THE DISAPPEARANCE FROM, EFFICACY IN AND EFFECT ON NON-TARGET ORGANISMS OF DIFLUBENZURON, METHOPRENE AND CHLORPYRIFOS IN A LENTIC ECOSYSTEM

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Douglas James Madder

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TABLE OF CONTENTS

Page

Page

LIST OF TABLES

Table

Table

LIST OF FIGURES

LIST OF APPENDICES

ABSTRACT

Madder, Douglas James. M.Sc., The University of Manitoba, November, 1977. The Disappearance From, Efficacy In and Effect on Non-Target Organisms of Diflubenzuron, Methoprene and Chlorpyrifos in a Lentic Ecosystem. Major Professor; W.L. Lockhart.

The disappearance from, efficacy in, and effect on non-target organisms of the mosquito larvicides Diflubenzuron, Methoprene and Chlorpyrifos was studied in a lentic ecosystem. Twelve sod lined pools of ca. 5,000 ℓ capacity (5.5 x 3.4 x 0.5 m) located at the University of Manitoba, Glenlea Research Station (ca. 15 km south of Winnipeg) were used for the study. Diflubenzuron as Dimilin WP-25 at 0.056 kg ai/ha, Methoprene as Altosid SR-10 at 0.056 kg ai/ha and Chlorpyrifos as Dursban 2.5G at 0.028 kg ai/ha were repetitively applied to 3 pools each leaving 3 as controls.

Bioassay using fourth instar Aedes aegypti (L.) (Culicidae) larvae, quantitative gas liquid chromatographic analysis of water from the treated pools, and qualitative observations showed that Diflubenzuron and Methoprene disappeared rapidly. No detectable residues (> 0.0005 mg/ℓ)attributable to the parent compounds were present after 2 days post-treatment and no bioactivity in evidence after 8 days post-treatment. Bioassay and qualitative observations showed the disappearance of Chlorpyrifos to be significantly slower than that of the insect growth regulators with bioactivity, determined by development of mosquito

larvae in the pools, present for 2 weeks post-treatment.

Emergence traps, semi-quantitative water samples (Van Dorne bottle samples) and qualitative observations were used to determine the effects of the insecticides on the target species Culex tarsalis Coquillett (Culicidae) and on non-target fauna. Chlorpyrifos was found to have the greatest efficacy producing control (reduced adult emergence) for > 22 days. Diflubenzuron produced control for 8 to 15 days and Methoprene for 0 to 16 days. Chlorpyrifos caused the most severe effects on the largest number of taxa including most Insecta and Crustacea. Diflubenzuron was found to be more selective affecting primarily Cladocera, Ephemeroptera and Diptera while Methoprene was the most selective compound deleteriously affecting only Diptera.

For the control of Culex tarsalis where non-target organisms are of little importance, Chlorpyrifos would be recommended. If non-target organisms were of significance Diflubenzuron would be preferred. Due to its low efficacy Methoprene could not be recommended for the control of this species.

INTRODUCTION

Recent research has shown that insect growth regulators are practical insecticides (Staal 1975; Anonymous 1974). Two of these compounds, Methoprene[1] (Altosid ; ZR-515; isopropyl-11-methoxy-3,7,11-trimethyl-2,4-dodecadienoate) and Diflubenzuron[2] [Dimilin ; PH-6040; TH-6040; 1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea] will be marketed in Canada. These compounds are expected to be used as mosquito larvicides; however, little information is currently available describing their effects on mosquito breeding habitats.

This study was designed to compare efficacies, residual lives, and effects on non-target organisms among Methoprene, Diflubenzuron, and a widely used organophosphate insecticide, Chlorpyrifos[3] [Dursban ; Lorsban ; O,O-diethyl-(3,5,6-trichloro-2-pyridyl) phosphorothioate] when used as mosquito larvicides. Effects of Chlorpyrifos on mosquito breeding habitats have been well documented, although only cursorily in Canada. It was used in this study to compare the effects of the growth regulators with a conventional insecticide.

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[1] Zoecon Corporation, Palo Alto, California, U.S.A.

[2] Thompson Hayward Company, Kansas City, Kansas, U.S.A. and Phillips Duphar B.V. Company, Holland.

[3] Dow Chemical Company, Midland, Michigan, U.S.A.

LITERATURE REVIEW

This review will be confined to literature pertaining to the disappearance, efficacy, and effects on non-target organisms of Diflubenzuron, Methoprene, and Chlorpyrifos in aquatic freshwater lentic ecosystems. Furthermore, discussion of the formulations of these compounds will be restricted primarily to those used in this study.

Diflubenzuron

Diflubenzuron was introduced as an experimental insecticide by the Phillips Duphar B.V. Co. of Holland in 1973 (Post and Vincent 1973) and its chemical properties have been described by Bijloo (1975). It is an insect growth regulator belonging to the group 1-benzoyl-3-phenyl ureas, which can cause the disruption of cuticle production in arthropods. The development of 1-benzoyl-3-phenyl ureas, including Diflubenzuron, as insect control agents has been reviewed by Bijloo (1975), Post and Mulder (1974), Elings and Dieperink (1974), Post and Vincent (1973), Mulder and Gijswigt (1973), Oliver *et al.* (1976), Wellinga *et al.* (1973a, 1973b), and van Daalen *et al.* (1972).

Disappearance[1]

The rate of its disappearance and the degradation pathways of Diflubenzuron in various situations have been studied by Schaefer and

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[1] Disappearance is used throughout as a general term for all phenomena which may have caused decrease in water residues.

Dupras (1976), Metcalf et al. (1975), and Ruzo et al. (1974).

Studies by Schaefer and Dupras (1976) using high pressure liquid chromatographic (HPLC) analysis determined that the primary factors causing degradation or disappearance of Diflubenzuron from water were hydrolysis of the parent compound and adsorption onto organic matter. Further, increasing water temperature and pH were found to promote degradation, while the presence of microorganisms and/or sunlight had no effect.

Field trials were also performed by Schaefer and Dupras (1976) to measure rates of disappearance of Diflubenzuron from mosquito breeding habitats. Application rates of 0.0224 to 0.112 kg active ingredient (ai)/ha resulted in residues that were less than 0.002 mg/ℓ or below detectable limits (< 0.001 mg/ℓ) within 3 days of treatment. Disappearance was found to be due in part to hydrolysis of the parent compound (confirmed by the presence of the p-chlorophenyl aniline metabolite after treatment), but adsorption appeared to be a more significant factor. Treated areas with large amounts of organic matter showed low efficacy and residues, and a high disappearance rate in comparison to areas with little organic matter. Thus adsorption onto organic material caused a loss of biological activity and was the primary factor removing Diflubenzuron from treated water.

From these data it was apparent that Diflubenzuron was degraded relatively quickly, yet was present for long enough to control floodwater and asynchronous mosquito larval populations. Despite these properties of Diflubenzuron itself, formulations have been developed to extend the life of this compound so that the duration of control can be extended. Formulations include silicate capsules (Sjogren and

Thies 1975), granules (Mulla and Darwazeh 1975c), sand and vermiculite (Rathburn Jr. and Boike Jr. 1975).

The formulation most commonly used in mosquito control is the Dimilin WP-25 formulation developed by Phillips Duphar B.V. It is a wettable powder (particle size < 5 μ) containing 25 percent active ingredient by weight (Anonymous 1975).

Water from field studies with Dimilin WP-25 at application rates of 0.0112 to 0.560 kg ai/ha have shown bioassay activity against mosquito larvae from 2 to 10 days post-treatment, and reduced emergence of mosquitoes from 4 to 14 days, with higher application rates acting for a longer period. Normal application rates (0.028 to 0.056 kg ai/ha) produced bioassay activity for 3 to 4 days, and reduced emergence for 6 to 10 days (Mulla and Darwazeh 1976, 1975c; Schaefer et al. 1976, 1975, 1974a; Mulla et al. 1974a; Pelsue et al. 1974).

At the application rate used in this study (0.056 kg ai/ha) residues would be expected to be below GLC detectable limits (< 0.0005 mg/ℓ) within 3 to 4 days of treatment and biological activity to be lost within 6 to 8 days of treatment.

Efficacy

Studies of morphological and physiological effects of Diflubenzuron on mosquitoes have been published by Miura et al. (1976) and Busvine et al. (1976). Lethal and sublethal effects on Culex tarsalis Coquillett are reported by Jakob (1973), Georghiou and Lin (1974) and Arias and Mulla (1975a, 1975b).

Diflubenzuron inhibits deposition of cuticle; its specific biochemical mechanism is beyond the scope of this review. However, some

information can be obtained from Sowa and Marks (1975), Yu and Terrière (1975), Ishaaya and Casida (1974), Post and Mulder (1974), and Mulder and Gijswijt (1973).

All mosquito species tested have shown sensitivity to Diflubenzuron (Gaaboub and Busvine 1976; Takahashi and Ohtaki 1976; Elings and Dieperink 1974; Steelman et al. 1975). Sensitivity of eggs (LC_{50}[1] = 0.025 mg/ℓ , Culex pipiens fatigans Wiedemann) is low in comparison with that of other immature stages. All larval stages are similar in sensitivity to Diflubenzuron (LC_{50} = 0.0013 to 0.0025 mg/ℓ, C. pipiens fatigans), although late third instar larvae tend to be the most sensitive. Sensitivity decreases substantially in the pupal stage, and is virtually nonexistent in the adult (Busvine et al. 1976). Death usually occurs during the first molt after exposure to the compound, but at lower concentrations several molts are required (Schaefer et al. 1974a).

Diflubenzuron eliminates larval populations present in the breeding area at the time of treatment. Due to its rapid disappearance the duration of control (lack of adult emergence) depends upon the presence of unhatched eggs and the speed of development of the subsequent mosquito population.

