area for mosquito control had been refused in the spring of 1970 because the application for a permit had been received too late to allow adequate time for pre-spray surveys. However, it may be possible to spray the area next spring, in which case we should be able to assess some of the effects of an operational application of a pesticide and possibly to trace its fate in the ecosystem.

The summer activities included:

1. Organization of laboratory facilities, library and other supporting services.

2. Selection and preliminary survey of a study area.

3. Assessment of possible analytical methods for the pesticides proposed.

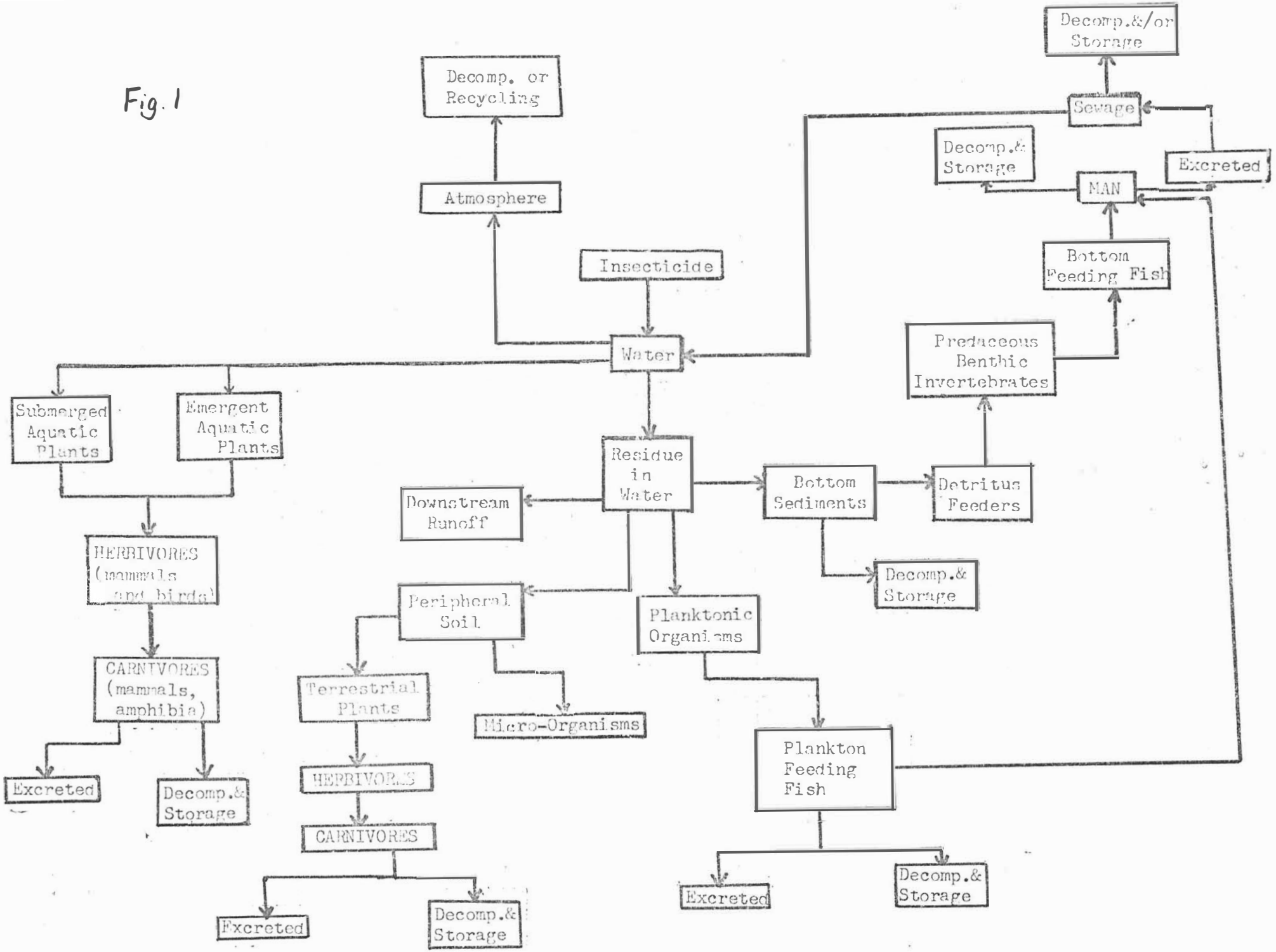
3. Preliminary study of the pesticides proposed through consultations, literature review, and laboratory tests to determine toxicity and other properties and to develop bioassay methods. The two compounds proposed are Dursban (O,O-diethyl O-3,5,6-trichloro-2-pyridylphosphorothioate) and Abate (O,O,O'O'-Tetramethyl O,O'-thiodi-p-phenylene phosphorothioate)

5. The preparation of a hypothetical model of the possible pathways involved in the transfer of pesticides or their breakdown products in an aquatic ecosystem. This model has been used in designing experiments. (Fig. 1.)

