THE UNIVERSITY OF MANITOBA

DISAPPEARANCE AND BIOACTIVITY OF DURSBAN INSECTICIDE IN TEMPORARY POOLS

BY

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ABSTRACT

Two formulations of Dursban insecticide (chlorpyrifos), emulsifiable concentrate (EC) and granular (G), were applied to outdoor sod pools at 0.056 kg ai/Ha. In the EC treated pools the peak chlopyrifos concentration occurred at zero hours post-treatment with 0.0162 ppm in the water compared to the maximum concentration of 0.0055 ppm at four hours in the water of the G treated pools. By 72 hours the chlorpyrifos concentrations in both the EC and G treated pools were the same (0.0010 ppm). The chlorpyrifos concentration from the EC treated pools had decreased rapidly and reached the detectable limit at 408 hours post-treatment. The G treatment resulted in a much lower initial chlorpyrifos concentration but maintained detectable residues until 720 hours. The EC treatment resulted in 100% mortality of Culex tarsalis bioassay for 96 hours and reached zero percent mortality by 408 hours. The G formulation, with a much lower initial chlorpyrifos residue than the EC provided 98% or better bioassay mortality for 168 hours while zero mortality did not occur until 720 hours post-treatment.

Dursban was applied at the rate of 0.028 kg ai/Ha to laboratory and field pools lined with sod, clay, or sand substrates. In the laboratory pools, G treatment resulted in an initial chlorpyrifos concentration of 0.0 ppm in the sod pools, 0.0057 ppm in the clay pools, and 0.142 ppm in the sand pools. As the result of the low chlorpyrifos concentration in the water, bioassay mortality in the sod pools was 0%, compared to 100% mortality in the clay and sand pools for 192 and 336 hours, respectively. The EC treated laboratory pools resulted in concentrations of 0.0081 ppm, 0.0086 ppm, and 0.0079 ppm chlorpyrifos in the water of the sod, clay, and sand pools, respectively and resulted

in 100% mortality for 48 hours, 96 hours, and 192 hours, for the sod, clay and sand, respectively. In field pools, the G Dursban treatment resulted in the chlorpyrifos concentrations of 0.0037 ppm for the sod pools, 0.0031 ppm for the clay pools, and 0.0045 ppm for the sand pools. This treatment maintained 100% bioassay mortality in the sod pools for 4 hours, in the clay pools for 48 hours, and in the sand pools for 96 hours. The EC treatment of outdoor pools resulted in 100% bioassay mortality for 4 hours, 48 hours, and 96 hours in the sod, clay, and sand pools, respectively. The initial chlorpyrifos concentrations in these pools were 0.0070 ppm, 0.0122 ppm, and 0.0125 ppm for the sod, clay, and sand, respectively.

In all trials, the lowest chlorpyrifos residue in the water, fastest chlorpyrifos disappearance rate, and shortest period of 100% bioassay mortality occurred in the water of the sod-lined pools while the highest residues, the slowest disappearance rate, and the longest control period occurred in the pools with sand substrate.

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I. INTRODUCTION

The mosquito Culex tarsalis Coquillett occurs throughout southwestern Canada and is an important vector of western encephalomyelitis. Birds are the preferred host, although C. tarsalis will take a blood meal from horses and humans which can result in the spread of the virus which causes disease in humans and horses.

C. tarsalis lays its eggs in temporary and permanent pools of water found, for example, in ditches, dugouts or containers that collect rainwater. It is while the mosquito larvae are in these pools that an effective program of control can be carried out using larvicide treatment.

One of the registered mosquito larvicides in Canada is Dursban (chlorpyrifos, O,O-diethyl O-2-(3,5,6-trichloro-pyridyl) phosphorothioate). The fact that it is registered means that it is effective but not necessarily always environmentally acceptable. Of environmental concern is the amount of insecticide residue present in the water after treatment and to what degree the residue level depends upon the formulation used in the control program. Residue levels are important in terms of predicting possible effects on non-target organisms in the water.

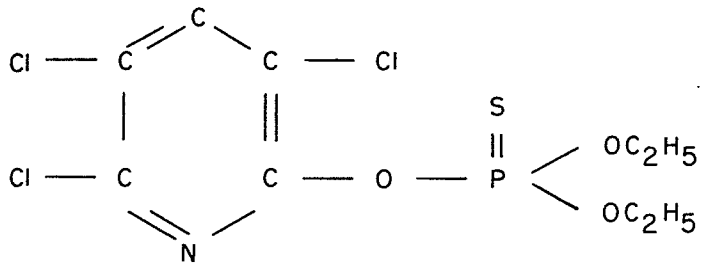
Research was undertaken to study the bioactivity and rate of disappearance of chlorpyrifos from temporary pools treated with either of two Dursban formulations (granular or emulsifiable concentrate). The bioactivity was measured by bioassays with laboratory-reared fourth instar C. tarsalis larvae while the chlorpyrifos concentration in the water was determined by gas liquid chromatography. Since many types of ground conditions exist that could influence the characteristics

of the infested pools, the possible effect of different substrates on the bioactivity and disappearance rates of chlorpyrifos, were determined for the two formulations in pools with sod, clay or sand substrates. Laboratory experiments were conducted in an attempt to predict the results that would occur in the field.

The object of this thesis is to achieve a better understanding of the activity and residues of Dursban insecticide in water when used for mosquito larval control.

II. LITERATURE REVIEW

Chlorpyrifos is an organophosphate insecticide developed by the Dow Chemical Company and is marketed under the tradename DURSBAN. Dursban is a broad spectrum insecticide effective against household pests, chinchbugs, cutworms, and mosquitoes (Gray 1965) and was first described by Kenega et al. (1965). Dursban has the molecular formula of $C_9H_{11}Cl_3NO_3PS$ and the following structural formula:



Chlorpyrifos is a colourless crystalline solid with a melting point of 41.5-43°C and a vapour pressure of 1.87×10^{-5} mm Hg at 25°C. Its molecular weight is 350.5. At 25°C chlorpyrifos is soluble in water to the extent of two parts per million (ppm); i.e., 2 mg/l (Smith 1966).

This review surveys the literature on the use of Dursban as a mosquito larvicide and its effect on the aquatic environment.

Biological Effects on Dursban

Effects of target organisms

Gray (1965) and Ludwig and McNeil (1966) determined the LD_{50} and LD_{95} for Dursban against several species of mosquito larvae (see Table I).

Table I. Dursban LD_{50} and LD_{95} for Mosquito Larvae

Larvae	LD_{50} (ppm)	LD_{95} (ppm)
Culex pipiens		0.0022
Culex fatigans	0.0003	0.0020, 0.0025
Aedes aegypti	0.0010	0.0040, 0.0028
Anopheles albamius*		0.0025

Ludwig and McNeil (1966) also noted that marshland treated with Dursban granules (G) at the rate of 2.2 kilograms active ingredient per hectare (kg ai/Ha) remained free of mosquito larvae for 11 weeks. An emulsifiable concentrate (EC) formulation applied to the marsh at the rate of 0.056 kg ai/Ha allowed the mosquito larvae to re-establish themselves 12 days post-treatment in one test and four weeks post-treatment in another test.

Mulla et al. (1966) determined the percent of control of Culex tarsalis larvae 24 hours post-treatment with Dursban EC-40 applied to pools at the rate of 0.001-0.01 kg ai/Ha (see Table II).

Table II. Percent Control of C. tarsalis 24 hours Post-Treatment

Dursban (kg ai/Ha)	Percent Control
0.001	63
0.002	83
0.005	100
0.010	100

* According to Stone et al. (1959) no such mosquito species exists. Gray probably means Anopheles albimanus.

Bailey et al. (1970) tested slow release (8.7% polyvinyl chloride (PVC) pellets) and EC formulations of Dursban for control of C. fatigans larvae in man-made pot-holes. Twenty-four hour bioassays were conducted by placing lab-reared larvae into paper containers floating in the pot-holes. Control with the PVC pellets ranged from zero days at five ppm to 70 days at 20 ppm. The EC gave 75 percent control for one day at 0.0025 ppm but more than 200 days at five, ten, and 20 ppm.

Tawfik and Gooding (1970) used field collected Aedes larvae to determine the LD_{50} of Dursban. The same information was also determined for DDT and Abate (see Table III).

Table III. LD_{50} for Dursban, DDT, and Abate Against Aedes Larvae

Insecticide	LD_{50} (ppm)
Dursban	< 0.0001
DDT	0.0010
Abate	0.0001

Dixon and Brust (1971) applied three formulations of Dursban to study the effectiveness of winter prehatch applications. Two EC formulations (41%) and one G (5%) were applied at 0.28 kg ai/Ha to frozen man-made pools in November. Bioassays with Aedes flavescens the following May revealed no residual control. Summer applications of Dursban at the same rate produced 90 percent control for three to four weeks. At the rate of 0.028 kg ai/Ha, one EC and the G formulation gave 100 percent control of Aedes vexans for one week while the second EC formulation gave 100 percent control for two weeks.

Womeldorf and Whitesell (1972) determined that various instars of Anopheles freeborni showed a differential susceptibility to Dursban (see

Table IV).

Table IV. Susceptibility of Various Instars of A. freeborni to Dursban

Location	Instar	LC_{50} (ppm)	LC_{90} (ppm)
1	2	0.00023	0.001
	4	0.0035	0.0055
2	2	0.00028	0.00045
	3	0.00071	0.0014
	4	0.0017	0.0029
3	1	0.00023	0.0004
	2	0.00082	0.0014
	4	0.014	0.031

Miller et al. (1973) compared the larvicidal effectiveness of a water emulsion and three polymer formulations of chlorpyrifos. The effectiveness of the formulations was monitored by in-pool bioassays with fourth instar lab-reared Culex fatigans larvae. The formulations were: polyethylene pellets, 9.9% chlorpyrifos; PVC pellets 10% chlorpyrifos; polyethylene pellets, 11.5% chlorpyrifos; and water emulsion, 0.48% chlorpyrifos. Their tests showed that the LC_{90} for fourth instar C. fatigans was 0.0009 ppm. The water emulsion, at a rate of 0.009 ppm or 0.028 kg ai/Ha controlled Culex restuans in the pools for less than two weeks. The polymer formulations were applied at five ppm or 15.5 kg ai/Ha and resulted in 100 percent control of C. restuans for the 24 weeks post-treatment period of the experiment.

Cooney and Pickard (1974) studied the effectiveness of Dursban on floodwater mosquitoes. Sites known to produce suitable larval populations after inundation from spring rains were treated with one percent Dursban clay granules at the rates of 0.01 and 0.056 kg ai/Ha. At the

higher rate, 100 percent control of Aedes sticticus and Aedes vexans lasted for 26 days even though the test plot was flooded four times and dried between each flood period. At the lower rate of 0.056 kg ai/Ha 100 percent control lasted for two days and then dropped almost to zero.

Nelson et al. (1976a) studied larval control of Psorophora confinnis with a polyethylene pellet of 10.6 percent chlorpyrifos. Treatment of rice pools at rates of 0.25, 0.50, 1.0, and 2.0 ppm resulted in average bioassay mortalities over an 11 week test period of 22, 58, 79, and 99 percent, respectively. In pools treated at 0.25 ppm the bioassay mortality at 11 weeks post-treatment was 14 percent and the highest chlorpyrifos residue of 0.0006 ppm was reached in the water. In the pools treated at 2.0 the bioassay mortality at 11 weeks post-treatment was 100 percent with a chlorpyrifos residue in the water of 0.0009 ppm. Of all the treatments, the pools treated at 2.0 ppm achieved the highest chlorpyrifos residues of 0.0022 ppm during the first week post-treatment.

Effects on non-target organisms

From laboratory tests, Ferguson et al. (1966) determined that Dursban was less toxic to fish than most chlorinated hydrocarbons but generally more toxic than other organophosphate insecticides. Tests on three species of fish from three different sites gave the following average median tolerated limit of Dursban in parts per billion (ppb) in water (see Table V).

Table V. Tolerated Limit of Dursban to Fish

Species	Dursban (ppb)
Notemiogonus crysoleucas	68
Gambusia affinis	347
Lepomis cyanellus	62

Hurlbert et al. (1970) conducted experiments to study the effects of Dursban on non-target organisms. Their test animals were mallard ducks, mosquitofish, corixids (Hemiptera) and several zooplankton species.

Four zooplankton species, ranked by increasing tolerance to Dursban were: Moina micrura (cladoceran), Cyclops vernalis (copepod), Diaptomus pallidus (copepod), and Asplanchna brightwelli (rotifer). Applications of 0.01 kg ai/Ha of 41% EC (four times at two week intervals) resulted in greater than 95 percent mortality for M. micrura and about 95 percent for C. vernalis. D. pallidus was unaffected at rates of 0.01, 0.05, and 0.1 kg ai/Ha but failed to develop in ponds treated at 1.0 kg ai/Ha. A. brightwelli population showed no evidence of Dursban susceptibility at any rates used in the experiments.