The toxicity of Diflubenzuron to mosquito larvae ranges from (LC_{50} values) 0.0003 to 0.005 mg/ℓ for Culex, Aedes and Anopheles spp. (Rathburn Jr. and Boike Jr. 1975; Busvine et al. 1976; Mulla et al. 1974a). Significant activity has also been shown against Psorophora and Culiseta spp. although no acute toxicity data have been published. The genus-specific sensitivity shown by Methoprene (cf. infra) is not evident with

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[1] $LC_{50(95)}$ is that concentration in mg/ℓ required to kill 50 (95) percent of a test population.

Diflubenzuron even though it binds to organic matter (Anonymous 1974; Mulla and Darwazeh 1975a, 1975c; Steelman et al. 1975). LC_{95} values range from 0.001 to 0.01 mg/ℓ for Aedes, Culex and Anopheles spp. (Jakob 1973; Rathburn Jr. and Boike Jr. 1975; Dame et al. 1976; Mulla et al. 1974a).

The recommended application rates for Diflubenzuron as Dimilin WP-25 are from 0.028 to 0.056 kg ai/ha or a concentration range of 0.01 to 0.02 mg/ℓ.

The duration of control and the efficacy exerted by Diflubenzuron in the present study would be expected to be only moderate due to the asynchronous nature of larval Culex tarsalis populations and their relatively rapid development rate under the experimental conditions.

Effects on Non-Target Invertebrate Fauna

Miura and Takahashi (1974a, 1974b) described effects of Diflubenzuron on non-target aquatic invertebrates in laboratory studies on 37 groups of organisms including algae, rotifers, crustaceans and insects. Mortality rates showed that Diflubenzuron is toxic to species of Cladocera, Conchostraca, Notostraca, Chironomidae, Ephemeroptera, Notonectidae and Odonata at concentrations of 0.02 mg/ℓ or lower. In artificial containers Diflubenzuron at 0.005 mg/ℓ suppressed Cladocera and copepod populations which required recovery times of at least 2 weeks for copepods and greater than 4 weeks for Cladocera. Field experiments at application rates of 0.062 to 0.124 kg ai/ha had similar results. Populations of Cladocera (Daphnia sp.), Conchostraca, Notostraca and Copepoda were significantly reduced due to treatment, but appeared to recover within 1 to 2 weeks of application. These treatments also resulted in a reduced chironomid and ephemeropteran emergence after

treatment.

Miura and Takahashi (1975) applied Diflubenzuron as Dimilin WP-25 to flooded pastures for mosquito control at rates between 0.025 and 0.062 kg ai/ha and studied subsequent abundance of 62 species or species groups. Reductions in numbers of Ephemeroptera nymphs and Cladocera were evident though these numbers recovered within 1 to 2 weeks of application. Similarly, numbers of copepods were reduced in some instances though not by large proportions and recovery occurred quickly. Immature insects such as dytiscid larvae, chironomid pupae, and hydrophilid larvae were also killed although no population reductions were found. Repeated applications at monthly intervals did not eliminate any of the organisms studied. Similar data are also reported by Miura and Takahashi (1974a) and Mulla et al. (1975a).

Several studies (Mulla et al. 1976, 1975b, 1974b) have been undertaken to determine the potential of Diflubenzuron for chironomid control (considered non-target organisms in this study). Applications of 0.056 to 0.280 kg ai/ha resulted in reduced emergence of chironomids from 4 days to 4 weeks after treatment. Higher application rates gave more prolonged reduction of emergence and also reduced densities of chironomid larvae. As previously mentioned, this apparent residual effect is primarily due to the presence of unhatched eggs and the developmental rate of the resulting target organism.

Steelman et al. (1975) studied the use of Dimilin WP-25 for the control of Psorophora columbiae (Dyar and Knab) in rice fields. Some field experiments included quantitative determination of effects of treatments from 0.001 to 0.280 kg ai/ha on non-target organisms. No significant differences were found between treated and control popula-

tions of adult or immature notonectids, corixids, or adult Thermonectus sp. (Dytiscidae), but a significant reduction in Tropisternus sp. adults (Hydrophilidae) and libellulid naiads was evident. A significant increase in baetid naiads and chironomid larvae was also reported that was attributed to the decrease in predators mentioned above.

Diflubenzuron selectively kills some aquatic invertebrates and is not as specific as Methoprene in respect either to developmental stage or to taxa affected. Generally, significant effects have been reported against certain taxa of Coleoptera, Ephemeroptera, Odonata, Diptera and Cladocera. Some effects on members of these taxa would be expected when applying Diflubenzuron at normal application rates for mosquito control.

Methoprene

Methoprene was introduced as an experimental growth regulator by the Zoecon Corporation in 1972 (Schaefer and Wilder 1972). Its chemical properties have been described in Anonymous (1973). Development of this and other juvenile hormone analogs as insect control agents has been reviewed by Staal (1975), Norland (1973), Slama (1971), Menn and Beroza (1972) and Slama et al. (1974).

Disappearance

One of the factors limiting commercial development of many juvenile hormone analogs has been their environmental instability.

Schaefer and Dupras (1973) first studied the degradation of Methoprene in water and reported a half-life of technical Methoprene in tap water placed in direct sunlight of about 2 hours.

Schooley et al. (1975) showed that this degradation was primarily

due to photoisomerism. Immediately upon exposure to sunlight more than 50 percent of Methoprene is isomerized to its 2Z isomer, an essentially biologically inactive compound. Further degradation studies reported by the same researchers showed that Methoprene, when placed in pond water containing a rich microbial population, also degraded very quickly, even in the absence of light. It was concluded that degradation of Methoprene by microorganisms and by photoisomerism is rapid and extensive though actual degradation pathways and rates in the field would depend upon meteorological conditions, water temperature and microbial community in the treated area.

Field studies using emulsifiable concentrate (EC) formulations applied to mosquito breeding habitats did show very rapid degradation rates (Schaefer and Dupras 1973; Schaefer and Wilder 1973; Steelman et al. 1975). Application rates of up to 0.56 kg ai/ha resulted in water residues which fell below gas liquid chromatographic (GLC) and mosquito bioassay detection limits (GLC detection limit 0.0003 mg/ℓ) within 3 days of treatment.

The short half-lives of technical Methoprene and its EC formulation are desirable environmentally, but they limit use as practical mosquito control agents. The active compound is not present long enough to affect all the larval mosquitoes present. In order to lengthen the effective period several slow release formulations have been tested e.g., polyurethane foam (Dunn and Strong 1973), sand and vermiculite (Rathburn Jr. and Boike Jr. 1975).

The Altosid SR-10 formulation registered for mosquito control in Canada (developed by the Zoecon Corp.) consists of a liquid suspension of polymer-Methoprene particles having a mean diameter of 1.0 μ with

the final product 10 percent active ingredient by weight.

Field applications of Altosid SR-10 at rates of 0.056 to 0.56 kg ai/ha to mosquito breeding habitats (flooded pastures) in California were described by Schaefer and Dupras (1973) and Schaefer et al. (1973). Application rates below 0.112 kg ai/ha resulted in residues that degraded below GLC detectable limits (0.0003 mg/ℓ) 2 days after treatment. Applications of 0.28 to 0.56 kg ai/ha resulted in low residues (< 0.01 mg/ℓ) at the end of the study, 3 days after treatment. Biological activity as shown by fourth instar mosquito larval bioassay was apparent for 4 to 5 days after treatment at an application rate of 0.112 kg ai/ha. Similar degradative rates, as monitored by bioassay and mosquito emergence, were reported for the Altosid SR-10 formulation at application rates of 0.01 to 0.1 mg ai/ℓ by Buei et al. (1975), Schooley et al. (1975), Mulla et al. (1974a), and Pelsue et al. (1974).

At the application rate used in this study (0.056 kg ai/ha) residues would be expected to degrade below GLC detection limits (< 0.0005 mg/ℓ) within 2 to 3 days of treatment and biological activity lost within 4 to 5 days of treatment.

Efficacy

The morphological and physiological effects of Methoprene on mosquitoes have been described by Hooper (1976), Paulov and Paulovova (1977), Judson and de Lumen (1976), Busvine et al. (1976), Naqvi et al. (1976), Downer et al. (1975) and Quistad et al. (1975). Lethal and sublethal morphological and physiological effects on the mosquito Culex tarsalis have been described by Arias and Mulla (1975a, 1975b) and Georghiou and Lin (1974).

The above-mentioned studies as well as those concerning Methoprene's

effects on other insect groups indicate that its mode of action is very similar to, if not the same as, juvenile hormone. The specific mode of action of juvenile hormone analogs is beyond the scope of this review, although information concerning it can be found in Menn and Beroza (1972).

All mosquito species tested have been susceptible to Methoprene, with the most sensitive stage being the fourth larval instar (Schaefer and Wilder, 1973, 1972; Naqvi et al. 1976; Busvine et al. 1976). Sensitivity increases as development proceeds from the egg (LC_{50} > 10 mg/ℓ, Culex pipiens fatigans) to the fourth instar larva (LC_{50} = 0.0014 mg/ℓ, C. pipiens fatigans), drops in the pupal stage and is almost ineffective in the adult (Judson and de Lumen 1976; Busvine et al. 1976). Despite the fact that the most sensitive developmental stage is the fourth larval instar, death does not occur until after pupation.

Studies of the toxicity of Methoprene to fourth instar mosquito larvae has produced LC_{50} values ranging from < 0.0001 to 0.01 mg/ℓ for Aedes, Anopheles and Culex spp. Activity has also been shown against Psorophora and Culiseta spp. although no LC_{50} data have been presented (Anonymous 1973; Mulla and Darwazeh 1975c; Steelman et al. 1975). LC_{95} values for Aedes spp. vary from 0.0001 to 0.01 mg/ℓ (Georghiou and Lin 1974; Rathburn Jr. and Boike Jr. 1975; Majori et al. 1977; Dame et al. 1976; Busvine et al. 1976; Wells et al. 1975; Buei et al. 1975).