THE STUDY AREA

The area chosen surrounds and is adjacent to "Lagoon City", a new cottage development near the shore of Lake Simcoe being constructed on land reclaimed from a swamp area characterized by a complex of alder, silver maple, white birch and cedar. Pasture and croplands lie on the higher ground beyond the wooded swamp. There are extensive temporary and permanent pools and marsh areas, many of which support a high population of mosquitos.

Fig. 1


Decomp.&/or Storage
Decomp. or Recycling
Sewage
Decomp.& Storage
Excreted
MAN
Atmosphere
Insecticide
Bottom Feeding Fish
Water
Predaceous Benthic Invertebrates
Submerged Aquatic Plants
Emergent Aquatic Plants
Residue in Water
Downstream Runoff
Bottom Sediments
Detritus Feeders
HERBIVORES (mammals and birds)
Decomp.& Storage
Peripheral Soil
Planktonic Organisms
CARNIVORES (mammals, amphibia)
Terrestrial Plants
Micro-Organisms
Excreted
Decomp.& Storage
HERBIVORES
Plankton Feeding Fish
CARNIVORES
Excreted
Decomp.& Storage
Excreted
Decomp.& Storage

The first survey was carried out in the last two weeks of May. At this time, the water in the roadside swamp areas was about two feet deep. Although the level fell progressively during the summer, the area was never completely dry. A large proportion of the water surfaces were covered with a dense growth of Lemna and the bottoms of the ponds with a dense mat of rotting leaves. As the water level lowered, the water became heavily laden with finely divided organic particles.

Samples of the aquatic fauna were taken, both from the surface water and bottom debris. An indication of the variety of organisms found is shown in the presence list in Table 1. No quantitative estimate of relative abundance of the species was attempted. Although few mosquito larvae were found at any particular sampling time, the suitability of the site for mosquitos was attested to by the large numbers of adults present.

Collections of living arthropods, molluscs and other invertebrates were made and brought to the laboratory for use in toxicity tests. The range of organisms was extended later by collecting additional forms not represented at Lagoon City from the Credit River near the Credit Forks.

TABLE 1

Presence List of Classes and Orders represented

Ciliata	-	Paramecium
Turbellaria	-	Planarian
Crustacea	-	
Order Cladocera	-	water flea
Podocopa	-	ostracods
Amphipoda	-	
Eucopedoda	-	copepods
Decapoda	-	crayfish
Mollusca	-	clams and snails
Oligochaeta	-	tubificids, leeches
Insecta:		
Order Plecoptera		stonefly nymphs
Hemiptera	-	corixids
Odonata	-	damselfly and dragonfly nymphs
Trichoptera	-	caddisfly larvae
Diptera	-	chironomid larvae
Coleoptera	-	beetle larvae

EXPERIMENTS

As chemical analysis for both Dursban and Abate are complex, time-consuming and expensive, it is essential to develop a bioassay for monitoring the ecosystem for level and presence of these compounds. It would be desirable, of course, to know something of the specific toxicity and behavioural response for the common aquatic invertebrates in the area so that in an operational application they themselves might serve as indicators

of the presence of pesticide. Further, it is important that some of the more obvious factors likely to influence the effect of the pesticide be examined in advance of operational spraying.

Accordingly a series of experiments were set up designed to:

1) test different species to determine which would be suitable for developing a standard bioassay to be used in future field experiments.

2) develop time/response curves for the species chosen, such that these could be used to monitor the presence of the insecticide in samples from the field, and its approximate concentration.

3) carry out tests to determine the effects of some ecological parameters on the toxicity of the insecticide.