Corisella decolor and Corisella edulis (Hemiptera: Corixidae) were the insect fauna in the pools. One day post-treatment the populations were drastically reduced. By using a nekton tow net the number of pre- and post-treatment corixids in the pools were estimated. At 0.05 kg ai/Ha the population was reduced by 92.5 percent, at 0.1 kg ai/Ha by 95.7 percent, and at 1.0 kg ai/Ha by 99.4 percent. The populations gradually recovered except in those pools treated at 1.0 kg ai/Ha which did not recover even after four weeks.

Prior to treatment, caged mosquitofish (Gambusia affinis) were

used to bioassay the pools. The effect of the first application of Dursban to the pools, on the caged mosquitofish is shown in Table VI. The fish increased in number and reproduced in all ponds except those at 1.0 kg ai/Ha.

Table VI. Mortality of G. affinis, 24 hours Post-Treatment

Dursban (kg ai/Ha)	Avg. Cumulative % Mortality
control	6
0.01	17
0.05	12
0.10	11
1.00	100

To study Dursban effect on vertebrates, five ducklings, each three to four weeks old and averaging 350 grams in weight, were placed on each pond three weeks before the first treatment. Two days before treatment the average weight had increased to 800 grams. Table VII shows the fate of the ducks used in these trials. Hurlbert concluded that the principal factor for the duck mortality was their exposure to Dursban.

Table VII. Duck Mortality on Pools Treated with Dursban

Dursban (kg ai/Ha)	Birds Surviving	Birds Dying
control	9	0
0.01	4	4
0.05	6	3
0.10	4	3
1.00	5	4

Brust et al. (1971) studied the effect of Dursban at levels from 0.08 to 1280 ppm in the drinking water of chicks. No adverse effects on the chicks were apparent at levels of Dursban below 80 ppm in the water. However, chick mortality reached 20 percent after exposure to 80 ppm Dursban and 100 percent mortality at 320 and 1280 ppm. At Dursban concentration of 80 ppm and above, cholinesterase activity in whole blood decreased whereas no decreased activity was observed at the lower concentrations.

Miyazaki and Hodson (1972) determined the toxicity of Dursban and a metabolite in chickens. The Dursban actue LD_{50} in two week old chickens was 34.8 mg/kg while its metabolite, 3,5,6-trichloro-2-pyridinol was found to have an acute LD_{50} of >1000 mg/kg.

Pimentel (1971) listed the LC_{50} for various arthropods to Dursban (see Table VIII). In his review, Pimentel stated that Dursban applied at 0.01 kg ai/Ha had no observable effect on mallards and pheasants.

Table VIII. Dursban LC_{50} for Arthropods

Species	LC_{50} (ppm)
Gammarus lacustris	0.00076
Pteronarcella badia	0.0042
Claassenia sabulosa	0.0082
Pteronarcys californica	0.0500

Hurlbert et al. (1972) applied Dursban (40% EC) to pools three times at two week intervals using rates of 0.028 and 0.28 kg ai/Ha. Twenty-four hour post-treatment samples after the second and third treatment showed a greater reduction of predaceous insects (Notonectidae, Dytiscidae, Coenagrionidae, and larval Hydrophilidae) than of the

herbivorous insects (Corixidae, Baetidae, and adult Hydrophilidae). The predaceous insect population generally recovered to control pond levels more slowly than the herbivorous insects. Five weeks after the last treatment the predaceous insect population in the pools dosed at 0.028 kg ai/Ha averaged only 45 percent of the total number found in the control pools while in the pools dosed at 0.28 kg ai/Ha only nine percent of the control population remained. In pools treated at both rates the herbivorous insect population was higher than in the control pools.

The population dynamics in the pools were upset by the impact of Dursban on the predator/prey relationship. Dursban removed the predators and the prey responded with dramatic increases in population. Cylcops vernalis and Moina micrura populations were destroyed by the Dursban which resulted in a 5-20 fold increase in the herbivorous rotifer population within one to three days.

Roberts et al. (1973) determined Dursban susceptibility levels (ppm) of some mosquito larvae and non-target organisms (see Table IX). The results show that the non-target organisms are more resistant to Dursban than the mosquito larvae tested. In the pools treated with a Dursban water emulsion at 0.009 ppm, a four week reduction in the population of gerrids (Gerris species) resulted and a two week reduction in the larval dytiscid population occured as well. Larval chaoborids and adult chironomids were unaffected. A polyethylene pellet formulation applied at 2.5 ppm reduced or suppressed the establishment of gerrids and larval chaoborids for nine weeks post-treatment while larval dytiscid population was reduced for 11 weeks.

Table IX. Dursban Susceptibility Levels (ppm) of Mosquito Larvae and Non-Target Organisms.

Insect	LC_{50} (ppm)	LC_{90} (ppm)
Culex fatigans (lab)	0.0005	0.0009
Culex fatigans (field)	0.001	0.0015
Laccophilus fasciatus	0.0021	0.0052
Chaoborus punctipennis	0.0054	0.0151
Notonecta undulata	0.0352	0.0488

Brown et al. (1976) determined the effect of analytical grade Dursban in xylene upon freshwater phytoplankton in a natural pond near Lake Huron. Of the seven species of phytoplankton tested, six species showed decreased growth rate in 0.0012 ppm Dursban while the seventh species, Ceratium, was not affected in concentrations as high as 0.240 ppm. Brown concluded that even in very low concentrations Dursban can have a considerable and long lasting effect upon freshwater phytoplankton.

Nelson et al. (1976b) studied diatom diversity as a function of insecticide treatment. Nelson interpreted higher diatom diversity to imply lower toxicity. Rice fields were treated with polyethylene pellets (10.6% chlorpyrifos) to give 0.25, 0.5, 1.0, and 2.0 ppm in the water. By six weeks post-treatment no substantial differences in population diversity between treated and control plots was observed. By 12 weeks post-treatment, significant decreases in diversity estimates occurred in the treated plots. During weeks six to 12 post-treatment, the diversity estimates in the control plots had actually increased.

Toxicity values for other non-target organisms are shown in Appendix A.

Dursban Degradation and Residues in an Aquatic Environment

In an aquatic system Dursban is rapidly adsorbed onto any soil particles or plant material which may be present in the water (Smith et al. 1966). In such a system Dursban undergoes both chemical and biological degradation (Smith 1966). The chemical degradation is the result of hydrolysis and photodecomposition while biological degradation occurs in the tissues of the plants and animals in the water.

The major chemical reaction is a slow hydrolysis of Dursban to 3,5,6-trichloro-2-pyridinol. In water the hydrolysis has a half-life of about 80-100 days (Smith 1966). Smith also stated that the rate of hydrolysis increased with an increase in pH or temperature. Smith (1968) found that at pH 7 about 50 percent of the insecticide was decomposed in 25 days, compared with 29 days at pH 5 and 108 days at pH 9. The work of Schaefer and Dupras (1970) also showed the effect of temperature on the stability of Dursban in water. At 10°C there was negligible decrease in concentration over a 16 hour period. After 16 hours at 24°C the concentration dropped from 0.1 ppm to about 0.074 ppm. At 38°C the Dursban concentration decreased from 0.10 ppm to 0.02 ppm over the same 16 hour period.

Dursban in aquatic systems is very sensitive to photodecomposition (Smith 1966). However, Smith stated that in large volumes of water the Dursban slowly settles out and is thus protected from the sunlight due to the water barrier. Under these conditions there is very little photodecomposition of the compound. Hydrolysis reduces Dursban to 3,5,6-trichloro-2-pyridinol which is very light sensitive and easily degraded by photo-dehalogenation (Smith 1968). The end result of the degradation is the liberation of CO_2 (see Appendix B) (Smith 1968).

Schaefer and Dupras (1969) also demonstrated the importance of

sunlight induced degradation. Two types of water, (distilled or collected from a mosquito breeding site) were placed in jars which, in turn, were placed in mosquito infested pools so that the conditions of water temperature and exposure to sunlight would be as close as possible to normal conditions. Under these conditions with an initial concentration of 0.10 ppm, Dursban in the field collected water decreased to 0.025 ppm in eight hours and in distilled water to 0.031 ppm. When the samples were placed in the dark and at constant temperature the concentration decreased from 0.1 ppm to 0.078 ppm over eight hours for both types of water.

Miller et al. (1973) used four different formulations of Dursban and found that residues were consistently higher in pools that were shaded than in pools exposed to direct sunlight.

The organic components of a pool have the ability to influence the degradation of an organophosphate insecticide in water. These components are soil particles, plants, aquatic animals, and bacteria. Smith et al. (1966) stated that since Dursban is relatively insoluble in water it is rapidly adsorbed onto any soil particles or plants which may be present in the water. In laboratory experiments, Smith showed that 70 percent of the Dursban was removed from the water within eight hours of application by adsorption of the chemical onto plants and soil particles. Residue studies by Hurlbert et al. (1970) confirm that Dursban concentration in the water remains low while the concentration on the vegetation is initially very high but declines rapidly.

Studies on the metabolism of Dursban by plants (Smith et al. 1967) demonstrated that only one percent of the Dursban enters the plants and whatever does enter is slowly metabolized. Smith et al. (1966) concluded that the soil and plants act as a reservoir for Dursban.

As the soluble insecticide in the water is degraded by hydrolysis or aquatic animals the Dursban is slowly liberated from the organic matter into the water. The metabolites are then readily absorbed by the plants which can further metabolize them to CO_2 (Smith et al. 1967).

Smith et al. (1966) found that Dursban was slowly absorbed from the water by fish but that they rapidly metabolized any such material. The metabolites were then liberated into the water. One of the major metabolites eliminated was 3,5,6-trichloro-2-pyridinol which is further degraded by sunlight or plants (Smith 1966; Smith et al. 1967).

Hirakoso (1968) determined that bacteria Bacillus subtilis and Pseudomonas aeruginosa could convert toxic parathion and fenitrothion to non-toxic amino-parathion and amino-fenitrothion. Using 27 bacterial species and a mosquito larvae bioassay, Hirakoso found that the activity of Dursban was not reduced by the bacteria. Whitney (1967) while studying the effects of soil microorganisms on Dursban found that there was no difference in its biological activity between soil that was and was not autoclaved prior to treatment. Schaefer and Dupras (1970) point out that the long residual action of Dursban in highly polluted habitats (dairy drains, sewage-holding ponds) could be due to the fact that bacteria do not readily degrade Dursban.

Ludwig et al. (1968) studied a salt marsh habitat for detectable Dursban residues. Their results showed that Dursban EC reached a maximum concentration in the water within one hour of application and then gradually decreased to non-detectable levels. At 0.028 kg ai/Ha, Dursban was not detectable in the water seven days post-treatment. At 0.056 kg ai/Ha there was a high initial peak of Dursban in the water followed by a decrease to non-detectable levels two weeks post-treatment. Silt samples collected one and two weeks post-treatment showed no Dursban

residues.

Hurlbert et al. (1970) observed similar results as Ludwig et al. (1968) with Dursban EC in freshwater ponds. Dursban concentration in the water reached a peak very quickly and decreased to a non-detectable level in seven days when applied at 0.056 kg ai/Ha. Maximum residues in mud occurred seven days post-treatment. Residues on vegetation were very high at four hours and one day post-treatment but by seven days post-treatment the residues on the vegetation had decreased 95 percent.

Mulla et al. (1973) studied Dursban residues in a warm-water lake treated at the rate of 0.22 kg ai/Ha (2% G). Maximum Dursban residues in water occurred one day post-treatment and declined to low levels within four weeks. Maximum residues in mud were obtained one week post-treatment and then declined over the subsequent four week period. Dursban residues in the mud were found in the top one inch section of the mud samples. In fish the maximum residues occurred two to three weeks post-treatment and declined to barely detectable levels within 25 days of treatment.

Dursban Analysis

Rice and Dishburger (1968) developed a technique for the analysis of Dursban in water. Extraction recovery from fortified samples averaged 92 percent from water samples. Residues as low as 0.0001 ppm in water were detectable by GLC using nonpolar stationary phase column and electron capture detector.

Dusch et al. (1970) used GLC to detect Dursban in water, mud, vegetation, fish, ducks, insects, and crustaceans. The detection limit was 0.5 nanograms.

Smith and Fischer (1967) developed a method for paper partition

and thin layer chromatography identification of Dursban and its metabolites. Seven compounds closely related to Dursban could be separated and identified. These included the pyridinol and the oxygen analogue metabolites of Dursban.

Fuzesi (1973) developed a method for GLC determination of Dursban concentration in either liquid or granular formulations. Fortified samples showed the accuracy of the technique to be 99.3-100.1 percent.

III. EXPERIMENTAL

Rearing of Culex tarsalis

The Culex tarsalis (Diptera; Culicidae) larvae used in the bioassays were laboratory reared. The colony originated from a field strain collected in 1974 at the University of Manitoba Research Station, Glenlea, Manitoba. The adults and larvae were reared in a growth cabinet at 25°C with a relative humidity of 73 percent and a light/dark cycle of 16/8 hours. The female adult mosquitoes were blood fed on Japanese Quail (Coturnix coturnix japonica) placed overnight in one cubic foot plexiglass cages. Five days post-blood meal the egg rafts were collected by placing styrofoam cups filled with water into the cages so that the females would oviposit on the water. Two days after oviposition the egg rafts were removed and allowed to hatch in water filled trays. Sixty mesh liver powder obtained from ICN Pharmaceuticals was used as larval food. After pupation, 12 days post-oviposition, the pupae were removed from the trays, transferred in styrofoam cups to plexiglass cages where the adults emerged. The egg to egg cycle for C. tarsalis was 22 days at 25°C. By having five separate emergence cages and four blood feeding cages it was possible to set up a routine cycle of adult blood feeding and oviposition. This cycle produced a daily supply of egg rafts, and, after rearing, a daily supply of fourth instar larvae which were used for a bioassay.