Culex spp. usually have LC_{50} values a factor of 10 greater than those of Aedes or Anopheles spp. Schaefer et al. (1974b) attributed this lower sensitivity to differences in feeding behaviour; Culex tarsalis is a filter feeder and shows low sensitivity to Methoprene while Aedes nigromaculis (Ludlow), a browsing feeder, shows high sensitivity. Research reported by Schaefer et al. (1974b) shows that Altosid SR-10

settles to the bottom of mosquito breeding pools, making it more available to browsing rather than to filter feeders. Mortality of Culex tarsalis was increased significantly when a charcoal formulation was used, to prevent settling.

Altosid SR-10 is registered for control of floodwater mosquitoes, primarily Aedes, Anopheles and Psorophora spp. The asynchronous nature of Culex and Culiseta larval populations combine with Methoprene's short residual life and lower toxicity to filter feeders to substantially reduce the duration that control is exerted over breeding habitats of these genera. As a result Methoprene is not registered for the control of Culex or Culiseta spp.

The registered application rates for the Altosid SR-10 formulation are 0.028 to 0.056 kg ai/ha or a concentration range of approximately 0.01 to 0.02 mg/ℓ.

The duration of control and overall efficacy of Methoprene in this study would be expected to be limited in view of the target species Culex tarsalis.

Effects on Non-Target Invertebrate Fauna

The first study to describe effects of Methoprene on aquatic invertebrate non-target organisms was that of Miura and Takahashi (1973). Laboratory studies were performed on 35 groups of organisms including algae, diatoms, protozoa, nematodes, platyhelminthes, rotifers, annelids, mollusks, crustaceans, and insects to determine their sensitivities to Methoprene. Mortality rates in these experiments showed that Methoprene was of very low toxicity to all organisms studied except Diptera. From these results significant mortality of organisms in actual breeding sites would only be expected in Diptera when registered application rates for

mosquito control are used.

In field studies (artificial containers, ponds and flooded pastures) the Altosid SR-10 formulation at application rates of 0.1 mg ai/ℓ caused no detectable effects in numbers of animals during the 3 to 4 week post-treatment observation period, with the exception of emerging Diptera (Chironomidae, Culicidae, Ephydridae, and Psychodidae) (Miura and Takahashi 1974a, 1973).

In laboratory studies Dunn et al. (1974) found that concentrations of Altosid SR-10 as low as 0.001 mg ai/ℓ delayed pupation of hydrophilid larvae, although concentrations as high as 1 mg ai/ℓ did not apparently affect the adult emergence rate. Some abnormal adults were present, however, as a result of the higher treatment rates.

Mulla et al. (1974b) evaluated Methoprene for control of chironomids. In laboratory studies LC_{50} values for Chironomus spp. were less than 0.01 mg/ℓ. Field studies using Altosid SR-10 at application rates of 0.112 to 0.280 kg ai/ha in 1.6 to 2.0 m deep water resulted in inhibition of chironomid emergence for 1 week, and reduced emergence for up to 2 weeks, although no effects on larval density were noted. Similar results have been reported by Mulla and Darwazeh (1975a, 1975b) and Pelsue et al. (1974).

Steelman et al. (1975) studied the use of Altosid SR-10 for the control of Psorophora columbiae in rice fields. Some experiments included quantitative determination of effects of Altosid SR-10 used at application rates of 0.001 to 0.28 kg ai/ha on non-target organisms. No statistically significant differences were found between treatment and control populations of adult or immature notonectids, adult or immature corixids, Thermonectus sp. adults (Dytiscidae), or immature baetids. Treatments did cause reduction in numbers of Tropisternus sp. adults (Hydrophilidae) in

contrast to Miura and Takahashi (1973) who reported no visible effects on Tropisternus lateralis (F.) when exposed to 1 mg/ℓ Methoprene for 120 hours. Neither do these results agree with field studies by Schaefer et al. (1974b) who reported no observable effects on members of this genus. Steelman et al. (1975) exposed Tropisternus sp. larvae to Methoprene and the effects were not observed until 80 days after treatment in the adults. Due to the relatively long developmental period in this genus, treatment effects were not apparent unless populations of both adults and immatures were monitored for an extended period. Steelman et al. (1975) also reported that Altosid SR-10 treatment caused a significant reduction of immature Libellulidae.

Steelman et al. (1975) also found an increase in chironomid immatures in Altosid-treated plots. They speculate that this may have been due to a treatment-related reduction of the above-mentioned predators. Similarly negative correlations were apparent for most prey-predator interactions in treated pools indicating mortality of predacious insects in general.

Norland (1973) and Norland and Mulla (1975) reported the effects of EC Methoprene applied to permanent mosquito breeding pools. Studies of the toxicity of Methoprene to Callebaetis pacificus Seemann (Ephemeroptera) nymphs showed 100 percent mortality when exposed to water from pools treated at 0.112 kg ai/ha and to undetermined residues in the same pools 5 days after treatment. Thus Methoprene shows a high toxicity to this species and possibly to other Ephemeroptera not previously observed.

Further field trials by the same authors studied the effects of repetitive applications (5 day intervals) of 0.112 kg ai/ha EC Methoprene on numbers and biomass of invertebrates in mosquito breeding pools.

Chironomid larvae were significantly reduced in numbers and biomass by the repetitive treatments. A reduction in numbers and an even greater reduction in biomass of Ephemeroptera nymphs and dytiscid larvae was also evident. This indicated that Ephemeroptera nymphs and dytiscid larvae in treated pools did not develop past their earlier larval instars. In contrast to Steelman et al. (1975) no significant effects were detected on populations of libellulid nymphs.

The studies discussed above show that Methoprene is selective in its toxicity in terms both of taxa and developmental stages affected. Conflicting results among the above studies may well be due to the various application rates used, specific taxa and developmental stages treated, and duration of the post-treatment observation periods. Generally significant effects have been reported against selected taxa of Coleoptera, Ephemeroptera, Odonata, and Diptera and these effects may be expected to occur to some extent at registered application rates.

Chlorpyrifos

Chlorpyrifos, an organophosphate insecticide developed by the Dow Chemical Co., was introduced as an experimental broad spectrum insecticide in 1965 (Gray 1965). It was first reported as a potent mosquito larvicide by Ludwig and McNeil (1966) and Mulla et al. (1966). Chemical properties of Chlorpyrifos are reported in Smith (1966). Due to the extensive literature published pertaining to the disappearance from and effects of Chlorpyrifos on mosquito breeding habitats only selected papers are discussed.

Disappearance

Shortly after the introduction of Chlorpyrifos several studies were

published showing its disappearance rate and degradative pathways in a variety of habitats. Laboratory studies reported by Smith (1966) and Smith et al. (1967, 1966) showed that the primary cause of disappearance of Chlorpyrifos from aqueous systems was its adsorption onto organic material rather than chemical decomposition. Hydrolysis of the parent compound would be expected in aqueous systems but it was thought to be of little importance. Degradation of Chlorpyrifos to 3,5,6-trichloro-2-pyridiol (the primary hydrolysis product) was promoted by increasing pH, temperature and ultraviolet light intensity. This degradation product is unstable in light and further degrades to a variety of compounds (Smith 1968).

Field studies by several authors determined residual life and factors that promote degradation of Chlorpyrifos in mosquito breeding habitats. One of the more extensive studies was that of Schaefer and Dupras (1970, 1969) who used GLC analysis to determine that the disappearance/degradation half-life of Chlorpyrifos applied at 0.112 kg ai/ha to flooded pastures (water temperature 24 to 40°C) was approximately 5 hours. From a series of laboratory and field studies it was confirmed that increasing temperature and the presence of light increased the disappearance rate of Chlorpyrifos, though the presence of a rich microbial community in treated waters did not affect the disappearance rate (Hirakoso 1969; Whitney 1967). The short residual life determined by Schaefer and Dupras (1970, 1969) did not agree with the residual efficacy of Chlorpyrifos reported to be greater than 3 weeks after treatment at 0.112 kg ai/ha in mosquito breeding pools. As a result of this apparent contradiction in results, and the laboratory evidence of Smith et al. (1967) showing adsorption to plant material, Schaefer and Dupras continued their studies to

determine whether the residual efficacy was due to the adsorption-desorption of Chlorpyrifos onto and from organic material.

They found that Chlorpyrifos did in fact adsorb to organic material in mosquito breeding habitats but it probably did so reversibly and its dissociation from the particulate matter was the cause of its extended residual efficacy. It was not determined whether the Chlorpyrifos was toxic when adsorbed onto particulate matter and ingested or whether indeed it had to be redissolved in water (at very low concentrations) to be toxic. Similar data have been shown by Mulla et al. (1973) and Hurlbert et al. (1970).

Technical Chlorpyrifos is degraded relatively quickly, yet due to its adsorption-desorption properties it shows extended efficacy. As a result it will control both floodwater and asynchronous mosquito larval populations. Many formulations have been developed for specific control situations; those primarily used for mosquito control are the EC and granular formulations. The EC formulation is usually used when floodwater mosquitoes are to be controlled and emergent vegetation is sparse. Granular formulations are used to penetrate vegetation and to provide a longer residual life for control of asynchronous mosquitoes. A granular formulation, Dursban 2.5G (2.5 percent Chlorpyrifos by weight adsorbed onto bentonite), was used in this study due to its wide use in mosquito control and because it is most suitable for the control of the target species in this study, Culex tarsalis.

The study most relevant to this one was that of Rawn (1977) showing the disappearance of Chlorpyrifos from mosquito breeding habitats. Rawn used GLC analyses and mosquito larval bioassays to determine the disappearance rates of EC and granular (Dursban 2.5G) formulations of Chlor-

pyrifos in 1 m^2 pools with either sand, clay or sod substrates. Rawn's study was also performed at the University of Manitoba Glenlea Research Station, approximately 100 m southeast of the pools reported herein. Dursban 2.5G was applied at 0.028 kg ai/ha to sod lined pools in similar fashion to that used here in terms of application rate, formulation, and substrate. It is evident from Fig. 1 (Rawn 1977) that residues disappeared below GLC detectable limits (< 0.0005 mg/ℓ) and below fourth instar Culex tarsalis larval bioassay detection limits 7 days after treatment.