Development of the Bioassay:

A selection of animals found at Lagoon City and the Credit Forks were tested for sensitivity to Dursban. Those tested included: ostracods, caddisfly larvae, stonefly nymphs, water beetles, snails, amphipods and tadpoles. LT_{50}'s were determined where possible, and the organism was evaluated as a potential candidate for bioassay.

The majority of organisms tested, such as the stoneflies and caddisflies, proved to be impractical for bioassay work. Differences in size of the insects collected in the field, the necessity of maintaining them under running water conditions, and the large numbers required for bioassay made their use impractical. The laboratory experiments did reveal the relative sensitivities of several organisms to Dursban. These results are summarized in Table 2.

TABLE 2

Effect of Dursban on various organisms

Conc. Dursban	Organism	Effect
0.001 ppm	caddisfly	slightly affected in 22 hrs.
0.01	amphipod	unaffected in 2 hours
0.01	ostracod	unaffected in 3 hours
0.03	amphipod	unaffected in 2 hours
0.05	caddisfly	22 hour LD_{50}
0.1	large snails	unaffected in 24 hours
0.1	small snails	loss of muscular co-ordination in 24 hours
0.1	stoneflies	LT_{50} - 205 minutes
0.1	corixids	LT_{50} - 143 minutes
0.5	tadpole	no reaction to stimuli after 24 hrs.
1	ostracod	LT_{50} - 155 minutes

The results of experiments with field collected organisms turned our attention to laboratory cultures from which more or less standardized animals would be available. Two were tried: the water flea (Daphnia magna) and larval mosquitos (Aedes aegypti). Details of rearing and the procedure for bioassay are found in Appendix A.

Time/response curves for Dursban using Daphnia and second instar Aedes larvae are shown in Figures 2 and 3.

LT_{50}'s or the time required for the pesticide to act were determined for the two species, rather than LD_{50}'s or some other parameter, in order that bioassays will be completed within one day in the field. For this reason, sensitive organisms such as D. magna and early instar mosquito larvae were required.

The shape of the graph for Dursban (Fig. 3) shows that at very low concentrations, a slight increase in the amount of insecticide has a relatively greater effect on the organism than the same increase at a higher concentration. The effect of the insecticide tends to level off at higher concentrations between .01 and 1 ppm. This means that increasing the dosage, at this concentration will not greatly shorten the time to kill the larvae. Whether or not Abate exhibits this same effect at low concentrations has yet to be determined.

Effects of some ecological parameters on toxicity

The ability of certain pesticides to codistill with water has been reported in the literature, especially with reference to DDT (Acree et al.,1963). Any pesticide possessing this property has a potential of becoming widely dispersed through the environment and affecting non-target organisms far from the point of application.

Experiments were carried out to test the toxicity of Dursban vapours emanating from a water surface, on hatchlings of the locust (Locusta migratoria migratorioides). The apparatus consisted of a chromatography jar containing 1000 ml. of 1 ppm Dursban. The hatchlings were placed in the apparatus, about 10 inches above the water, as shown in the diagram in Fig. 4, held in place by a fine mesh screening taped to the sides of the jar. A glass plate cover maintained a closed system. After periods of from 18 to 24 hours, the mortality of the locusts was noted. Variations of this experiment, included placing water plants in the water, along with the insecticide to determine the effect of the presence of living plants on volatilization of the compound. Suspensions of detritus, prepared in a Waring blender, were also added to the solution in some experiments to test the effect of finely divided, decomposing organic matter on volatility. One ppm solution which had been left exposed outdoors overnight and in sunlight for several hours was also tested for the effects of heat, light, and exposure to the atmosphere on the toxicity of the vapours.

Hatchlings exposed to Dursban vapours from a 1 ppm water solution exhibited 100% mortality within 24 hours. Addition of detritus resulted in lower mortality over the same time interval, as did the addition of plants.