Experiment Organization

(i) Outdoor temporary pools - 1975

During the summer of 1975, five artificial pools were set up at Glenlea, Manitoba. Each pool consisted of a wooden frame one metre square and 30 centimetres high, lined with four mil polyethylene.

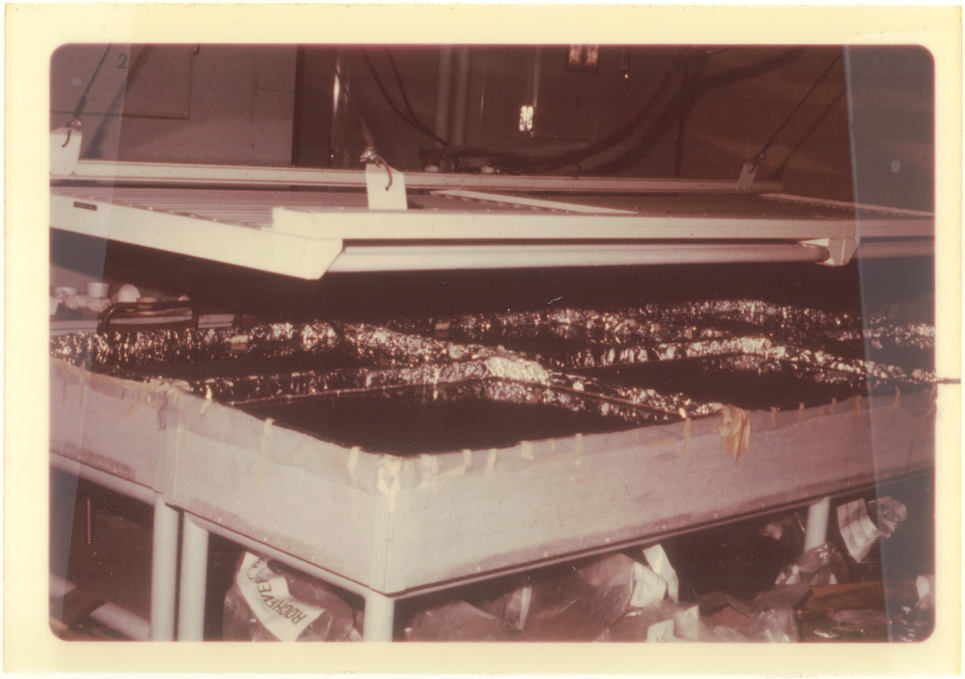
The pools were constructed at ground level with a fine sand base under the polyethylene. The inside of each pool was lined (bottom and sides) with five centimetres of grass sod which was obtained from a commercial sod grower. The sod on the sides of each pool was held in place by wood braces. Water was added until the sod was saturated; this meant that no free water could be seen in the bottom of the pools. To each pool, 150 litres of water was added (Figure 1). The water was obtained from a nearby polyethylene lined dugout which traps snow-melt water in the spring. After the water was added, the level in each pool was marked so that the volume of free water in the pools could be maintained at 150 litres by daily additions to the pools.

(ii) Laboratory temporary pools

In the laboratory, three pools were constructed in each of four box benches. The dimensions of each pool were 110.5 cm long X 76.2 cm wide X 21.6 cm high. Each pool was lined with four mil polyethylene. To prevent direct contact between the water and the polyethylene the sides of each pool were lined with aluminum foil. The bottom of each pool was lined with one of three substrates (sod, clay or sand). The sod was obtained from the same source as for the outdoor temporary pools in 1975. The clay was dug from the banks of the Red River and the sand was purchased from a local supply company. The substrate composition is shown in Table X. The pools were maintained at a volume of 100 litres using tap water (Figure 2). There were four pools of each substrate, three received experimental treatments and the fourth was a control. At the conclusion of an experiment all the water, substrate, foil and polyethylene was removed and replaced for the next experiment. By adjusting the height of fluorescent light banks above

Figure 1. Outdoor Temporary Pools - 1975

Figure 2. Laboratory Temporary Pools



the benches it was possible to provide 24 hours continuous light at 1000 foot-candles to all the benches.

(iii) Outdoor temporary pools - 1976

In June 1976, 12 one metre square pools were set up at Glenlea. The pools were constructed in the pattern of the laboratory pools; i.e., they were lined with polyethylene with aluminum foil sides and one of three substrates (sod, clay or sand) on the bottoms of the pools (Figure 3).

Sampling

In each experiment all the pools were sampled following the same time intervals post-treatment: 4, 24, 48, 96, 144, ..., 288 hours. Sampling continued until two consecutive bioassays on the water from the treated pools gave zero mortality.

Water samples were collected for analysis at each sample period. To collect the water samples, a 100 ml glass beaker was used as a dipper. Five water samples of 100 ml each were dipped from five different locations in each pool. The locations were the four corners plus the centre of the pool, just below the water surface. The five 100 ml samples from each pool were combined in a 600 ml glass beaker from which a 200 ml aliquot was taken and poured into a cabinet oval bottle. The bottles were tightly capped with teflon lined screw caps and returned to the laboratory for bioassay and gas liquid chromatograph analysis.

All sampling, storage, and analytical glassware was silanized to prevent possible adsorption of chlorpyrifos to the glass. The silanizing procedure consisted of coating dry glassware (pre-cleaned with chromic acid) with a 15 percent Dri-Film-toluene solution for one minute, followed by three hexane washes. The glassware was allowed

Table X. Substrate Composition

Substrate	pH	Organic Matter (%)	Component Analysis		
			% Sand	% Silt	% Clay
Sod	6.0	75.1	--	--	--
Clay	7.6	0.6	16	42	42
Sand	8.8	0.2	99	1	0

Figure 3. Outdoor Temporary Pools - 1976

to air dry completely before use. Dri-Film was obtained from Chromatographic Specialties Ltd., P.O. bag 1150, 300 Laurier Blvd., Brockville, Ontario.

Bioassay

To determine the presence of bioactive compounds in the water samples, bioassays were conducted in the laboratory using fourth instar larvae of laboratory reared C. tarsalis. Twenty larvae were added to 100 ml of sample water contained in cabinet oval bottles. After the introduction of the larvae, the bottles were stored in the dark at room temperature for 24 hours. After the exposure period the percent mortality of the larvae was recorded.

Treatments

Two commercial formulations of Dursban insecticide (Dow Chemical of Canada) containing chlorpyrifos as the active ingredient were tested and compared for bioactivity and chlorpyrifos disappearance rates in water. Dursban 2.5G was a granular (G) formulation with the chlorpyrifos impregnated on bentonite clay at a concentration of 2.5 percent. The second formulation was an emulsifiable concentrate (EC) (Dursban M) which contained chlorpyrifos at a concentration of 48 percent.

(i) Outdoor temporary pools - 1975

During the summer of 1975 the Dursban 2.5G and EC formulations were applied to the Glenlea pools at the maximum recommended rate of 0.056 kg ai/Ha (0.05 lbs ai/A). This rate of application would produce a maximum theoretical initial concentration of 0.038 ppm in the water. Two pools were treated with each formulation and one untreated pool was kept as a control. The substrate in each pool was sod. Water samples

were taken periodically post-treatment and returned to the laboratory for analysis. All pools were analyzed individually.

(ii) Laboratory temporary pools

Laboratory experiments were conducted to compare the bioactivity and disappearance of chlorpyrifos in pools with different substrates (sod, clay, or sand). In the first experiment, the pools were treated with Dursban 2.5G at the rate of 0.056 kg ai/Ha (0.038 ppm in the water). In the second experiment the pools were treated with Dursban 2.5G at the rate of 0.028 kg ai/Ha (0.019 ppm in the water). This was the minimum recommended rate for larval control. In the third experiment the pools were treated with EC Dursban at the rate of 0.028 kg ai/Ha. In the laboratory experiments the bioassays were conducted on water samples from each pool. However, for GLC analysis the water samples from replicate pools were combined and 100 ml was used for extraction.

(iii) Outdoor temporary pools - 1976

In the summer of 1976 the laboratory experiments in which Dursban was applied to the pools at 0.028 kg ai/Ha were duplicated outside. The outdoor pools were constructed in the pattern of the laboratory pools. Pools were bioassayed individually while the samples for GLC analysis were combined according to substrate (sod, clay or sand).

Sample Extraction and Chlorpyrifos Analysis

To determine the concentration of chlorpyrifos in the water, 100 ml water samples were extracted by partitioning with methylene chloride. The samples were extracted the day they were collected. One hundred ml of water was pipeted into a silanized 250 ml separatory funnel. The sample was subjected to four successive partitionings with 25,

20, 15, and 10 ml of methylene chloride. The methylene chloride (lower phase) from the separatory funnel was collected in a silanized glass bottle and stored at -40°C until GC analysis. Chlorpyrifos stored under these conditions did not degrade (Webster and Reimer 1976). Extraction efficiency tests were run by adding known amounts of chlorpyrifos to pool water and determining the percent recovery as determined by GLC analysis. The extraction efficiency averaged 34 percent (Webster and Reimer 1976).

The methylene chloride extracts were removed from storage and allowed to warm up to at least 0°C. A small amount of sodium sulfate was added to each bottle and was vigourously shaken. The methylene chloride extract was decanted into a silanized 100 ml round bottom flask and reduced in volume to about 20 ml on a rotary evaporator. The sodium sulfate in the bottles was washed twice with 15 ml of hexane. The hexane washings were decanted into the round bottom flask. The hexane/methylene chloride extract was evaporated to dryness and two ml of hexane was pipeted into the round bottom flask. This was the final sample volume from which a two microlitre aliquot was taken for GLC analysis.

All solvents used in the preparation of test solutions, extractions, and analysis were distilled in glass (Caledon Laboratories Ltd.)

Chlorpyrifos in the solvent extracts was measured using gas liquid chromatography (GLC). GLC analyses were performed using a Varian 2400 gas chromatograph fitted with a titanium tritide electron capture detector. A 1.2 m Pyrex, 2 mm i.d. column was packed with 80-100 mesh Gas Chrom Q coated with five percent DC 200 and 15 percent QF1 in a one to one ratio (w/w); temperatures, injector, 211°C; detector, 220°C; column, 180°C; flow rate, nitrogen carrier, 50 ml/min; retention time 2.83 min.