Selected papers concerning the disappearance rate of granular formulations of Chlorpyrifos from mosquito breeding habitats as measures of duration of control and bioassay are discussed below.

Cooney and Pickard (1974) studied the application of Chlorpyrifos on clay granules at 0.112 and 0.056 kg ai/ha to floodwater mosquito breeding habitats. They found mosquito larval bioassay mortality (> 75% mortality) for more than 25 days after treatment when treated at 0.056 kg ai/ha. Dixon and Brust (1971) determined that a 5 percent ai granular formulation applied at 0.028 kg ai/ha to sod lined pools resulted in biological activity as shown by mosquito larval bioassay for 2 weeks after treatment. Tawfik and Gooding (1970) also studied the residual activity of a granular formulation (attapulgus clay) applied at 0.056 kg ai/ha to mosquito breeding pools. They found significant residual activity for at least 4 weeks as shown by failure of re-establishment of the target species. Further data concerning disappearance rates of Chlorpyrifos applied as granular formulations at application rates greater than 0.112 kg ai/ha are shown in Mulla et al. (1973) and McNeill et al. (1968).

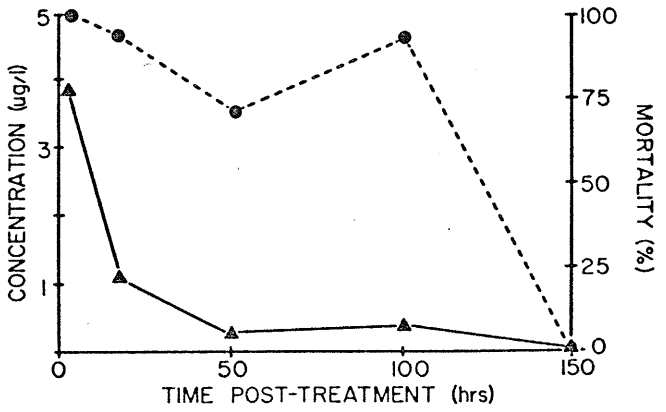


Figure 1. Residues as determined by GLC and bioassay (fourth instar Culex tarsalis bioassay) resulting from treatment of sod lined pools with Chlorpyrifos (Dursban 2.5G) at 0.028 kg ai/ha (Rawn 1977).

Legend: GLC residues ——

Bioassay -----

At the application rate used in this study (0.028 kg ai/ha) residues and disappearance should be similar to that shown by Rawn (1977) (Fig. 1).

Efficacy

Studies of the mode of action and the physiological effects of organophosphate insecticides in general are beyond the scope of this review but these topics have been recently reviewed by Wilkinson (1976) and Matsumura (1975).

All mosquito species tested have been sensitive to Chlorpyrifos (Cooney and Pickard 1974; Womeldorf and Whitesell 1972; Nelson et al. 1976a; Miller et al. 1973; Bailey et al. 1970). Sensitivity of eggs was low in relation to other immature stages. First instar larvae were highly susceptible to Chlorpyrifos (LC_{50} = 0.00023 mg/ℓ, Anopheles freeborni Aitken) though sensitivity decreased with larval development (fourth instar larvae LC_{50} = 0.0035 mg/ℓ, A. freeborni). Sensitivity continued to decrease in pupae and adults (adult LC_{50} = 0.18 mg/ℓ, A. albimanus Wiedemann) (Womeldorf and Whitesell 1973; Mulla et al. 1966). Death usually occurs within 24 hours of treatment.

Chlorpyrifos eliminates mosquito larval populations present at the time of treatment. Residual action is a function of death of the highly sensitive first instar larvae and the development time required for the new immature stages after residues drop below toxic levels.

The acute toxicity of Chlorpyrifos to mosquito larvae is (LC_{50}) from < 0.0001 to 0.014 mg/ℓ for Aedes, Culex, and Anopheles spp. (Womeldorf and Whitesell 1972; Tawfik and Gooding 1970; Sinegre et al. 1975; Washino et al. 1972; Georghiou et al. 1969; Steelman et al. 1967; Lofgren et al. 1967). Toxicity to Psorophora and Culiseta spp. has also been

reported though no LC_{50} data are stated (Craven and Steelman 1968; Lewis et al. 1966). No genus-specific sensitivity differences are apparent though in some areas of high pesticide use resistance to Chlorpyrifos has appeared. LC_{90} values vary from 0.001 to 0.03 mg/ℓ for Aedes, Culex, and Anopheles spp. (Gray 1965; Ludwig and McNeil 1966).

The recommended application rates for Chlorpyrifos as Dursban 2.5G are from 0.028 to 0.056 kg ai/ha or a concentration range of approximately 0.01 to 0.02 mg/ℓ.

The duration of control and efficacy exerted by Chlorpyrifos in this study would be expected to be very high; in fact, it should be the most effective among the 3 chemicals tested.

Effects on Non-Target Invertebrate Fauna

Despite 10 years of operational use of Chlorpyrifos as a mosquito larvicide, few laboratory toxicity studies have been reported concerning aquatic non-target invertebrates. Those limited data available show high toxicity i.e., $LC_{50} < 0.01$ mg/ℓ for Notonectidae, Hydrophilidae and Chaoboridae (Federle and Collins 1976; Roberts et al. 1973).

Field studies performed by Hurlbert et al. (1970) determined effects of Chlorpyrifos on non-target fauna in a mosquito breeding habitat. One study concerned the effects of 3 applications of EC Chlorpyrifos at 2 week intervals to a series of freshwater ponds. The application rate used was 0.028 kg ai/ha, similar to that used here, and significant effects were observed on almost all fauna in the habitat. Predacious notonectid and dytiscid populations generally increased in control pools throughout the 10 week experiment whereas populations in treated pools were much reduced after each treatment and had not recovered at the end of the experiment 5 weeks after the last treatment. Belostomatids were

similarly affected but had almost recovered to control populations by the end of the experiment. Tipulid larvae were never caught in treated pools whereas they were caught in large numbers in control pools. Zygopteran populations were reduced severely in treated pools but had recovered by 3 to 4 weeks after the last treatment. Adult corixids showed little adverse response to the treatment while populations of corixid nymphs declined to 0 with each treatment but recovered within 2 weeks of the treatments with the exception of the third one. By 4 weeks after the last treatment hydrophilid larval populations had returned to control levels. Ephemeroptera nymphal populations were reduced to 0 after the first treatment and showed little recovery until the end of the experiment.

The authors state that effects on the invertebrate populations can be related to trophic levels. In control ponds the total number of predacious insects increased throughout the experiment while populations of herbivorous insects decreased until halfway through the experiment and thereafter remained relatively stable. In treated ponds, the first treatment caused about the same mortality in both predacious and herbivorous populations (40-90 percent) while subsequent treatments caused less evident reductions in herbivorous populations than with predacious organisms. After cessation of treatments populations of predacious insects were significantly lower than those of herbivorous insects in treated pools. In control pools the opposite was true. This is attributed by Hurlbert et al. (1970) to the general hypothesis that for herbivorous insects, the insecticide treatment partially replaced one mortality factor (predacious insects) with another (Chlorpyrifos), whereas for predacious insects the treatment added an additional morta-

lity factor. Populations normally experiencing high mortality (herbivores) could respond to treatment more efficiently (recolonization by immigration, high fecundity, short life cycles) than those populations which normally experience lower mortality rates (predators).

Hurlbert et al. (1972, 1970) also studied the effects of the treatment on phytoplankton and zooplankton in the pools. As their discussion is extremely detailed and since Cladocera and copepods were the only planktonic organisms studied here, only the effects on those groups are discussed. Copepods were substantially reduced by the treatments but usually recovered above control population levels within 2 weeks of treatment while Cladocera were more severely affected and recovered more slowly. It was apparent that phytoplankton and zooplankton were affected in complex ways by the treatments. Either through primary or secondary effects the treatments did result in increased phytoplankton which may have helped the recovery of herbivorous insect populations discussed above. Other studies of the effects of Chlorpyrifos on bacteria, phytoplankton, and zooplankton are offered by Butcher et al. (1977), Nelson et al. (1976b), Brown et al. (1976), and Steelman et al. (1967).

Washino et al. (1972) studied the effects of EC Chlorpyrifos applied at 0.0125 to 0.2 kg ai/ha to rice fields for mosquito control. Their data indicated deleterious effects against belostomatids, anisopterans, hydrophilid larvae, dytiscid larvae, ephemeropteran nymphs and notonectids. No effects were noted, however, on corixids or on hydrophilid adults. The authors state that on a seasonal basis, there was no obvious impairment of most non-target organisms in the field.

Roberts et al. (1973) studied the effect of Chlorpyrifos applied as a water emulsion at 0.0125 kg ai/ha on non-target organisms in artificial

field pools. This treatment resulted in a 5 week reduction in gerrid populations and a 2 week reduction in larval dytiscids. Populations of chironomid adults emerging from the pools and chironomid and chaoborid larvae were apparently not affected by the treatment.

Chlorpyrifos has been applied at 0.112 to 0.280 kg ai/ha for chironomid control with a significant decrease in adult emergence for 2 to 12 weeks (Ali and Mulla 1976; Mulla and Khasawinah 1969; Mulla et al. 1975b, 1973). Results most applicable to those reported herein are those of Mulla et al. (1975b) who applied 0.112 and 0.280 kg ai/ha to a 1.5 m deep lake. These treatments reduced the numbers of chironomid larvae present for 2 and 4 weeks respectively with reduction in adult emergence of 3 and 5 weeks respectively.