Fig. 2

LT_{50} vs concentration of Dursban for *Daphnia magna* in water with and without detritus

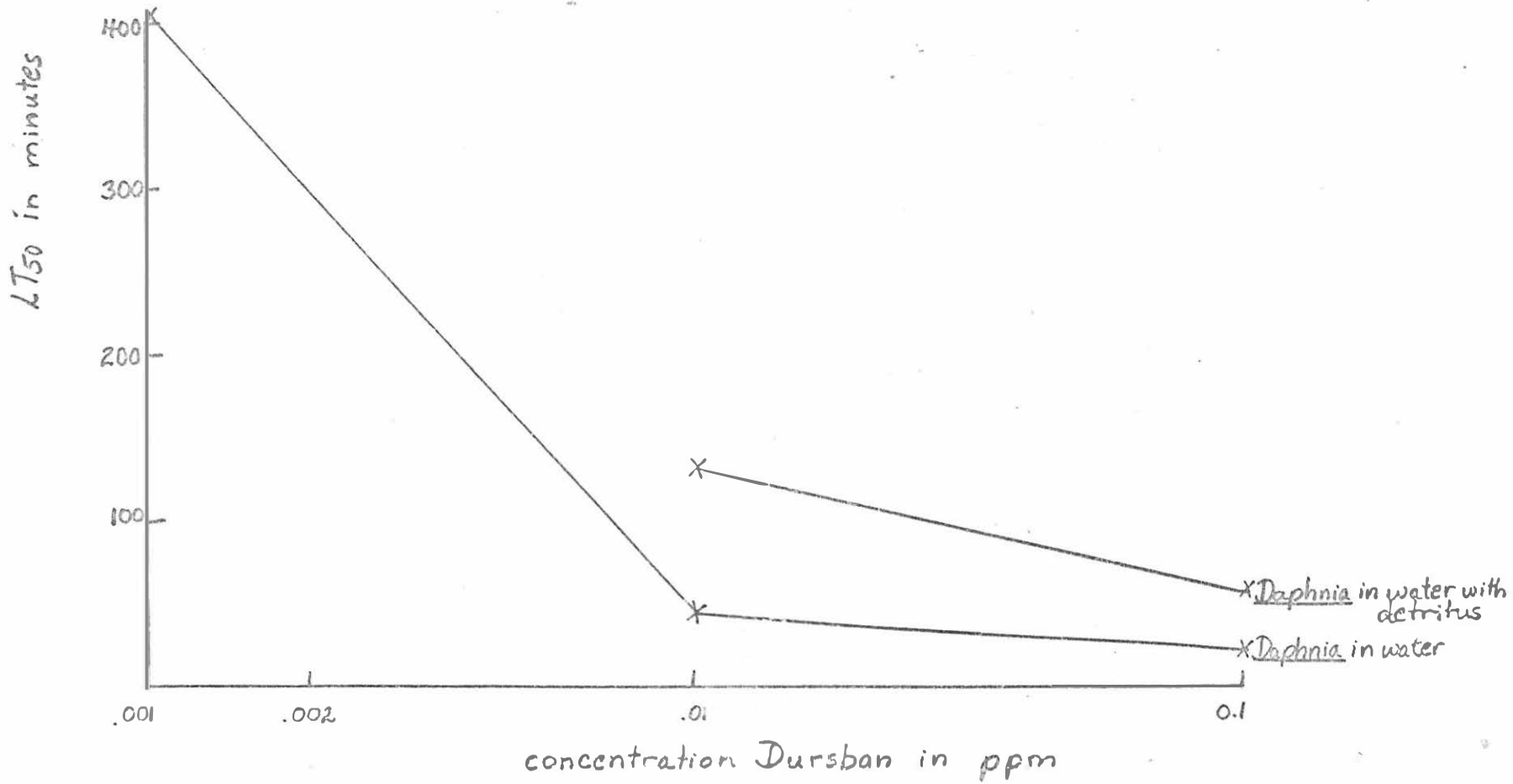


Fig. 3

LT_{50} vs concentration of Dursban or Abate for second instar larvae of *Aedes aegypti*

