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.

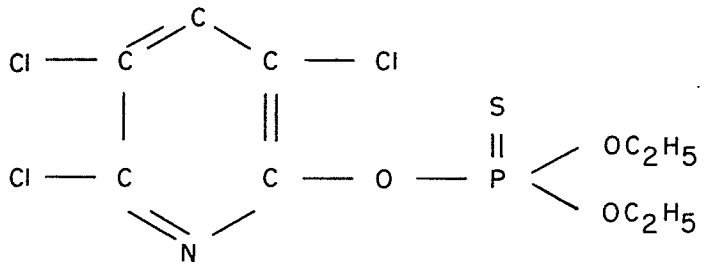
Research was undertaken to study the bioactivity and rate of disappearance of chlorpyrifos from temporary pools treated with either of two Dursban formulations (granular or emulsifiable concentrate). The bioactivity was measured by bioassays with laboratory-reared fourth instar C. tarsalis larvae while 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.