From these data, it is apparent that significant reductions in most aquatic invertebrate non-target organisms could be expected for 2 or more weeks when Chlorpyrifos is applied at normal application rates for mosquito control.

MATERIALS AND METHODS

Construction of Pools

Having decided to perform a study to compare effects of three insecticides on aquatic invertebrates in mosquito breeding habitats, a suitable experimental location was sought. It was considered impractical to use naturally occurring breeding sites for the following reasons:

(a) Lack of uniformity between natural ponds in respect to substrate, fauna and flora (numbers and composition), and water quality;

(b) Lack of control over water levels in naturally occurring breeding sites;

(c) Lack of electrical power at most breeding sites (required for various instruments); and,

(d) Originally this experiment was intending to use radiolabelled insecticides to follow degradation rates and pathways. Their use would necessitate as closed an ecosystem as possible. Thus pools were located in an access-controlled area such that the water and substrate could be controlled and disposed of easily if necessary.

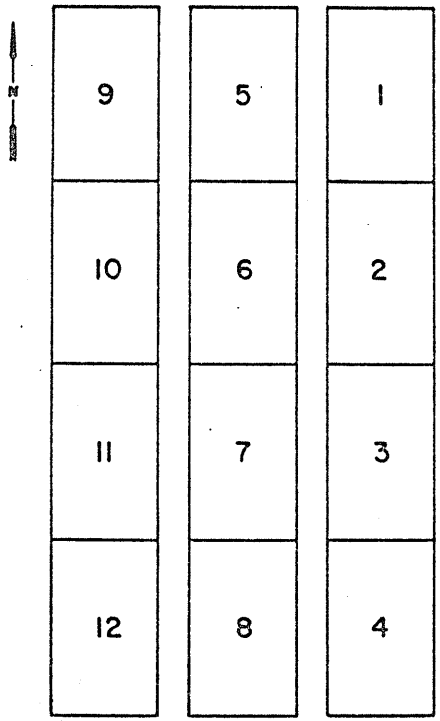
As a result a series of mosquito breeding sites was constructed at the University of Manitoba Glenlea Research Station. Twelve pools were constructed during the summer of 1975, 1 year prior to the experiment. They were built in the sequence shown in Fig. 2 to the specifications (Figs. 2 and 3) and manner (Fig. 4) described below.

A series of 12 dugouts approximately 5 x 4 x 1 m were dug by a

Figure 2. Experimental pools.

a) Numbering system
b) Outside dimensions (dimensions in meters)

a



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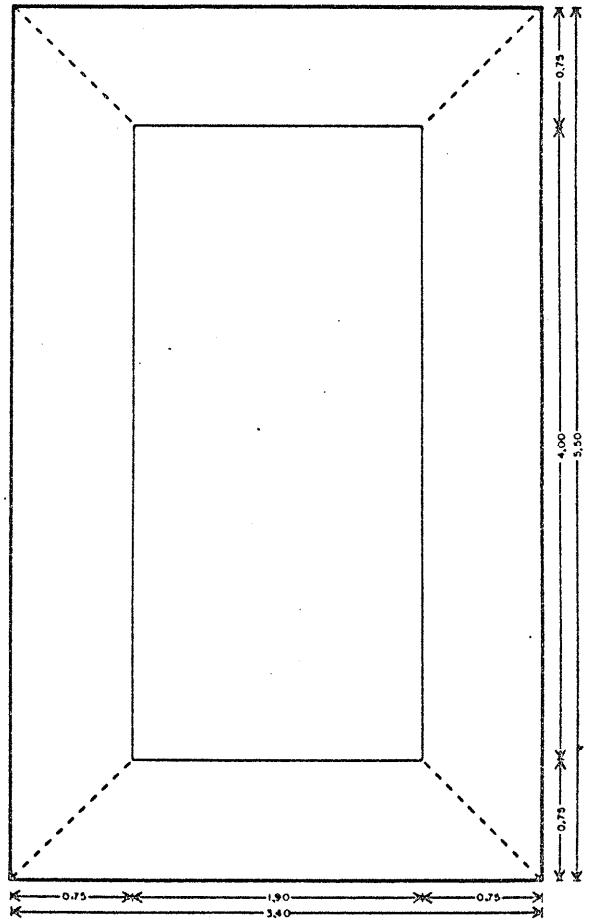


Figure 3. Cross-section of experimental pool (dimensions in meters).

Legend: A - Water
B - Sod
C - Sand
D - Earth
E - Polyethylene
F - Frame board

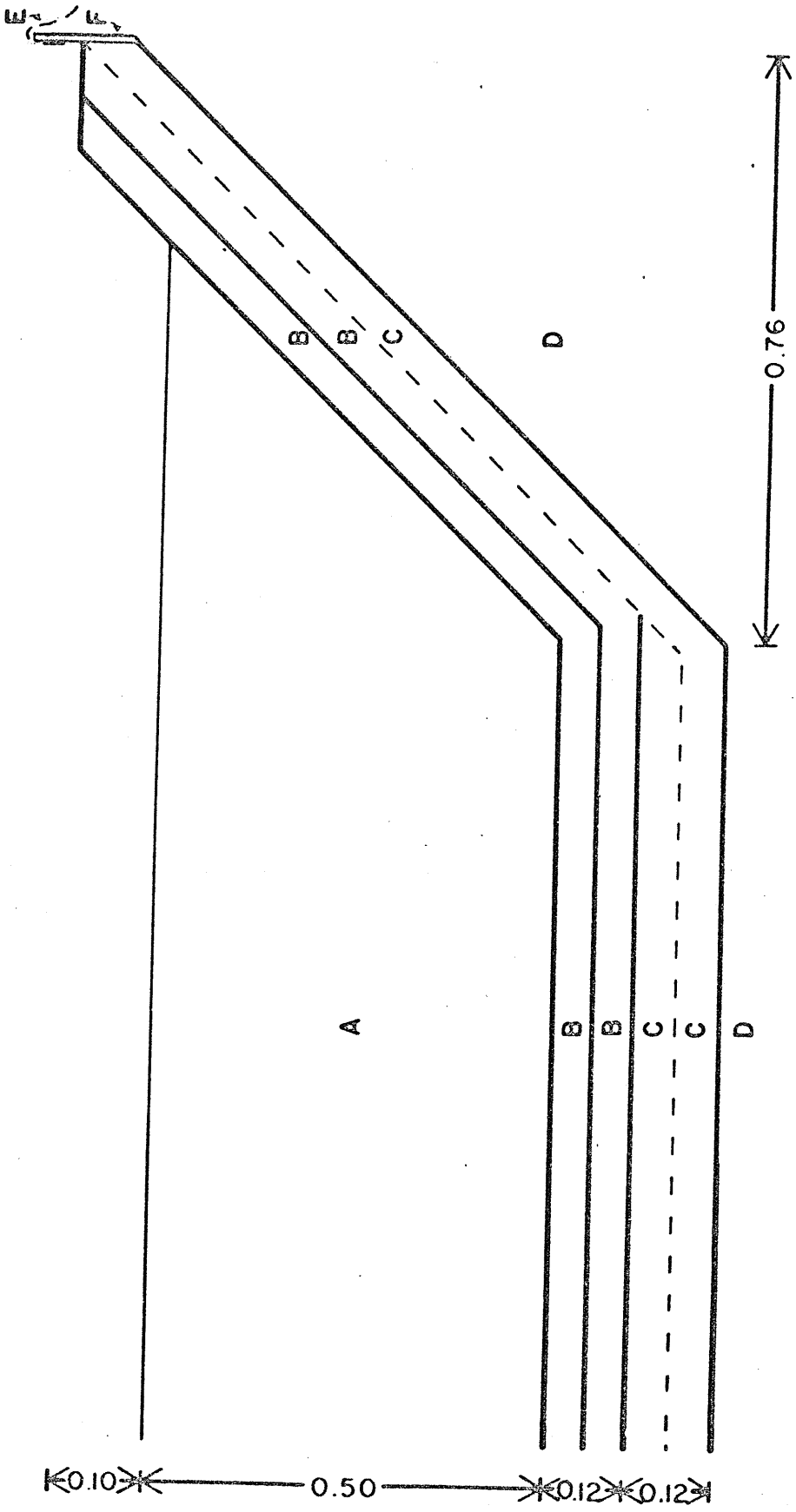
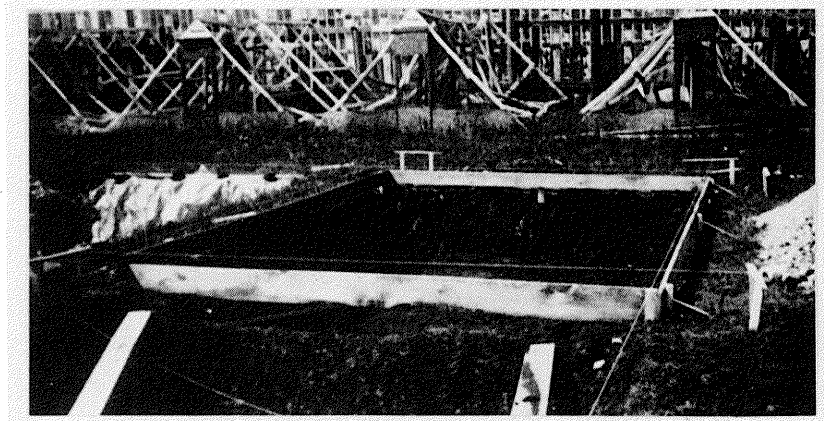

E
B
B
C
D
0.76
A
B
B
C
C
D
0.10
0.50
0.12
0.12

Figure 4. Construction of pools.