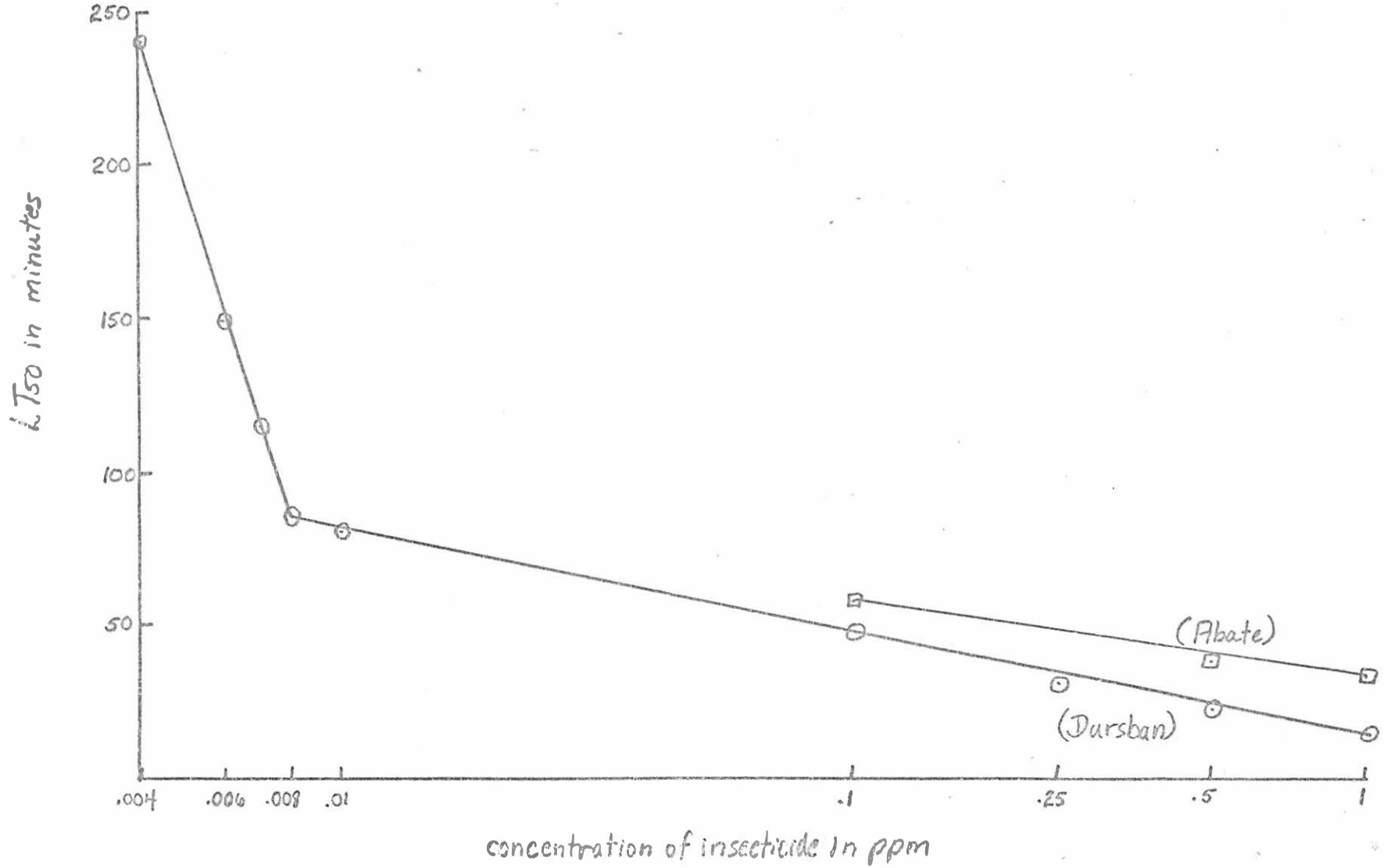
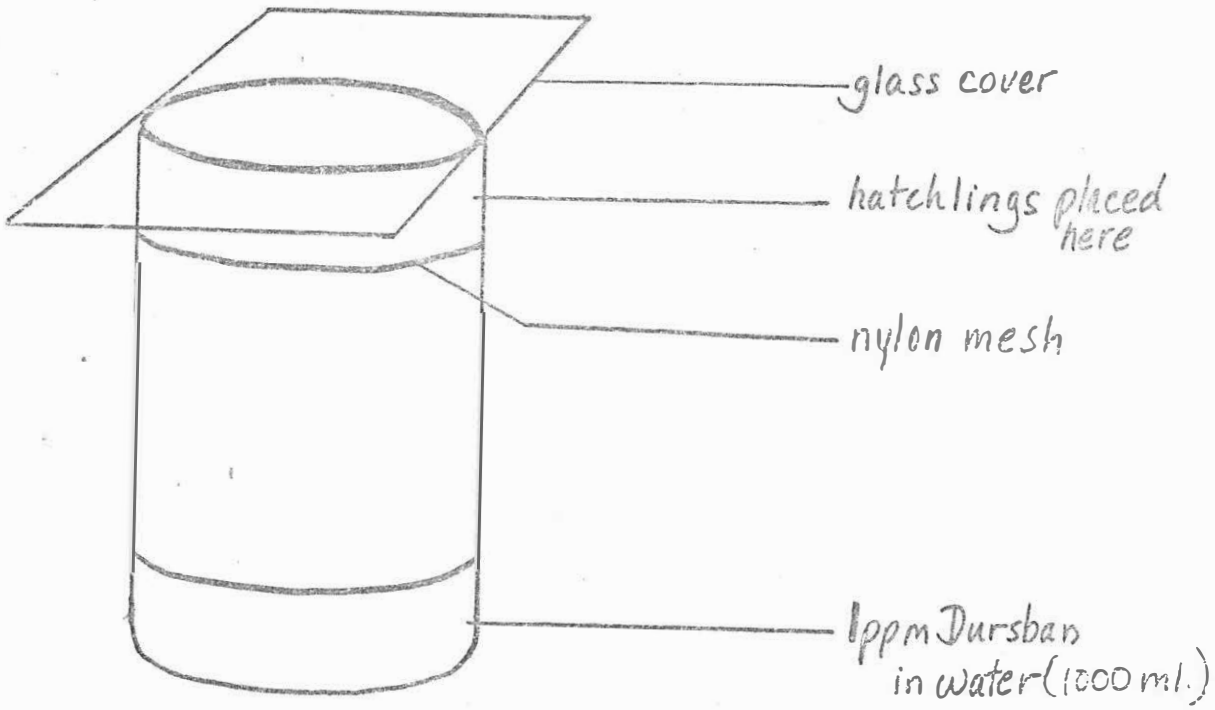