IV. RESULTS AND DISCUSSION

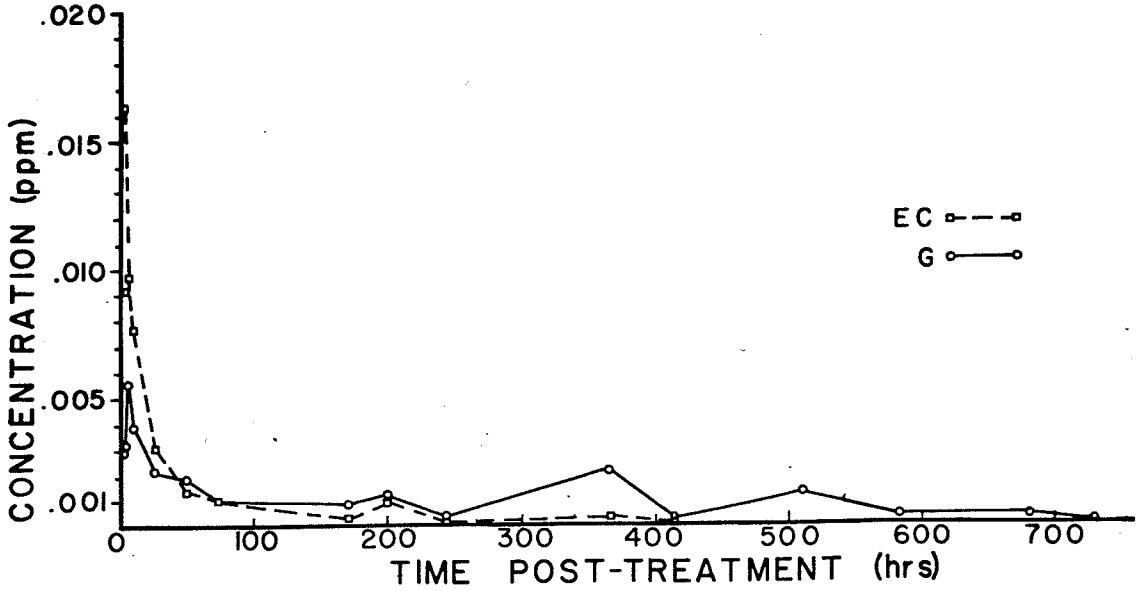
(i) Outdoor temporary pools - 1975

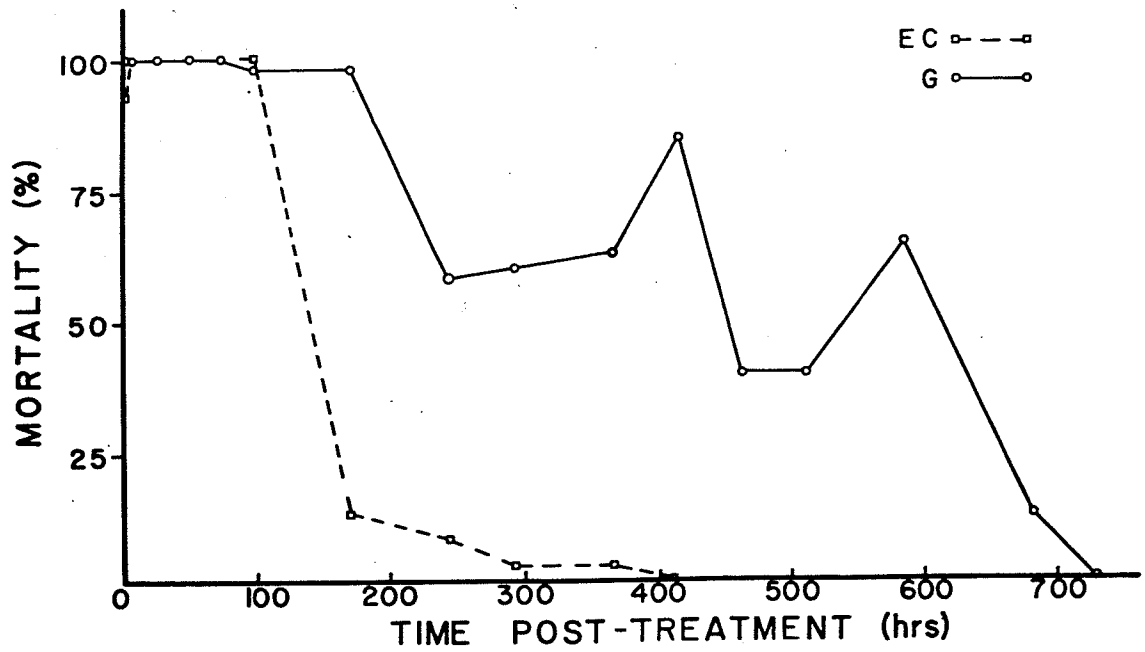
The results from the summer of 1975 indicated considerable variation in the bioactivity and disappearance of Dursban for both the emulsifiable concentrate (EC) and the granular (G) formulations in sod-lined pools (Figures 4 and 5).

The time zero EC concentration in the water was high (0.0162 ppm) indicating an immediate release and dispersal of the chlorpyrifos throughout the water. Within eight hours post-treatment only 46.9 percent of the initial concentration of chlorpyrifos could be detected in the water and by 24 hours, only 18.5 percent (0.0030 ppm). The concentration continued to decrease until 72 hours before leveling off at a relatively constant but low concentration until 408 hours post-treatment when no chlorpyrifos could be detected. Smith et al. (1966) reported that under laboratory conditions, 70 percent of the chlorpyrifos had disappeared from the water within eight hours post-treatment. This rate of chlorpyrifos disappearance from the water is slightly faster than occurred in the 1975 temporary pools. This difference could be due to the comparison of field and laboratory data or to the effect of aquatic organisms like fish used in Smith's study. EC Dursban at the rate of 0.056 kg ai/Ha was applied to a salt-marsh habitat by Ludwig et al. (1968). The highest Dursban residue detected in the water was 0.0054 ppm at one hour post-treatment which was only about one third of the highest concentration achieved in the sod pools with the EC Dursban in 1975. By 24 hours Ludwig could only detect 0.0004 ppm and at 312 hours post-treatment no residue was detected in the water. Thus it is apparent that the chlorpyrifos in the water quickly disappears after treatment

Figure 4. Chlorpyrifos Water Concentration in Outdoor Sod Pools Treated with G or EC Dursban at 0.056 kg ai/Ha.

Figure 5. Percent Bioassay Mortality in Water Samples From Outdoor Sod Pools Treated with G or EC Dursban at 0.056 kg ai/Ha.


.020
.015
.010
.005
.001
CONCENTRATION (ppm)
EC
G
0
100
200
300
400
500
600
700
TIME POST-TREATMENT (hrs)


100
75
50
25
MORTALITY (%)
EC
G
0
100
200
300
400
500
600
700
TIME POST-TREATMENT (hrs)

with an EC formulation.

In the pools treated with the G Dursban, the maximum concentration in the water did not occur until four hours post-treatment. The lag in the release of the chlorpyrifos to the water as compared to the EC formulation was due to the release properties of the granules. The maximum concentration of chlorpyrifos achieved with the G was 0.0055 ppm in the water or only 34 percent of the level achieved with the EC. After reaching peak concentration, there was a rapid decrease in concentration in the G treated pools. By 72 hours post-treatment the concentration had decreased to 0.001 ppm or 18.2 percent of peak concentration. At 360 and 504 hours post-treatment an increase in the chlorpyrifos concentration in the water was detected. These increases might be attributed to either secondary release of the active ingredient from the granules or desorption from the organic matter in the pools. At 720 hours post-treatment, no chlorpyrifos was detected in the pools with G Dursban.

The C. tarsalis bioassays showed that the EC gave 100 percent control for 96 hours post-treatment. This dropped to 13 percent by 168 hours and to zero by 408 hours post-treatment. The sudden drop in mortality indicates that there is a very fine line between the concentration producing mortality and that allowing survival.

The G formulation gave 100 percent control for 72 hours and 98 percent control for 168 hours post-treatment. As with residue data, the mortality data for G revealed two secondary peaks (408 and 576 hours post-treatment). However, the secondary mortality peaks both occurred one sample period (48 hours) after the GC peaks. An explanation for the lag in mortality could be that at these low concentrations the most important route of entry of the chlorpyrifos into the larvae

was through ingestion of contaminated food particles. The lag could be the result of the time required for the particles to adsorb the chlorpyrifos that was recently released into the water. Zero control in the G pools occurred 720 hours post-treatment.

In comparing the bioassays for both the Dursban G and EC (Figure 5) it is evident that the G provided longer residual control. Cooney and Pickard (1974) compared Dursban clay granules and EC formulations. At a rate of 0.11 kg ai/Ha the G controlled Aedes vexans for 30 days while the EC was effective for only 14 days. Except for the longer period of control as a result of a higher application rate, the results of Cooney and Pickard compared with the present research in that the G Dursban provided longer mortality than the EC. However, Dixon and Brust (1971) tested two EC and one G Dursban formulation at a rate of 0.028 ka ai/Ha and their results indicated that one EC formulation provided 100 percent control of Aedes vexans for two weeks while the second EC and the G formulation were effective for only one week. The G formulation used by Dixon and Brust had five percent active ingredient on a corn cob carrier and thus differed from the G formulation tested in 1975 which was 2.5 percent active ingredient on clay granules. This difference in formulation could explain why the EC formulation was more effective than the G in the work of Dixon and Brust and why their data was opposite to the results in Figure 5. Bailey et al. (1970) applied Dursban in polyvinyl chloride pellets and an EC formulation to potholes to provide a chlorpyrifos concentration in the water of 10 ppm. The EC provided 238 days with control above 75 percent mortality while the pellets lasted only 28 days. The 238 days of control with the EC Dursban noted by Bailey et al. (1970) was achieved as a result of an application rate of 10 ppm compared to only 0.038 ppm in the 1975

outdoor temporary pools. From these results it can be concluded that residual control with Dursban is very dependent not only on the type of formulation but also the carrier utilized in the formulation.

Due to the large population of C. tarsalis and C. restuans at Glenlea it was possible to monitor natural infestation of the test pools. Twenty-four hours after the flooding of the sod pools (the morning of Dursban treatment) egg rafts were observed in all the test pools. By 168 hours post-treatment, third instar larvae were collected from the control pool. The larvae were identified as C. tarsalis and C. restuans (Barr 1958). In the treated pools the eggs hatched and first instar larvae survived for only a short period of time. It was not until 360 hours post-treatment that second and third instars were observed in the EC treated pools. By the end of the experiment (840 hours post-treatment) only a few specimens of first instar larvae had been collected from the G pools. It was concluded from these observations that even though the bioassay with fourth instar larvae indicated little or no control, there was still enough active ingredient in the water to control the more sensitive first instar larvae. The susceptibility of various instars of Anopheles freeborni was studied by Womeldorf and Whitesell (1972) (Table IV). Different instars from three populations were tested and in each population the latter instar larvae was found to be more resistant to the Dursban than the younger larvae.

By comparing Figures 4 and 5 it is apparent that the EC with an initial release three times greater than the G (0.0162 and 0.0055 ppm, respectively) had a shorter period of efficacy. Therefore, within the first 24 hours post-treatment with the EC there was a massive overkill with the excess chlorpyrifos. The G formulation, which slowly releases

the active ingredient, provided a longer period of larval control with less insecticide in the water. Nelson et al. (1976a) treated pools with a controlled-release formulation of chlorinated polyethylene pellets of 10.6 percent chlorpyrifos at the rate of two ppm in the water. Bioassays with Psorophora confinnis resulted in greater than 11 weeks of 100 percent control. During the 11 weeks, the highest chlorpyrifos residue they detected in the water was 0.0022 ppm. In terms of effect on non-target organisms, slow-release of the chlorpyrifos can be very important. In Table IX, Roberts et al. (1973) demonstrated that Dursban was less toxic to non-target organisms than to the target mosquito larvae. Thus the non-target organisms would have a better survival rate with the low concentration from the G rather than the very high residues resulting from EC application.

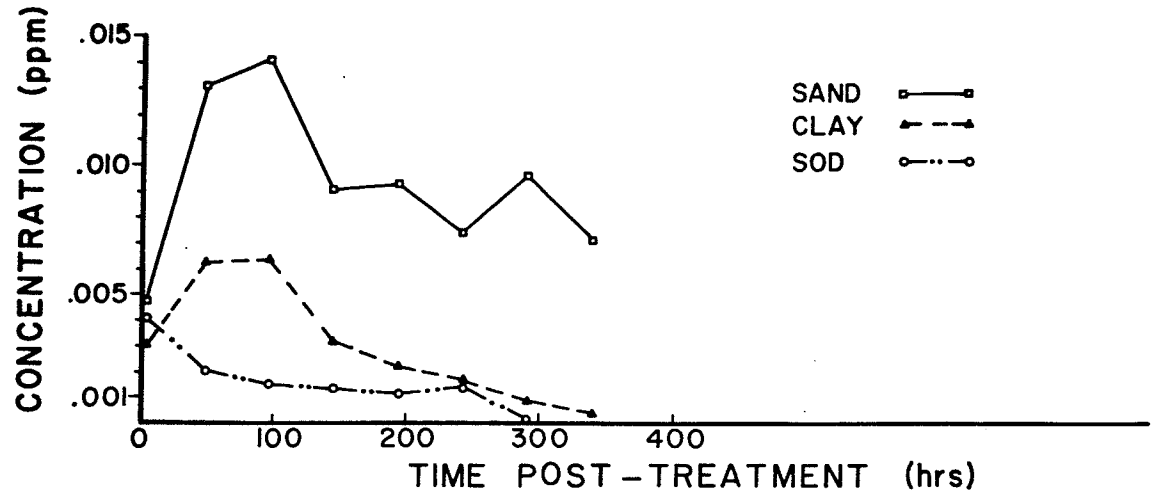
(ii) Laboratory temporary pools

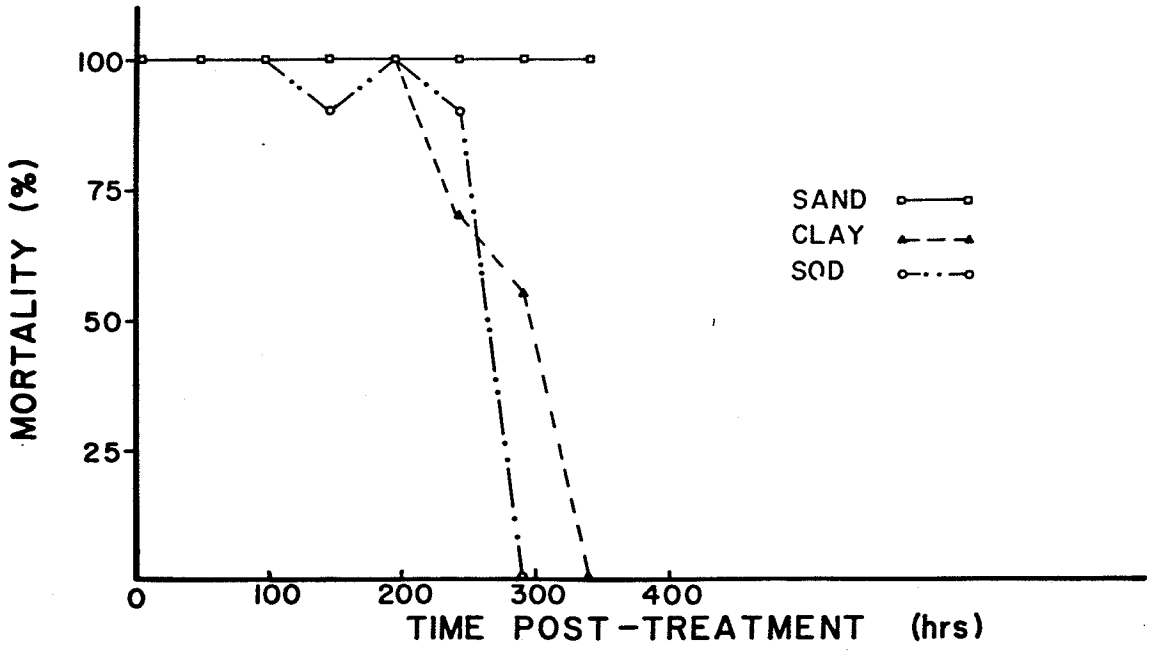
Three substrates (sod, clay or sand) were established in laboratory pools to determine if the substrate can alter the effectiveness of Dursban. The bioassay and GC data revealed that the substrates could exert an influence on the efficacy and disappearance of Dursban applied at 0.056 kg ai/Ha (0.038 ppm in water) (Figures 6 and 7).