a) "Roughed-Out" pool and frame
b) Pool with sand overlayed with polyethylene and sand
c) Sod lined pool
d) Flooded pool

a



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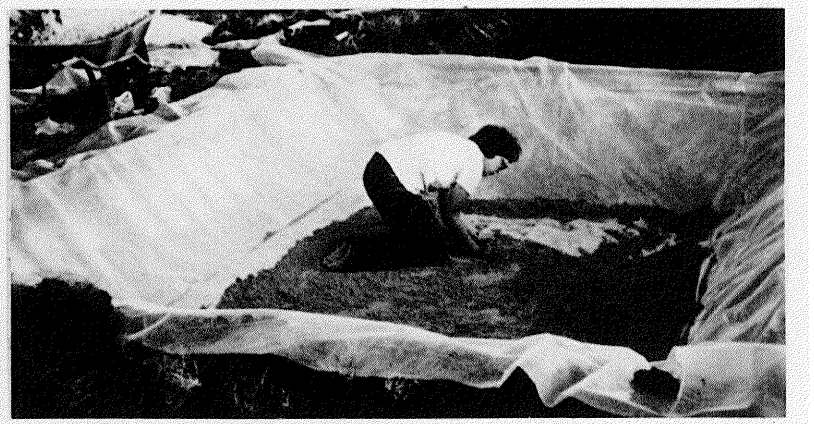


c

d

backhoe. They were dug in 3 rows with 4 per row, in a north-south orientation with a 1 m walking area left between each row of pools. A wood frame was constructed around the edge of each dugout extending at least 8 cm above ground level. Frames were constructed of 12 x 2 to 24 x 2 cm pine boards and anchored by 4 x 7 cm stakes. Final dimensions of frames around each pool were 5.5 x 3.4 m. The frames were constructed to raise the sides of the pools above ground level to prevent water from running into pools from adjacent areas and to provide a foundation for planks that would extend across the pools during sampling.

After the frames were constructed the sides of the dugouts were altered to their exact dimensions i.e., slope and size as shown by Figs. 2 and 3. This was done by either building up the sides with earth and pieces of sod or trimming the sides down if the original dugout was too small.

A 2 to 5 cm layer of sand ("Playsand" - Supercrete Company Ltd., Winnipeg) was spread over the earth bottom and sides of the pools. The sand provided a base on which a polyethylene sheet (10 mill, 0.25 mm thickness) was placed to prevent water loss through the bottom of the pools, so that at the end of construction all of the pools could hold water to the top of the frames. This depth was reduced in some pools due to slumping of the sides during the spring and summer of 1976.

A 2 to 5 cm layer of sand was placed over the polyethylene on the bottom of the pool. It was found impractical to place sand up the sloping sides over the polyethylene as it would slide down to the pool's bottom. The sand was placed both under and on top of the poly-

ethylene to reduce the possibility of its puncture by pieces of earth, stone, or the pressure of ice in winter.

On top of the polyethylene and sand 2, 6 cm-thick layers of sod were laid. The sod was cut from the area where the pools were dug, as well as from the area immediately south thereof. A chemical analysis of this sod as determined by the Manitoba Provincial Soils Laboratory, Ellis Building, University of Manitoba, is shown in Appendix A. The sod was laid with its grass side up and such that there were no gaps between adjacent pieces. The top layer, wherever possible, covered seams between pieces of sod in the lower layer. As a result there were no areas where water could reach the polyethylene without passing through the sod. This construction minimized the possibility of adsorption of the chemicals onto the polyethylene. Also, the thickness of the substrate (approximately 12 cm) provided suitable conditions for development of such organisms as chironomids and tipulids.

As pools were completed each was flooded to a depth of ca. 50 cm. This left ca. 15 cm from the water level to the top of the frames so that the pools could accept 15 cm of rain without overflowing. This also provided a substantial land-water interface, with emergent vegetation for oviposition and emergence of insects.

All water used in filling and topping the pools during 1975 and 1976 was pumped from a large dugout located east of the experimental pools. The water added in 1975 was not filtered in order to aid colonization of the pools by aquatic invertebrates occurring in the large pond. The water level in the pools was maintained at ca. 50 cm until permanent ice formed in the fall of 1975.

Prior to the experiment during 1976 the water was also maintained

at ca. 40 cm until chemical treatment was initiated. All water added in 1976 following the commencement of the experiment was filtered at the pump's inlet valve through a 20 liter pail filled with chicken grit and at the exit valve by a "Y" filter (Sutherland Plumbing and Heating Supplies Ltd., Winnipeg). Filtration was used to minimize disturbance of the ecosystem and/or alteration of treatment-related effects by adding organisms to the pools. Water was not added during the first week after treatment and was added after that time only if the pool's water level had dropped to such an extent as to make water sampling difficult or the emergence traps ineffective (cf. infra). This procedure was followed in an effort to maintain natural conditions in the pools, and to reduce the possibility of dilution of the insecticides in the pools after treatment. The days upon which water was added are shown as notes in Appendix B.

Monitoring of Environmental Parameters

Monitoring of atmospheric weather conditions was performed by the staff of Glenlea Research Station at a weather station ca. 800 m north of the experimental site. Maximum and minimum air temperature, rainfall, and hours of insolation are shown in Appendix C.

Water quality analysis of pool water was performed several times during the fall of 1975 and the summer of 1976. Samples were taken by the author and analyzed by the Water Quality Laboratory, Freshwater Institute, Environment Canada, Winnipeg, using methods described by Stainton et al. (1977). In addition, the pH of the water in each pool was determined immediately before each treatment using a battery-operated Radiometer pH meter. These data are reported in Appendices D and E.

A series of 21 temperature probes connected to a Honeywell continuous reading thermograph was used to monitor water temperature ± 0.2°C in the pools as well as water temperature stratification in 1 pool. One probe was located at the bottom centre of each pool, approximately 15 cm away from the base of the south slope. Ten probes were placed at various locations and depths in pool 12 to note temperature stratification. Temperature at each probe was recorded every 15 minutes from June 23 to Aug. 23, 1976, although only daily maximum and minimum temperatures of selected probes are reported (Appendix B).

Treatment of Pools

As 12 pools were available for 4 treatments, 3 pools were treated identically with each regime (Diflubenzuron, Methoprene, Chlorpyrifos and Control). Diflubenzuron was applied as Dimilin WP-25, Methoprene as Altosid SR-10, and Chlorpyrifos as Dursban 2.5G. These formulations were chosen because they are registered and used for mosquito control. The application rates used for the chemicals were 0.056 kg ai/ha for Diflubenzuron and Methoprene, and 0.028 kg ai/ha for Chlorpyrifos which would produce initial concentrations of 0.02 mg/ℓ (6.5×10^{-8} M), 0.02 mg/ℓ (6.5×10^{-8} M) and 0.01 mg/ℓ (2.9×10^{-8} M) respectively if all active ingredient was released and dissolved upon treatment. These application rates are within the range of rates used for these chemicals for mosquito control. (The lower rate was used for Chlorpyrifos to compare the results of this experiment with those of G. Rawn [1977] who was performing his experiments at the lower rate.) Thus the results of these experiments should be similar to those resulting from normal mosquito control operations.

Using the numbering system of Fig. 2 the pools were randomly

selected for treatment as shown in Table 1. The treatments were performed by determining the water volume of each pool (based on theoretical dimensions and measured water depth), and measuring (weight - Dimilin, Dursban; volume - Altosid) the corresponding amount of chemical. The chemicals were then added to 350 ml pond water to produce a slurry and immediately poured into the pools on east-west and north-south transects. The criterion of retreatment was the presence of mosquito adults in the emergence traps of that treatment group for 2 consecutive emergence trap sampling days. (This procedure was difficult to follow after the second treatment with Diflubenzuron or Methoprene due to their low efficacy). Some criterion had to be set in this manner as it was impossible to determine whether control had been lost in Methoprene and to a lesser extent Diflubenzuron treated pools, until adult emergence had occurred. This criterion for retreatment would result in only a relative measure of efficacy as actual control programs could not allow adult mosquito emergence.

Monitoring Disappearance of the Insecticides

The disappearance of the insecticides from the pools was monitored by GLC for Methoprene and Diflubenzuron, and by mosquito larval bioassay for all 3 compounds. GLC analysis was not used to monitor Chlorpyrifos degradation as another project was underway to evaluate that (Rawn 1977). The sampling procedures for GLC and bioassay samples for both Methoprene and Diflubenzuron are outlined below. Four sets of glassware were used in the sampling, 1 set designated and used for only 1 treatment group.

Gas Liquid Chromatography - Methoprene

Prior to sampling a 100 ml glass beaker attached to the end of a

TABLE 1. Treatment regime.

Treatment	Pool		
Diflubenzuron	7	10*	12
Methoprene	2*	5	11
Chlorpyrifos	3	4*	6
Control	1	8*	9

*Pools used for bioassay.

1 m aluminium rod by a steel clamp was rinsed with water from the pool to be sampled. This apparatus was then used to take 5 ca. 100 ml samples from the pool. One sample was taken ca. 60 cm from each of the 4 corners of the pool with the fifth taken from the centre of the pool. At each site the beaker was lowered to a depth of ca. 30 cm. After each sample was taken it was poured into a pre-rinsed 600 ml glass beaker. A 100 ml glass pipette pre-rinsed with pool water was used to quantitatively deliver 100.0 ml of the pool water from the 600 ml beaker to a prepared 250 ml glass sample bottle. The sample bottle was prepared by rinsing it with pesticide grade n-hexane and air drying. Ten ml of pesticide grade n-hexane were then quantitatively delivered into the sample bottle, thus preparing it for the pool water. After the pool water was pipetted into the sample bottle, the bottle was tightly sealed with aluminium foil and a metal or bakelite cap. The bottle was then rigorously hand-shaken for 2 minutes to aid in the partitioning of the Methoprene from the pool water into the n-hexane. The sample was then placed in the refrigerator ($+5^{o}C$) at the experimental site for temporary storage, i.e., 1 to 2 hours. GLC samples were the last taken during any sampling day so that there would be a minimum delay between sampling and analysis or low-temperature storage.