Fig. 4

Apparatus for testing toxicity of Dursban vapours to hatchlings of *Locusta migratoria migratorioides*



Exposure of the toxic solution to the atmosphere overnight resulted in lower toxicity in tests the next day. When the solution was exposed to sunlight for a short time, the temperature of the water was raised, thereby increasing the volatility of the insecticide, and 100% mortality was reached in a shorter time. Table 3 summarizes this information.

TABLE 3

Effect of Dursban vapours on locusts:(Dursban concentration = 1ppm.)*

Time of exposure	1 ppm Dursban	% Mortality detritus in water	vegetation in water	outdoors overnight	in sunlight 6 hrs.
18 hours	89.5%	5.0%		67.0%	
18 1/2 hrs.	85.0%		47.2%	85.0%	100%
19 hrs.	91.0%	40.0%			
23 hours		84.5%			
24 hours	100.0%	90.0%		71.6%	

*Note: all figures are average of at least 2 replicates.

Affinity of Dursban for Organic Matter:

The observation of an apparent reduction of toxic vapours when detritus or plants were present in the water to which Dursban was added led to questions on the extent of the affinity of Dursban for organic matter. Three soil columns were prepared; two of dry swamp soil of 12 and 14 cms. and one of soil from dense woods of 15 cms. Eighty ml. of 1 ppm Dursban was allowed to percolate through the soil columns, and the eluate tested for Dursban using mosquito larvae. The columns were then washed with dechlorinated water and the eluates tested for Dursban by means of a bioassay with second instar A. aegypti. It was found, when solutions of 1 ppm Dursban were percolated through columns of soil that:

1) Samples from 80 ml percolated through the 14 cm. column of swamp soil, failed to kill mosquito larvae, suggesting that there was a minute amount of pesticide left.

2) 70 ml. of the 80 ml. percolated through the 12 cm. column of dry swamp soil were estimated to contain 0.005 - 0.006 ppm. Dursban. Three 80 ml. washings of the column with dechlorinated water each contained about 0.005 ppm. Dursban after passing through the soil column.

3) 80 ml. of 1 ppm Dursban percolated through the 15 cm. column of dense woods soil contained an estimated 0.005 ppm Dursban.

Further evidence of the affinity of Dursban for organic matter was obtained by comparing the mortality of D. magna exposed to Dursban in water containing finely divided organic matter and in that containing

no organic matter. Fig. 2 illustrates that the LT_{50} for D. magna is greater in water and Dursban when organic particles are present.

DISCUSSION AND PROPOSALS FOR FUTURE

Most of the work to date has been done to gain familiarity with the site, and to develop techniques which will be required to monitor pesticidal effects following a spray programme. Experience with the effects of two organophosphate insecticides, one of which will almost certainly be used in the spray programmed has been gained, and their toxicity for several organisms at different concentrations has been tested in the laboratory.