The GC data revealed that at four hours post-treatment the chlorpyrifos concentration in the water for all pools was fairly similar. In the sod, clay, and sand pools the concentration was 0.0040, 0.0030, and 0.0047 ppm, respectively. The highest chlorpyrifos concentration in the water was achieved and maintained in the sand pools. In the sod pools the concentration of chlorpyrifos in the water decreased to the limit of detectibility 288 hours post-treatment.

Figure 6. Chlorpyrifos Water Concentration in Laboratory Pools with Sod, Clay or Sand Substrate Treated with G Dursban at 0.056 kg ai/Ha.

Figure 7. Percent Bioassay Mortality in Water Samples From Laboratory Pools with Sod, Clay or Sand Substrates Treated with G Dursban at 0.056 kg ai/Ha.


CONCENTRATION (ppm)
.015
.010
.005
.001
0
100
200
300
400
TIME POST-TREATMENT (hrs)
SAND
CLAY
SOD


MORTALITY (%)
100
75
50
25
0
100
200
300
400
TIME POST-TREATMENT (hrs)
SAND
CLAY
SOD

At the conclusion of this experiment (336 hours) the chlorpyrifos concentration in the water from the clay pools was 0.0004 ppm and from the sand pools was 0.0070 ppm.

The difference in chlorpyrifos water concentration can be attributed to the various adsorptive qualities of the substrates. Smith (1966) demonstrated that chlorpyrifos rapidly adsorbs to any organic matter present in water. Thus the sod (75.1 percent organic matter) could act as a major adsorptive sink for the chlorpyrifos, resulting in a low concentration of chlorpyrifos in the water of the sod pools. Hurlbert et al. (1970) treated ponds with EC Dursban at 1.121 kg ai/Ha and at four hours post-treatment detected only 0.2 ppm Dursban in the water while the vegetation residue reached 26 ppm. The difference in rates of chlorpyrifos disappearance in the water of the clay and sand pools must be attributed to some factor other than organic matter since both the clay and sand had similar organic matter content (0.6 and 0.2 percent, respectively). Therefore, the clay and silt components of the clay substrate (Table X) must have been responsible for the faster reduction of the chlorpyrifos residues in the water of the clay pools.

Harris (1966) stated that with many insecticides, the substrate component is very important in establishing the adsorptive capacity of the soil. He noted that insecticide treated sand maintained higher bioassay mortality than clay or high organic matter content soils. Harris concluded that the difference in bioassays was the rate of adsorption of the pesticides and that with the sand, the active ingredient was not adsorbed onto the sand particles and thus was still biologically available. Whitney (1967) also found that Dursban activity was reduced by organic matter content in soils. Lower efficacy was reported in muck and potting soil compared with sandy soils. Miller et al. (1973)

detected chlorpyrifos adsorption by the pool substrate by determining that the chlorpyrifos water residues were consistently lower in polyethylene pools with soil substrates than in pools without soil.

Greenland (1970) stated that the amount of pesticide which can be adsorbed (the adsorption capacity) was determined by the number of adsorption sites, which is usually closely related to the specific surface area of the substrate. Bailey and White (1970) found that organic matter had a surface area of 500-800 square metres per gram (sq m/g) compared to the surface area of clay which ranged from a low of 7 sq m/g to 800 sq m/g depending upon the type of clay mineral present in the soil. Thus a greater number of adsorption sites are available in soils with high organic matter content due to the larger surface area. The adsorption of molecules to a substrate is primarily determined by the nature and concentration of exchangeable cations in the substrate (Greenland 1970). Bailey and White (1970) determined that the cation exchange capacity for organic matter was 200-400 milliequivalents/100 g compared to 3-150 milliequivalents/100 g for various clay minerals. Thus the organic matter would have the greater ability to hold adsorbed molecules than the clay. These two factors, surface area and cation exchange capacity could explain the faster rate of disappearance of chlorpyrifos from the water of the sod pools rather than from the clay pools.

In the presence of excess water, all the adsorption sites on the clay and sand would be filled with water. Thus, in order to adsorb a pesticide, the water molecules must first be desorbed from the active sites (Greenland 1970). When the water is displaced from the interlamellar region of the clay particles and replaced with a pesticide, there is a net gain in entropy. Due to this entropy gain, relatively

inert molecules can be adsorbed by clays. Since sand does not have a layered structure like clay there is no gain in entropy after water displacement and thus less opportunity for adsorption of the pesticide onto the sand. With less adsorption onto sand, a higher chlorpyrifos concentration in the water of the sand pools would be expected and did indeed occur.

The bioassay confirmed the GC data in that the larval efficacy of Dursban was substrate dependent. One hundred percent bioassay mortality was obtained in both the sod and clay pools for 192 hours post-treatment. Zero mortality was achieved 288 hours post-treatment in the sod pools and 336 hours in the clay pools. The sand pools maintained 100 percent mortality to 336 hours post-treatment, the end of the experiment.

In the remaining experiments the application rate was reduced to the minimum recommended rate (0.028 kg ai/Ha) in order to shorten the residual effect of the Dursban and thus reduce the chance of the pools becoming too stagnant to work with.

Dursban G was applied to the three flooded substrates (sod, clay or sand). Because of the high capacity of the sod for the adsorption of insecticides from the water, 100 percent mortality for the shortest period of time was expected in the sod pools. However, the bioassay mortality in the sod pools was lower than expected while 100 percent mortality occurred in the clay and sand pools. Since the formulation was active in the clay and sand pools, it was assumed that the granules were still active and had not deteriorated on the shelf. To remove the possibility that the low mortality in the sod pools was due to error in application of the granules to the pools, the sod pools were reconstructed and retreated at the same rate. The results of this treatment on the sod as well as the results from the clay and sand

pools are shown in Figures 8 and 9.

Extremely low mortality again occurred in the sod pools. The highest mortality was 6.7 percent at 192 hours post-treatment. The GC substantiated the fact that there was little or no chlorpyrifos in the water of the sod pools. In order to determine if Dursban would act as a larvicide in these sod pools, the pools were retreated at 216 hours at twice the previous rate (0.056 kg ai/Ha). At 220, 240, and 264 hours post-treatment, 100 percent bioassay mortality occurred. Analysis of a sample taken at 220 hours post-treatment by GLC showed a chlorpyrifos concentration in the water of the sod pools of 0.0035 ppm.

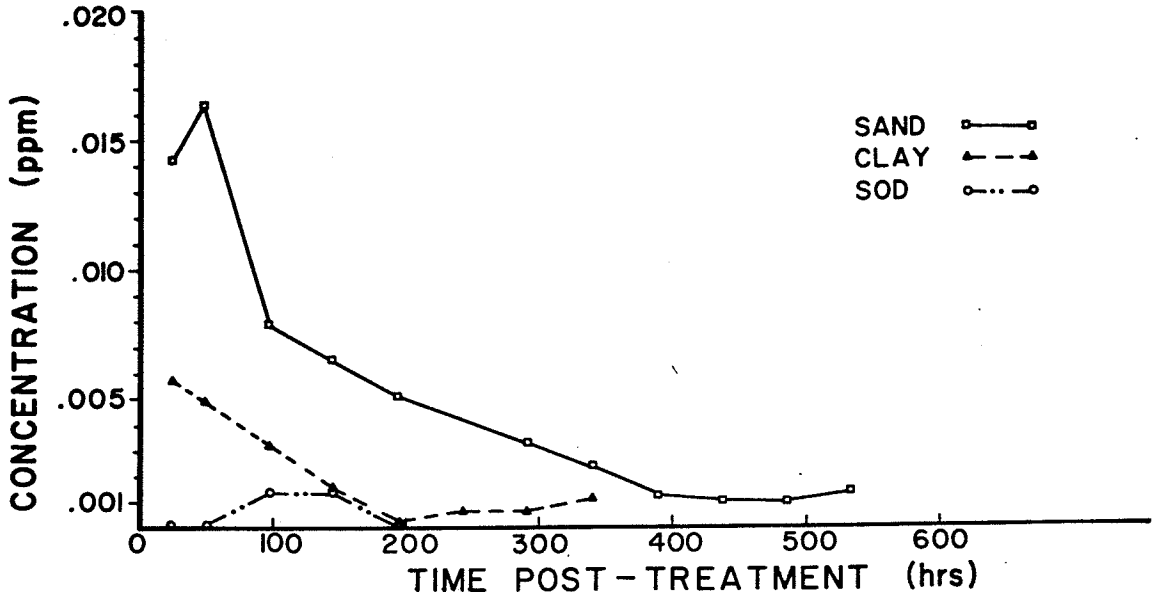
The granules had obviously released their chlorpyrifos in the sand and clay pools and thus it was assumed that the chlorpyrifos was also released in the sod pools. An explanation for the low concentration and mortality in the sod pools could be that, at the lower rate of application, the organic matter was able to adsorb the chlorpyrifos from the water as fast as it was released from the granules. At the higher rate of re-application the sod was not able to adsorb all the chlorpyrifos and thus the concentration in the water became high enough to exert 100 percent bioassay mortality for at least 48 hours.

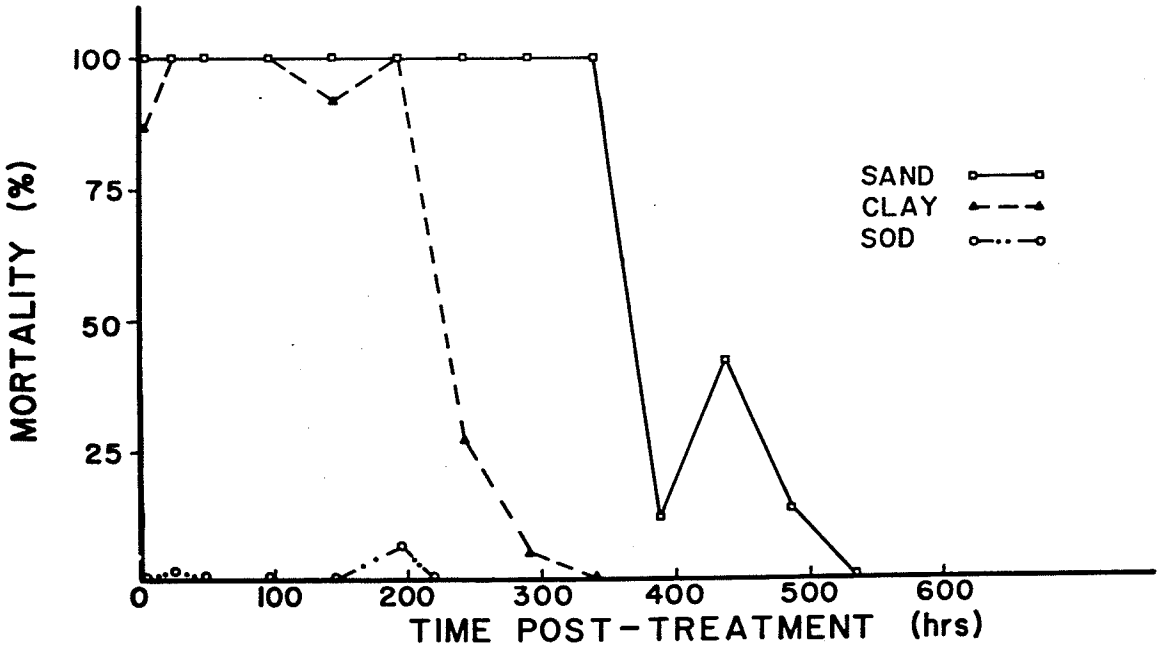
The bioassay data indicated 100 percent larval mortality in the clay pools for 192 hours and in the sand pools for 336 hours post-treatment. Mortality decreased to zero at 336 and 528 hours post-treatment in the clay and sand pools, respectively.

The GC data confirmed the bioassay data in that the concentration of the chlorpyrifos in the water was considerably higher in the sand pools than in the clay pools, especially during the period 0 to 336 hours post-treatment. For example, at 48 hours post-treatment, the

Figure 8. Chlorpyrifos Water Concentration in Laboratory Pools with Sod, Clay or Sand Substrate Treated with G Dursban at 0.028 kg ai/Ha.