After samples were taken they were returned to Winnipeg for either immediate analysis or storage at $-20^{o}C$ for up to 48 hours before analysis was performed. Analysis of spiked samples treated in a similar manner as that described above, showed no degradation of the Methoprene after partitioning with n-hexane (J. Solomon, Freshwater Institute, Environment Canada, Winnipeg, unpublished data). A Florisil column was used to clean the extract, after which a 1 to 5 µl aliquot was

injected into a Tracor MT 220 GLC fitted with a glass column (1.7 m x 4 mm i.d.) with 3% OV 225 on 80/100 mesh Chromosorb W, a 20 ml/min prepurified N_2 gas flow and a ^{63}Ni electron capture detector. Temperature conditions were as follows: Injector 200°C, oven 180°C, detector 300°C. This analysis was developed by J. Solomon and analyses were performed by the author and J. Solomon at the Freshwater Institute, Environment Canada. The detection limit of this analysis from pool water was 0.0005 mg/ℓ.

Gas Liquid Chromatography - Diflubenzuron

Sampling for Diflubenzuron GLC analysis was identical to that for Methoprene, with the following exceptions. In the first experimental run no partitioning solvent was used with Diflubenzuron samples. Subsequently the sample bottle containing the pool water was not shaken and was stored without a partitioning solvent. Pesticide grade ethyl acetate was used as a partitioning solvent for the subsequent runs in the same manner as n-hexane was used for the Methoprene samples. Once the samples were returned to Winnipeg they were stored at -20°C for a period of 5 to 6 months before being analyzed. A storage study showed that samples taken and stored with or without the partitioning solvent were stable during this storage period (Appendix F). The analysis was done by the author using the method of Worobey and Webster (1977a, 1977b) in the Pesticide Research Laboratory, Department of Soil Science, University of Manitoba. A Varian 2440 GLC fitted with a glass column (1.7 m x 2 mm i.d.) with 5% OV 101 on 80/100 mesh Chromosorb W, a 10 ml/min prepurified N_2 gas flow and an ^{3}H electron capture detector was used to perform the analyses. Temperature conditions used were as follows: Injector 179°C, oven 122°C, detector 182°C. The detection

limit of this analysis from pool water was 0.0005 mg/ℓ.

Some Diflubenzuron analyses were performed in the non-linear range of the ^{3}H detector used for the analysis. To correct for this non-linearity in detector response a computer program designed by J. Reimer (Pesticide Research Laboratory, Department of Soil Science, University of Manitoba) was used to determine true water concentrations corresponding to the detector response.

Bioassay Sampling

The bioassay study focused on 1 pool in each treatment group. The pools were randomly chosen within each treatment group as indicated in Table 1.

Bioassay sampling was similar to that of GLC sampling with 5 *ca.* 100 ml samples taken from the locations previously described and poured into a 600 ml glass beaker. An aliquot (100-150 ml) of this composite sample was then poured into a 250 ml sample jar. The jar was then tightly sealed with a bakelite or metal cap and stored (1 to 2 hrs) at ambient temperature until returned to Winnipeg. Three independent replicate samples were taken from each pool in this manner.

Fourth instar *Aedes aegypti* (L.) larvae were used to determine the toxicity of the water samples. Mosquito larvae were chosen as the test organism as they are target organisms for these insecticides and as a result are sensitive to low concentrations. *A. aegypti* were specifically chosen for their ease of culture. Fourth instar larvae were used because they are highly sensitive to Methoprene and Diflubenzuron and show significant though lesser sensitivity to Chlorpyrifos.

Eggs of a 20^{+} year laboratory strain (undetermined origin) of *A. aegypti* were obtained from Dr. R.A. Brust of the Department of Entomology,

University of Manitoba. A culture of this strain supplied sufficient larvae for bioassays. One hundred and fifty to 200 eggs were placed in each of several covered plastic pans (6 x 22 x 30 cm) containing ca. 1 liter of City of Winnipeg tap water. After hatching the larvae were fed a suspension of finely ground liver powder (ICN Pharmaceuticals Ltd., Cleveland, Ohio, U.S.A.).

When water samples were taken 240 fourth instar larvae were removed from the rearing pans using an eye-dropper and placed in styrofoam cups with 20 per cup. All excess water was removed from the cups and 20 larvae were then washed with < 5 ml water into each of the 3 bioassay samples for each treatment. A small amount (< 1 ml) of the ground liver powder suspension was added to each bottle to provide food for the larvae. The number of larvae successfully developing into adults was noted with daily observations. Total detachment of the adult from the pupal exuvium was used as the criterion of successful emergence.

Mortality due to treatment was determined for each treatment and corrected for control mortality using Abbott's Formula (Abbott 1925).

Monitoring Effects on Invertebrates

Colonization of pools by a variety of aquatic organisms including mosquito larvae throughout the fall of 1975 and spring of 1976 resulted in pools similar to mosquito breeding sites. Populations of the aquatic invertebrates were monitored for treatment effects using an aquatic sampling device, emergence traps, and qualitative observations.

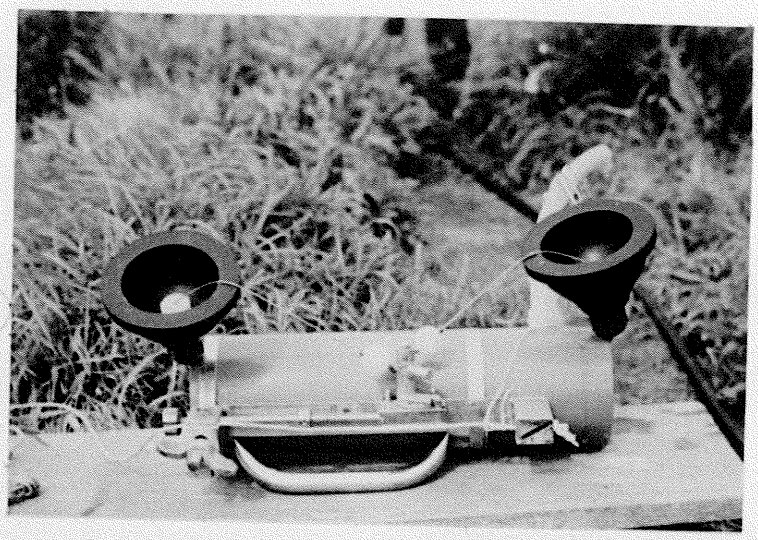
Aquatic Sampling

Populations of aquatic invertebrates in the pools were monitored using 4 modified 2 liter Van Dorne bottles (VDB) obtained from Wildlife

Figure 5. Van Dorne bottle sampling.

a) VDB readied for sampling
b) VDB lowered into pool at selected sampling position
c) Draining VDB

a



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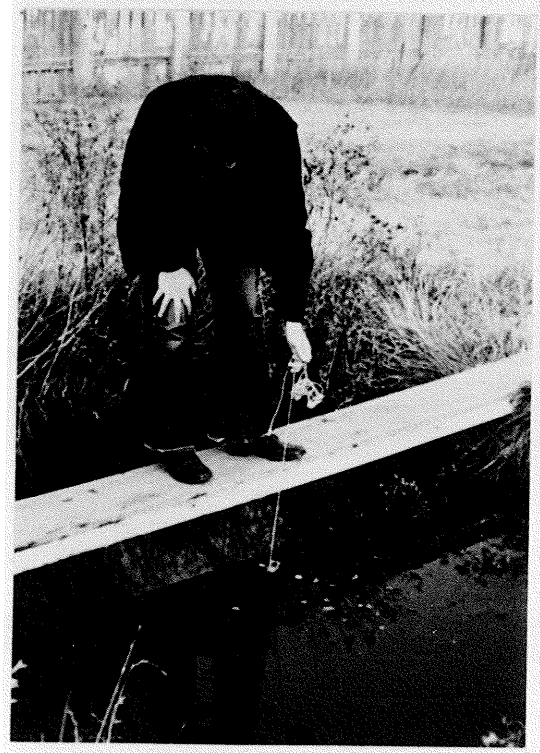

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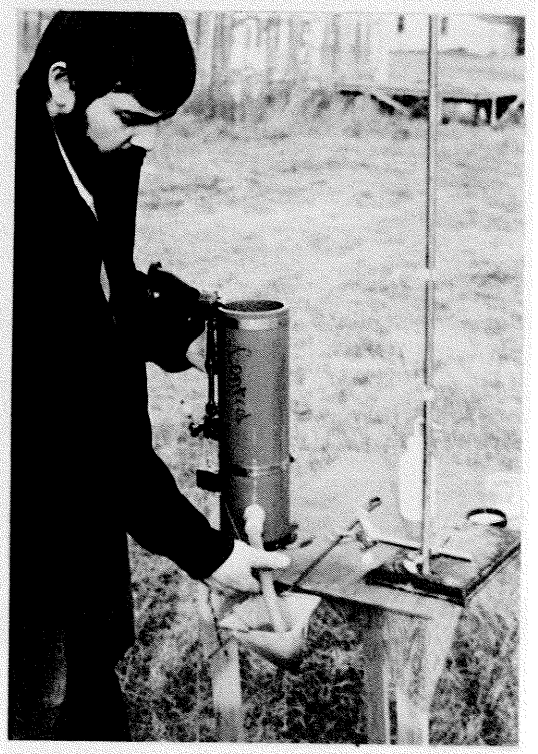


Supply Co., Michigan, U.S.A. (Fig. 5). The original drainage system of the VDB did not allow passage of larger invertebrates from the cylinder and as a result the original cylinders were replaced with a comparable section of PVC 12 cm diameter pipe (2 ℓ volume) which had a 2 cm diameter drainspout located 6 cm from one end. A 16 cm section of 2 cm diameter Tygon tubing was placed on the drain and the flow of water from the VDB regulated with a pinch clamp.