The action of the insecticides under different environmental conditions has been studied in the laboratory, and the acquisition of C-14 labelled compounds will permit these experiments to be expanded and refined.

With respect to the activities in the field, to be carried out in conjunction with the spraying operation, the following outline is proposed:

1) Early April or late March, immediately before application of the pesticide, assessment of fauna present and recording of the amount of water cover at the site.

2) Modification of bioassay procedures according to compound to be applied and method of application.

3) Setting up of field monitoring stations, using method of Gillies et al., (1968).

4) Organization of a field station at which bioassays can be carried out(optional).

5) Collection of aquatic animals for analysis by GLC, at intervals post-spray.

6) Analysis of detritus samples from swamp bottom for presence of pesticide. This will be continued throughout the summer if initial results are positive.

7) Monitoring by GLC of certain organs from organisms higher in the food chain.

8) Determination of effectiveness of spray programme by assessing abundance of target organisms before and after spraying, and at intervals post-spray.

9) Monitoring changes in populations different from those noted in 1970.

Personnel Required:

Two or three engaged in field work and bioassays. They should be familiar with bioassay technique before spraying begins. One person capable of GLC analysis.

APPENDIX A

1. Rearing Daphnia

D. magna were reared according to the method of Bond (1934) Frear & Boyd (1967), being fed on the blue-green alga, Anabaena flos-aquae, with weekly additions of yeast suspension. The cultures were maintained at room temperature, 20°C, and aerated continually. When animals were required for an experiment, the larger ones were selected, but no attempt to standardize the animals according to age was made. The water was removed by straining through a wire sieve, and the Daphnia were then introduced into the test medium.

2. Rearing mosquitos.

Aedes aegypti was the species used in all experiments as it is easy to maintain in laboratory cultures (Needham, 1937). Adults were raised in wooden frame cages covered with fiberglass mesh. Eggs were collected by placing paper strips on the sides of a dish half-filled with water, at the air-water interface. These strips with eggs were removed each day, and placed in an aerated pan maintained at 25°C. by means of an incandescent 100 watt lamp. The various larval stages of the mosquito were maintained separately in this way. Pupae were returned to the cages in water to keep the adult population at a high level. Adults fed twice weekly on blood from live rats. Glucose solution was available in the cages for males and supplementary feeding. Larvae were fed on yeast and ground Purina chow.

Locust rearing

Locusts were reared according to the method of Tobe and Loughton. (1969) Locusts were used within 24 hours of hatching in all experiments. Egg cases were obtained from adults maintained in a large culture by placing plastic cups filled to within one-half inch of the top with sand. After egg laying the cups were removed and covered with plastic held in place by an elastic band. The eggs were incubated at 37°C. for nine days, after which hatchlings to be used for experimentation were released into a plastic bag containing bran. Some bran was made available to the hatchlings during experiments.

Description of Experiments:

LT_{50}'s were determined for D. magna at varying concentrations of Dursban (0.1, 0.01, 0.001 ppm). This was done by setting up 50 ml. beakers containing the required concentration of insecticide and adding ten daphnids to each one. Death was designated as the state where swimming movements could no longer be provoked in the animals by swirling the water in the beaker. When 50% of the daphnids in a beaker had reached this state (died), the time was noted. An average time was obtained from the five replicates and this plotted on the graph of time versus concentration of insecticide. (Fig. 2) LT_{50}'s were determined as above for second instar larvae of Aedes aegypti. These are graphed in Fig. 3.

LITERATURE CITED

Acree, F.Jr.,Benza, M. Bowman, M.C. 1963 Codistillation of DDT with water J. Agr. Fd. Chem. 11:278-280

Bond, R.M. 1934. A culture medium for daphnids. Science 79:60

Frear, D.E.H., Boyd, J.E. 1967. Use of Daphnia magna for the microbioassay of pesticides. I. Development of standardized techniques for rearing Daphnia and preparation of dosage-mortality curves for pesticides. J. Econ. Ent. 60 (5): 1228-1236.

Gillies, P.A., Womeldorf, D.J., Walsh, J.D. 1968. A bioassay method for measuring mosquito larvicide depositions. Mosquito News

Needham, J.G. 1937 Culture methods of invertebrates. New York. 590 pp.