Figure 9. Percent Bioassay Mortality in Water Samples from Laboratory Pools with Sod, Clay or Sand Substrate Treated with G Dursban at 0.028 kg ai/Ha.


.020
.015
.010
.005
.001
CONCENTRATION (ppm)
SAND
CLAY
SOD
0
100
200
300
400
500
600
TIME POST-TREATMENT (hrs)


100
75
50
25
MORTALITY (%)
SAND
CLAY
SOD
0
100
200
300
400
500
600
TIME POST-TREATMENT (hrs)

chlorpyrifos concentration in the water of the sand pools was 0.0163 ppm while in the clay pools it was 0.0049 ppm. Even though the concentrations were different, the pattern of chlorpyrifos disappearance in the clay and sand pools was similar (Figure 8). Initially, both substrates had a period of steady and relatively linear degradation, followed by a period of almost constant concentration with very slow loss of chemical.

At 432 hours a large increase in mortality (11.7 to 41.7 percent) was observed in the sand pools. The reason for the increased mortality was not determined since no corresponding increase in chlorpyrifos concentration was detected by the GC.

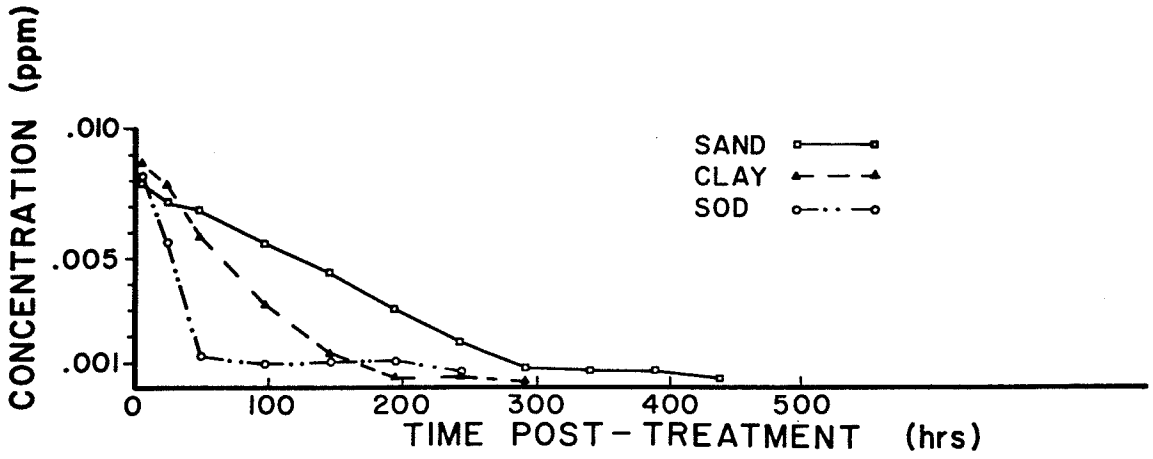
At the conclusion of the G experiments the laboratory pools were relined with sod, clay, and sand and treated with EC Dursban at 0.028 kg ai/Ha. The results of this experiment are shown in Figures 10 and 11.

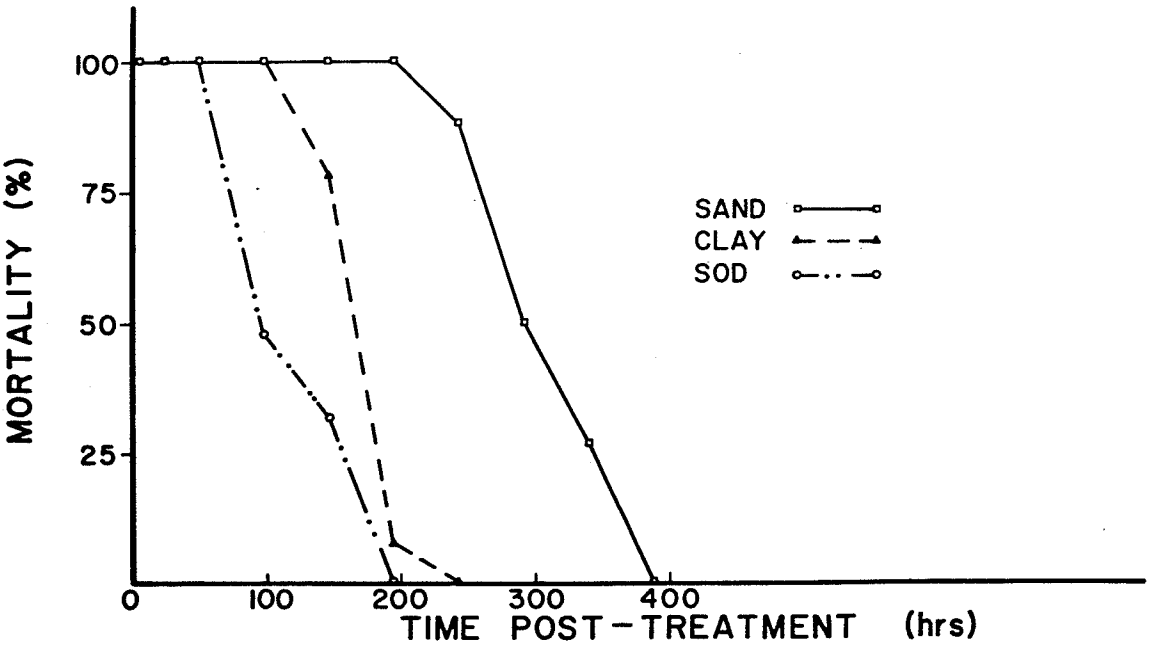
The bioassays indicated that the active ingredient was released to the water very quickly. At four hours post-treatment 100 percent bioassay control was achieved in all the pools. Even the sod pools, which had little or no control with the G at the same rate of application, recorded 100 percent control by four hours post-treatment and maintained 100 percent control for 48 hours compared with 96 hours for the clay pools and 192 hours post-treatment for the sand pools. Zero mortality was reached first by the sod pools at 192 hours, clay pools at 240 hours, and the sand pools at 384 hours post-treatment.

The GC results indicated that the most rapid rate of decrease in chlorpyrifos concentration occurred in the sod pools. In the clay pools, chlorpyrifos disappeared at an intermediate rate and the slowest occurred in the sand pools. At four hours the concentration in the water ranged from 0.0079 ppm in the sand, 0.0081 ppm in

Figure 10. Chlorpyrifos Water Concentration in Laboratory Pools with Sod, Clay or Sand Substrate Treated with EC Dursban at 0.028 kg ai/Ha.

Figure 11. Percent Bioassay Mortality in Water Samples from Laboratory Pools with Sod, Clay or Sand Substrate Treated with EC Dursban at 0.028 kg ai/Ha.


CONCENTRATION (ppm)
.010
.005
.001
0
100
200
300
400
500
TIME POST-TREATMENT (hrs)
SAND
CLAY
SOD


MORTALITY (%)
100
75
50
25
0
100
200
300
400
TIME POST-TREATMENT (hrs)
SAND
CLAY
SOD

the sod, and 0.0086 ppm in the clay pools. A larger variation in concentration was expected due to the different adsorptive qualities of each substrate. By 24 hours post-treatment, the chlorpyrifos concentration in the water of the sod pools was considerably lower than in either the sand or clay pools. By 48 hours post-treatment the sand pools, which had the lowest concentration at four hours, exhibited a higher concentration than the clay and sod and maintained a higher chlorpyrifos concentration throughout the remainder of the experiment.

To see if there was a statistically significant difference in the rates of disappearance between the substrates, an analysis of covariance (Snedecor and Cochran 1967) on the slope of the disappearance lines was tested. For the test, the sod curve was based on 4 to 96 hours, the clay on 4 to 192 hours, and the sand on 4 to 288 hours post-treatment. The test is shown in Appendix C. The results indicated that there was a statistically significant difference (1%) in the disappearance rates of the chlorpyrifos between the sod/sand and clay/sand pools. There was no significant difference between the sod/clay pools.

The results of the experiments on the two formulations indicated that at 0.028 kg ai/Ha the EC Dursban could control the larvae in the sod pools whereas the G Dursban failed to do so. In the clay pools 100 percent bioassay control lasted for 192 hours post-treatment with G and only 96 hours as a result of the EC treatment. The G controlled the larvae in the sand pools for 336 hours while the EC was totally effective for only 192 hours post-treatment. Zero mortality was reached sooner in the clay and sand pools treated with the EC Dursban than in the same pools treated with G Dursban.

In the sod and clay pools, the GC results indicated that a higher

initial chlorpyrifos concentration was achieved in the EC pools than in the G pools of the previous experiment. This result was expected and corresponds to the results obtained the previous summer (Figure 4). However, in the sand pools a higher concentration resulted from the G treatment, and not from the EC, as expected. The G sand pools reached 0.0163 ppm while the EC sand pools reached only 0.0079 ppm.

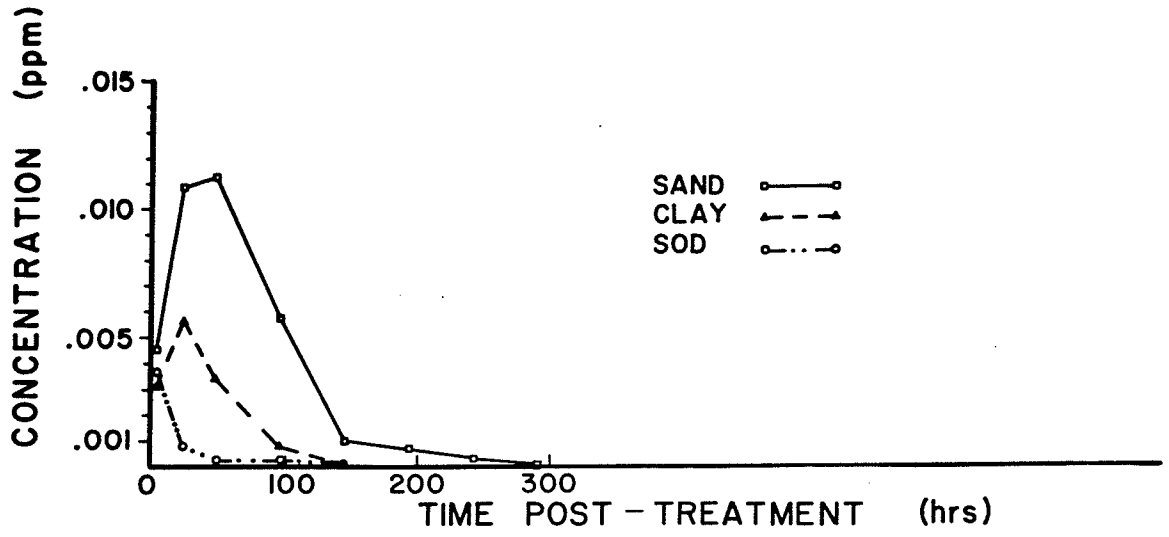
(iii) Outdoor temporary pools - 1976

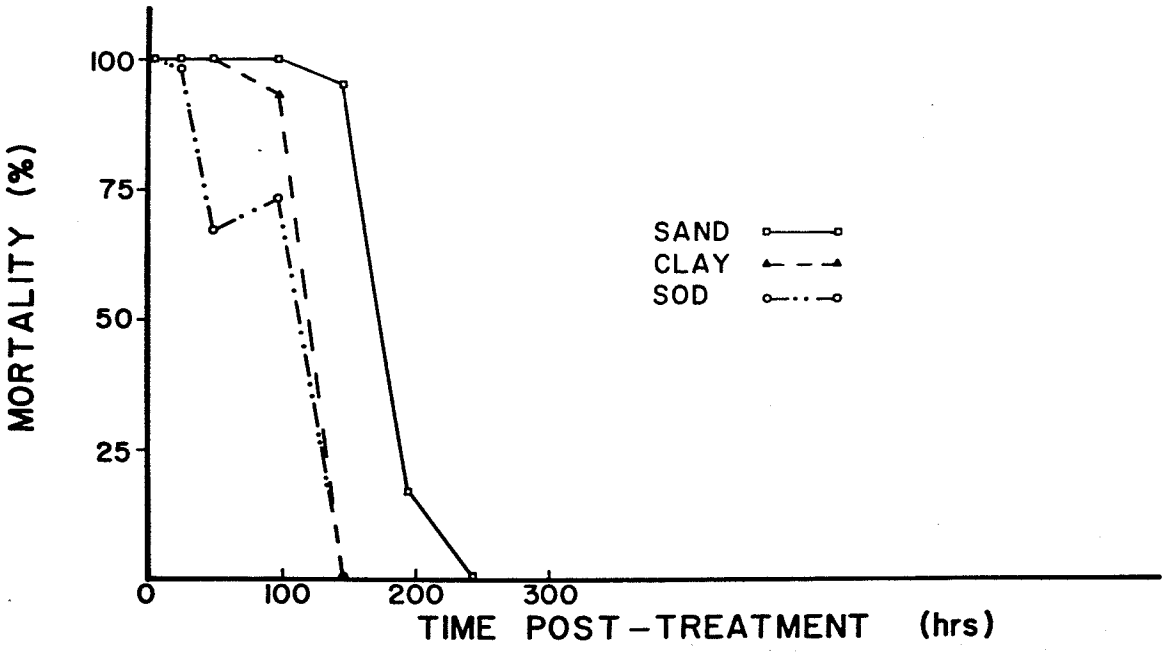
Figures 12 and 13 show the results obtained from outdoor Glenlea pools with three substrates (sod, clay or sand) treated with G Dursban (0.028 kg ai/Ha). In the sod, clay, and sand pools 100 percent mortality was achieved four hours post-treatment. By 24 hours the control in the bioassay of the sod pools had dropped to 98 percent whereas the clay pools maintained 100 percent for 48 hours and the sand pools continued 100 percent control for 96 hours post-treatment. Zero mortality for both the sod and clay pools occurred at 144 hours post-treatment and the sand pools reached zero percent at 240 hours post-treatment. Prior to sampling at 192 hours, rain raised the level of the water in the sand pools three centimeters higher than normal (about a 1/3 dilution). This dilution probably shortened the long residual control expected in the sand pools.

The GC data indicated that the chlorpyrifos was released from the G into the water in the pools of all three substrates. The peak concentration for all the substrates occurred at different times, the sod pools at 0.0037 ppm at four hours, the clay pools at 0.0056 ppm at 24 hours, and the sand pools at 0.0112 ppm at 48 hours post-treatment. Initially the clay pools had a higher chlorpyrifos concentration than the sod pools, but at 144 hours post-treatment chlorpyrifos was undetectable

Figure 12. Chlorpyrifos Water Concentration in Outdoor Pools with Sod, Clay or Sand Substrate Treated with G Dursban at 0.028 kg ai/Ha.

Figure 13. Percent Bioassay Mortality in Water Samples from Outdoor Pools with Sod, Clay or Sand Substrate Treated with G Dursban at 0.028 kg ai/Ha.



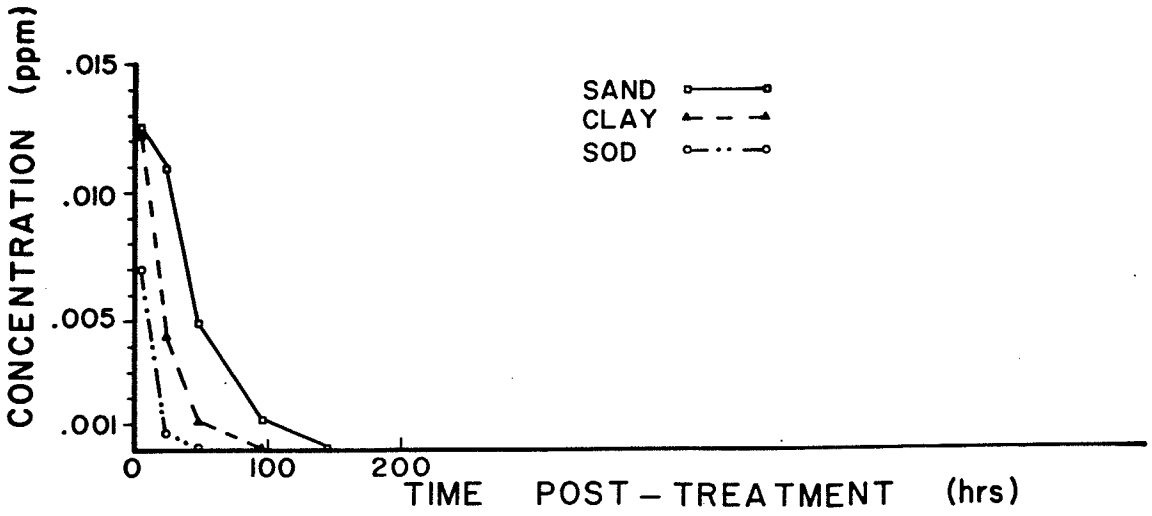


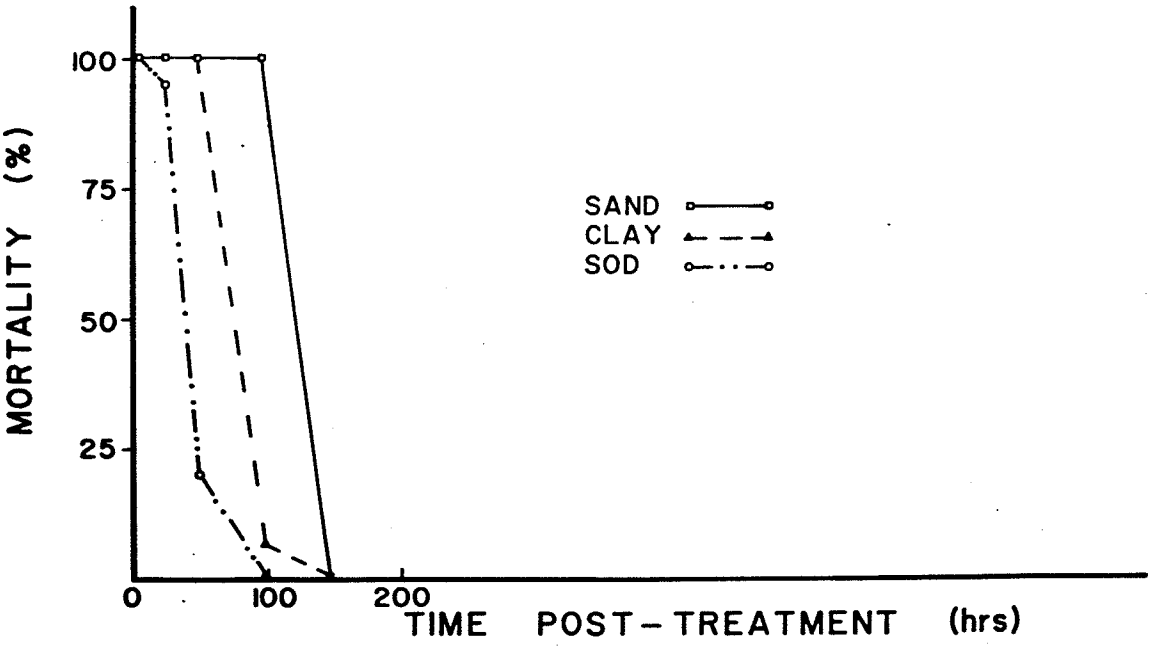
in the water from either pool. It was not until 288 hours post-treatment that the chlorpyrifos concentration in the water from the sand pools decreased below the minimum detectable limit. Thus, as in previous experiments, the lowest concentration and shortest residue period occurred in the sod pools.

The addition of the rainwater between 144 and 192 hours had little effect on the chlorpyrifos concentration in the sand pools. There was a decrease in concentration (0.0010 to 0.0007 ppm) in the water after the rainfall but this would have been expected as the normal disappearance pattern. The small change in concentration corresponding to a significant change in mortality (95 to 17 percent) between pre and post-rainfall further emphasizes the fact that there is a very narrow margin between the 100 percent control and the no effect level of chlorpyrifos in the water.

Figures 14 and 15 show the results for the EC Dursban trial at Glenlea (0.028 kg ai/Ha) using sod, clay or sand pools. The mortality curves followed the pattern established in the previous experiments. The sod pools had the shortest control period while the sand had the longest. One hundred percent control was maintained for four hours post-treatment in the sod pools, 48 hours in the clay pools, and 96 hours in the sand pools. The sod pools reached zero control by 96 hours. The clay and sand pools both reached zero control at 144 hours even though the sand pools had a longer period of 100 percent control. The drop in mortality in the clay and sand pools was rapid. In the clay pools during a 48 hour period (48 to 96 hours post-treatment) the bioassay mortality dropped from 100 to 7 percent. For the 48 hour period of 96 to 144 hours in the sand pools a decrease from 100 to zero percent was recorded in the bioassay.

Of the three substrates the lowest maximum chlorpyrifos concentration


CONCENTRATION (ppm)
.015
.010
.005
.001
0
100
200
TIME POST-TREATMENT (hrs)
SAND
CLAY
SOD


MORTALITY (%)
100
75
50
25
0
100
200
TIME POST-TREATMENT (hrs)
SAND
CLAY
SOD

in the water was detected in the sod pools at 0.0070 ppm at four hours post-treatment. The concentration in the clay and sand pools at four hours was 0.0122 and 0.0125 ppm, respectively. The chlorpyrifos in the sod pools decreased to the minimum detectable limit by 48 hours post-treatment. The chlorpyrifos concentration in the clay pools, which was about equal to the sand pools at four hours, decreased more rapidly than the sand pools and reached the minimum detectable limit by 96 hours post-treatment. The sand pools maintained the highest concentration of any of the pools throughout the experiment. At 140 hours post-treatment, the chlorpyrifos in the water of the sand pools reached the detectable limit.

The comparison of the outdoor G and EC Dursban results indicated that the period of 100 percent larval control was independent of formulation. One hundred percent control was maintained in the sod pool for four hours, the clay pool for 48 hours, and the sand pool for 96 hours post-treatment regardless of formulation. However, the residual control in the G pools was generally longer than found in the EC pools. In the sod pools, zero mortality was reached 144 hours after treatment with the G and only 96 hours with the EC. Zero mortality in the clay pools occurred at 144 hours post-treatment with both the G and EC formulations. With the EC, zero mortality in the sand pools occurred at 144 hours and with the G at 240 hours post-treatment. Except for the clay substrate the results corresponded with the results obtained the previous summer from sod pools treated at twice the rate in which the G Dursban provided longer residual larval control than the EC Dursban.

The GC data showed that the G resulted in lower chlorpyrifos concentrations in the water of the sod and clay pools than did the EC. In the sod and clay pools the G achieved the maximum chlorpyrifos concentration

of 0.0037 and 0.0056 ppm, respectively while the EC resulted in 0.0070 and 0.0122 ppm, respectively. The maximum chlorpyrifos concentration achieved in the sand pools was similar for both formulations (G at 0.0112 ppm and EC at 0.0125 ppm).

The reason the sand concentration is similar for both formulations and the sod and clay are significantly different can be explained by the adsorptive capacities of the various substrates. For both the sod and clay the variation in concentration as a result of G or EC treatment is due to the release nature of the particular formulation. The chlorpyrifos in G is released slowly and the chlorpyrifos in EC is released very quickly to the water. In either case the sod or clay immediately began to adsorb the chlorpyrifos from the water. It is the lack of adsorption in the sand pools that results in similar chlorpyrifos concentrations occurring with both formulations. The maximum chlorpyrifos concentration in the water of the sand pools was achieved at different times post-treatment with each formulation. The EC peaked at four hours post-treatment while the G reached its maximum chlorpyrifos concentration at 48 hours post-treatment. The G, without the loss of chlorpyrifos from the water through adsorption was able to continue to release its active ingredient for 48 hours and therefore increase its concentration in the water and thus match the concentration of the initial and only release of chlorpyrifos from the EC formulation. In the sod and clay pools the chlorpyrifos from the G would have been adsorbed during that critical 48 hours period and thus a lower chlorpyrifos concentration would have resulted from the G treatment.

Chlorpyrifos in the water was also detected for a longer period of time with the G Dursban. After treatment with the EC, the chlorpyrifos decreased to the minimum detectable limit at 48 hours, 96 hours,

and 144 hours for the sod, clay, and sand pools, respectively. In the G pools this limit was not reached until 144 hours for the sod and clay and 288 hours for the sand pools.

To determine the value of the laboratory pools in predicting results later obtained in the field, the data for the laboratory and outdoor pools treated at 0.028 kg ai/Ha Dursban were compared and considerable differences were noted.

Comparison of the bioassay graphs (Figures 9 and 13) of the laboratory and outdoor pools treated with G Dursban shows two major differences. The first difference was the fact that treatment of the outdoor sod pools with the G formulation was able to achieve 100 percent mortality, whereas in the laboratory sod pools, less than 10 percent bioassay mortality was obtained. The higher mortality in the outdoor pools could be the result of better active ingredient distribution within the pool water as a result of greater water movement due to the wind action or the formation of convection currents. These environmental actions would have been very much reduced within the confines of the laboratory. The second difference was the length of 100 percent control and the time required to reach zero mortality in the clay and sand pools. In the laboratory, 100 percent mortality lasted for 192 hours for the clay and 336 hours for the sand, while in the field this level of control lasted only 48 hours for the clay and 96 hours for the sand. Indoors, the clay and sand pools reached zero mortality at 336 and 528 hours, respectively, and outdoors at 144 hours and 240 hours, respectively.

The pattern established by the bioassay was confirmed by the GC data on G Dursban. There was more chlorpyrifos in the water of the outdoor sod pools (0.0037 ppm) than was detected in the laboratory sod pools (0.0014 ppm). Residues in the clay and sand pools were more

persistent in the laboratory than out.

The shorter control period in the outdoor versus laboratory pools is consistent with loss of chlorpyrifos by photodecomposition in the water. Smith (1966) stated that photodecomposition plays an important part in the breakdown of chlorpyrifos (Appendix B). In the outdoor pools the sunlight was very effective in speeding up the rate of disappearance of chlorpyrifos as shown by the shorter control period. The fluorescent light banks used in the laboratory could not match the light intensity of the sun and thus had less effect on initiating photodecomposition of chlorpyrifos. The importance of sunlight was substantiated by Miller et al. (1973) in that he found chlorpyrifos water residues were higher in the outdoor pools that were shaded from sunlight than in pools exposed to direct sunlight.

The bioassay for the EC Dursban followed the G pattern in that the control period was longer in the laboratory than in the field. One hundred percent mortality lasted in the laboratory for 48, 96, and 192 hours for the sod, clay, and sand pools, respectively. In the field 100 percent mortality was maintained in the sod, clay, and sand pools for 4, 48, and 96 hours, respectively. In the sod pools zero mortality was reached at 192 hours in the laboratory and 96 hours in the field. In the laboratory, the clay pools reached zero mortality at 240 hours versus 144 hours outdoors. Zero mortality was reached in the laboratory sand pools at 384 hours and in the field sand pools at 144 hours post-treatment.

No anomalies were found in the GC data for the laboratory and outdoor EC pools. Chlorpyrifos residues were detected longer in the water of the laboratory pools than in the water of the field pools and the rates of chlorpyrifos disappearance were as expected. The fastest rate

was observed in the sod pools, an intermediate rate in the clay pools and the slowest rate of chlorpyrifos disappearance from the water occurred in the sand pools.

It appears from these experiments that laboratory pools can be used to determine the bioactivity and disappearance patterns of insecticides but not to predict definite length of larval mortality in the field.

A summary of the data is presented in Tables XI and XII. Table XI compares the hours of 100 percent bioassay mortality for each set of experimental conditions. At 0.056 kg ai/Ha the EC formulation was more effective than the G. The G provided larval control for longer periods in the laboratory pools; however, in the outdoor pools (0.028 kg ai/Ha), the control period was independent of formulation. Of the treated outdoor pools, the sod pools maintained 100 percent larval mortality for the shortest period of time.

Table XII shows the percent chlorpyrifos remaining in water 48 hours post-treatment. In most cases the rate of chlorpyrifos disappearance was faster in the EC treated pools than in the G treated pools. It is also apparent that chlorpyrifos is removed faster from the water by the sod substrate than by either the clay or sand substrate.

Table XI. Hours of 100 Percent Bioassay Mortality With Dursban.

		Pool Substrate		
		Sod	Clay	Sand
Outdoor 0.056 kg ai/Ha	EC	96	-	-
	G	72	-	-
Outdoor 0.028 kg ai/Ha	EC	4	48	96
	G	4	48	96
Laboratory 0.056 kg ai/Ha	G	192	192	336
Laboratory 0.028 kg ai/Ha	EC	48	96	192
	G	0	192	336

Table XII. Percent Chlorpyrifos Remaining in Water 48 Hours Post-Treatment.

		Pool Substrate		
		Sod	Clay	Sand
Outdoor 0.056 kg ai/Ha	EC*	8.0	-	-
	G*	62.1	-	-
Outdoor 0.028 kg ai/Ha	EC**	0.0	9.8	39.2
	G**	8.1	109.7	248.9
Laboratory 0.056 kg ai/Ha	G**	50.0	206.7	276.6
Laboratory 0.028 kg ai/Ha	EC**	14.8	67.4	86.1
	G***	0.0	86.0	114.8

* Percent remaining from 0 hours post-treatment.
** Percent remaining from 4 hours post-treatment.
*** Percent remaining from 24 hours post-treatment.

V. SUMMARY

Dursban insecticide is one of the common chemicals used for the control of mosquito larvae. To study the bioactivity and disappearance of Dursban, mosquito pools were established in both the field and the laboratory. Treatment of the pools took place with Dursban in either granular (G) or emulsifiable concentrate (EC) formulations. Sod, clay, and sand were used as pool liners to determine the effect of these substrates on Dursban activity.

The outdoor sod pools treated with the G formulation (0.056kg ai/Ha) provided longer residual mortality of C. tarsalis bioassays than the EC formulation. The maximum chlorpyrifos concentration achieved in the water was three times higher in the EC pools than in the G pools and occurred immediately post-treatment. The slow-release G formulation resulted in a lower and somewhat delayed maximum concentration in the water.

Laboratory studies at 0.028 kg ai/Ha Dursban resulted in longer residual bioassay mortality from both formulations (G and EC) than those obtained in the field at the same rate of application. One exception was the G formulation in the laboratory sod pools which failed to control the bioassay mosquitoes while the EC formulation did.

In all trials the pool substrate greatly influenced Dursban activity. The sod substrate resulted in the shortest larval control period and the lowest chlorpyrifos concentrations in the water. The rate at which Dursban was removed from the water was also highest in the sod pools. The sand pools provided the highest residue levels, longest period of control, and the slowest disappearance of chlorpyrifos from the water.

VI. CONCLUSIONS

Control of Culex tarsalis is undertaken to reduce the nuisance population and to prevent the spread of western encephalomyelitis, a disease vectored by C. tarsalis. An effective control method is the application of Dursban larvicide to the mosquito breeding sites.

EC Dursban (0.056 kg ai/Ha) prevented establishment of Culex species in experimental sod pools up to 360 hours post-treatment compared with 840 hours for the G. The EC achieved a maximum chlorpyrifos concentration of 0.0162 ppm in the water at zero hours post-treatment whereas, 0.0055 ppm at four hours post-treatment was the maximum amount of chlorpyrifos in the water of the G treated pools.

Residual control of Dursban was affected by the pool substrate. Sand and clay pools had longer periods of larval mortality and higher chlorpyrifos residue levels than the sod pools. The varying degrees of control with each substrate were consistent with the different adsorptive capacities of each substrate.

The laboratory experiments failed to predict the field efficacy of Dursban. For example, treatment of indoor sod pools with G Dursban (0.028 kg ai/Ha) failed to control the larvae while complete control was achieved in the field at the same application rate. One hundred percent bioassay mortality lasted for only four hours in the field sod pools. Thus, this rate of application is not recommended in pools with high organic matter content which can adsorb the chlorpyrifos from the water and reduce its residual effectiveness.

Mosquito breeding sites treated with G Dursban (0.056 kg ai/Ha) will provide effective larval control, lasting longer than the EC formulation. The lower residue level in the water resulting from the

G application does not affect the efficacy of the treatment but can increase the chance of survival of the non-target organisms present in the mosquito pools.

To obtain longer residual mortality of mosquito larvae without increasing the application rate of the insecticide, a new formulation to overcome the adsorptive capacity of the substrate is required. Such a formulation would not result in high chlorpyrifos concentrations in the water immediately post-treatment, but would continue to release the active ingredient and thus maintain a low and constant concentration of insecticide in the water.

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Mosq. News. 32: 364.

APPENDIX A

Toxicity Values for Dursban on Non-Target Organisms

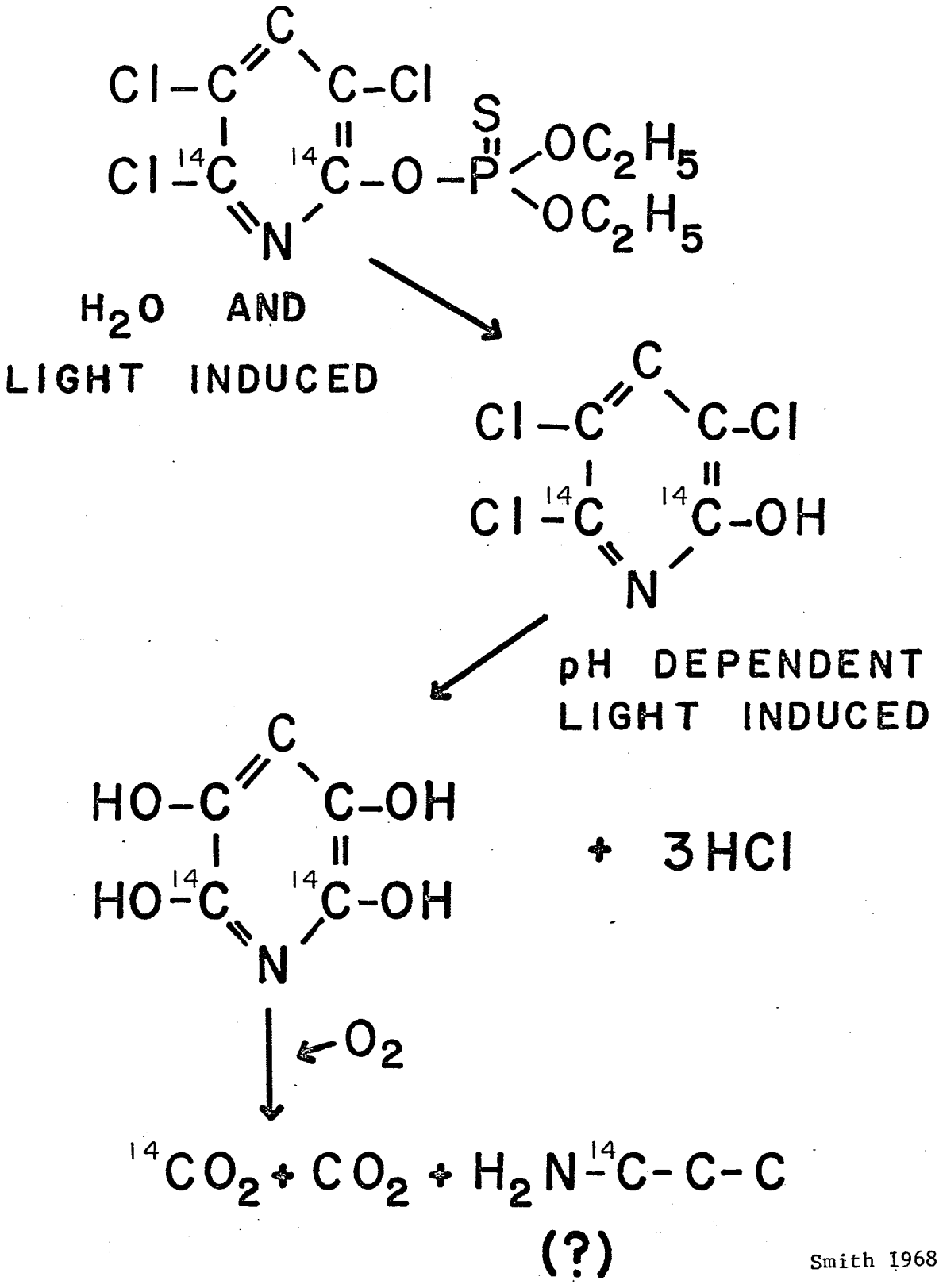
Organism		Acute oral LD_{50} (mg/kg)
female rats[1]		135
male rats[1]		163
guinea pigs[1]		500
chicks[1]		32
rabbits[1]		1000-2000
mallards[2]		70-80
pigeons[2]		26.9
house sparrows[2]		21
Canada geese[2]		80
rainbow trout[2]	LC_{50}^{48}	20 (ug/l)

[1] Gray (1965)

[2] Pimentel (1971)

APPENDIX B

PHOTODECOMPOSITION OF ^{14}C DURSBAN



Smith 1968

APPENDIX C

Analysis of Covariance of Disappearance Rates of Chlorpyrifos in Pools of Different Substrate

Sod, n = 4		Clay, n = 6		Sand, n = 8	
TIME (Hr) X	CONCENTRATION (ppm) Y	X	Y	X	Y
4	0.0081	4	0.0086	4	0.0079
24	0.0056	24	0.0078	24	0.0071
48	0.0012	48	0.0058	48	0.0068
96	0.0009	96	0.0032	96	0.0055
		144	0.0013	144	0.0044
		192	0.0004	192	0.0030
				240	0.0017
				288	0.0007

Sand vs Clay

	df	X^2	XY	Y^2	Deviation Regression df	SS	MS
Within							
Sand	7	76094	-1.9	0.0000480	6	0.0000006	
Clay	5	26701	-1.2	0.0000576	4	0.0000037	
					10	0.0000043	0.00000043
Pooled	12	102795	-3.1	0.0001056	11	0.0000122	
	Difference between slopes				1	0.0000079	

Comparison of slopes: F = 0.0000079/0.00000043
= 18.37 (df: 1, 10) Stat. sig. 1%

(Cont'd)

Sand vs Sod

Within Sand	7	76094	-1.9	0.000048	6	0.0000006	
Sod	3	4716	-0.37	0.0000367	2	0.0000077	
					8	0.0000083	0.000001
Pooled	10	80810	-2.27	0.0000847	9	0.000021	
		Difference between slopes			1	0.0000127	

Comparsion of slopes: F = 0.0000127/0.000001
= 12.7 (df: 1, 8) Stat. sig. 1%

Clay vs Sod

Within Clay	5	26701	-1.2	0.0000576	4	0.0000037	
Sod	3	4716	-0.37	0.0000367	2	0.0000077	
					6	0.0000114	0.0000019
Pooled	8	31417	-1.57	0.0000943	7	0.0000159	
		Difference between slopes			1	0.0000045	

Comparison of slopes: F = 0.0000045/0.0000019
= 2.37 (df: 1, 6) Not sig.

Snedecor and Cochran 1967
pp: 432