A sampling grid was set out for each pool so that the VDB samples could be taken at random locations within a pool. No sampling positions were located within 75 cm of the pool's frame so that the sampling device would not strike the sloped sides of the pools. Also, no samples could be located within 1.3 m of the south frame board or at positions 8A and 8B as the emergence traps (cf. infra) occupied this area.

Numbered markers from 1 to 8 were placed on the east and west frame boards at 30 cm intervals to provide the north-south portion of the 48-position grid. The east-west markings at 36 cm intervals (A to F) were marked with spray paint on the 2 x 24 cm pine boards which extended across the pools in an east-west direction. These boards were then used as sampling platforms. The VDB sampling positions in each pool used on any specific sampling day were randomly chosen the day before sampling and a sample sheet showing the sampling locations in each pool was drawn up.

Samples were always taken from the south side of the sampling boards, starting at the most northerly sampling position in a pool, and moving south to subsequent sampling locations.

Four VDB's were used, 1 per treatment. The pools were sampled in

3 groups of 4, each group containing 1 pool of each treatment. The pools were sampled in the order shown in Table 2 with 5 samples per pool.

The VDB's were readied prior to sampling by opening their ends and attaching the cable releases to the spring-loaded lock (Fig. 5). The pre-determined sampling location within the first pool was then read from the sampling sheet and the sampling platform was moved so that its south side was at the specified marker on both the east and west frame boards of the pool. The author then walked along the sampling platform to the sampling site and lowered the VDB into the water by its rope until it was completely submerged (Fig. 5). The firing pin at the top end of the bottle was then pressed by hand, releasing the spring-loaded lock and closing the ends of the sampler. The process of lowering the sampler into the water, pressing the firing pin and closing the ends of the sampler required 1 to 2 seconds. The sampler was then removed from the water and given to an assistant for emptying, whereupon the author would obtain the next VDB sample. After each pool had been sampled once, the first pool in the group would be returned to, and the cycle repeated until 5 samples were taken in each of the 4 pools in the first group of 4 treatments. Groups 2 and 3 were then sampled in a similar manner.

To empty the VDB's the top (the end opposite the drain) of the VDB was opened and held open by attaching the release cable to the spring-loaded lock (Fig. 5). The pinch clamp on the Tygon tubing drain would then be opened and the contents of the bottle drained out through a 50 μ mesh Nitex net. This net size was chosen because it retained all organisms to be studied and still allowed rapid drainage of the VDB. After drainage of the sample, 50 to 100 ml of tap water and a brush were

TABLE 2. Van Dorne bottle sampling regime.

Sample Group	Pool			
1	7	6	1	2
2	8	11	10	4
3	3	9	12	5

Pools were sampled from group 1 to group 3 and within groups from left to right.

used to rinse any material remaining out of the VDB. The contents of the net, after the excess water was drained off, were washed into a 250 ml jar using 95 percent ethanol and, if necessary, a small stiff brush. Ethanol was then added to the jar so that it was at least half full. The empty VDB would then be readied for re-use by opening the bottom end of the sampler and setting its release cable. The sample jars were sealed with bakelite or metal lids and stored until their contents could be identified and counted.

Pools were sampled with the VDB's every Monday, Wednesday and Friday from June 18 to August 23, 1976 with the exceptions of August 2nd and 20th, 1976. Sampling usually commenced between 1000 hr and 1030 hr and was always complete by 1230 hr.

Organisms in the jars were identified and counted as outlined below. Jar contents were emptied and rinsed onto a petri dish with a series of parallel lines drawn on the bottom to aid in the counting. A binocular microscope (x 160) was used to count and identify, to as low a taxa as was practical, the organisms in the sample.

Emergence Trap Sampling

Methoprene, and to a lesser extent Diflubenzuron, do not usually result in obvious deleterious effects to insects until they attempt to molt to their adult form. As a result it was considered necessary to employ an emergence trap to determine numbers and types of emerging insects.

Twelve emergence traps, 1 per pool, were constructed as outlined below. Trap frames were of 2 x 2 cm pine boards. North and south faces of the trap were constructed as shown in Fig. 6 and were covered with white nylon drapery lining. The east and west faces of each trap were

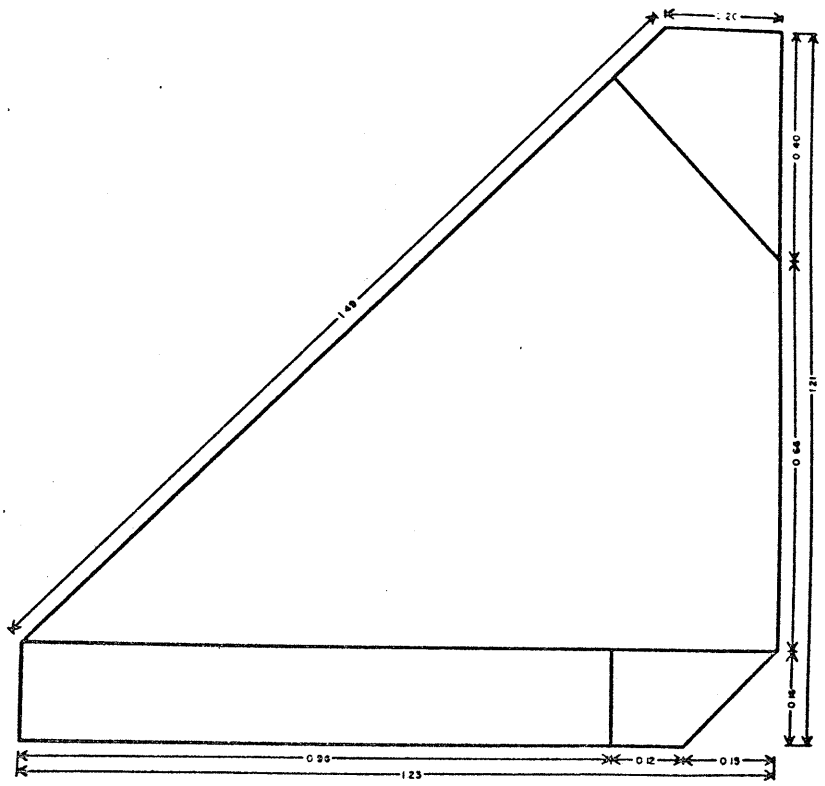


Figure 6. Dimensions of north and south faces of the emergence traps (dimensions in meters).

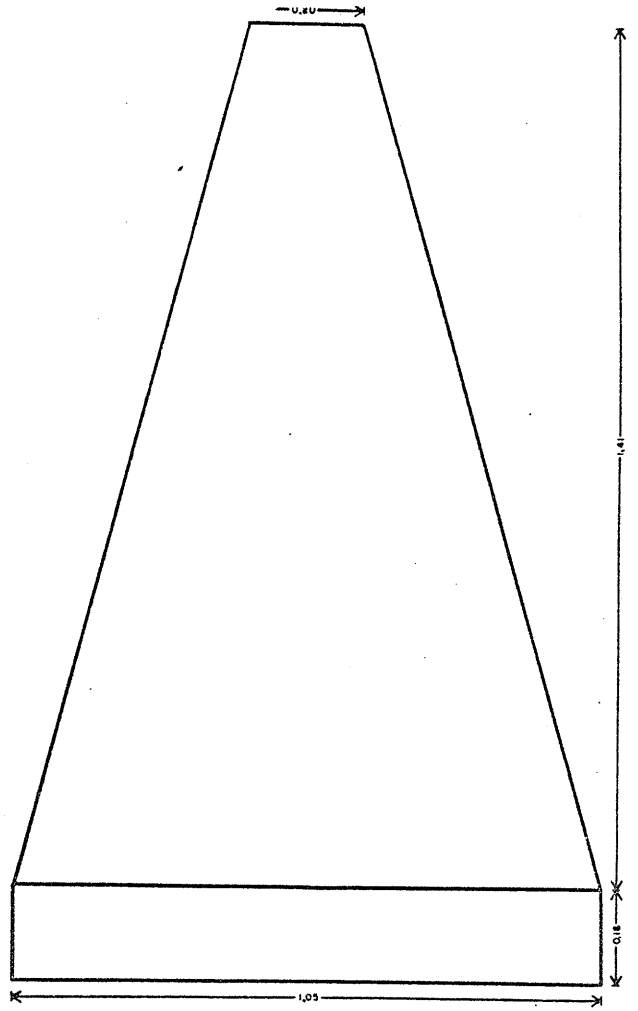
constructed as shown in Fig. 7 and covered with 4 mill (0.1 mm thickness) clear polyethylene. Drapery lining was used on 2 sides (north and south) in order to allow some movement of air through the traps with a subsequent reduction of heat and condensation inside the trap. The polyethylene was used on the other 2 sides (east and west) to allow maximum penetration of light into the traps and to facilitate viewing into the trap's interior, this being necessary for emptying the traps.

The bottom of the trap covered 1 m^2 of water although the actual area varied slightly, due to fluctuations in water level during the experiment. The area under the trap included portions of the pool's flat bottom, its sloping side and a section of the land-water interface. The inclusion of the land-water interface ensured that emergent vegetation would be available to those organisms requiring it or preferring it for their successful emergence. The top of the trap was covered with a 20 x 20 cm piece of 0.5 cm plywood in the centre of which a 9 cm diameter hole was cut. An 11 cm diameter metal lid (the lid fitting a 4 liter glass jar) with an 8 cm diameter hole (corresponding to that in the plywood) was nailed onto the plywood in an inverted position. This arrangement allowed a 4 liter jar to be screwed onto the top of the trap. A polyethylene cone was positioned in the mouth of the jar to funnel insects into the jar, and reduce the chance of insects returning to the main body of the emergence trap. Ideally, insects after emergence would fly upward to the top of the trap, and into the glass jar and be trapped. Not all of the insects emerging under the trap were caught in the glass jar. In order to prevent those insects not entering the trap's top from being included in subsequent days' catches, the main body of the emergence trap was vacuumed out before the removal of the sampling jars. To this

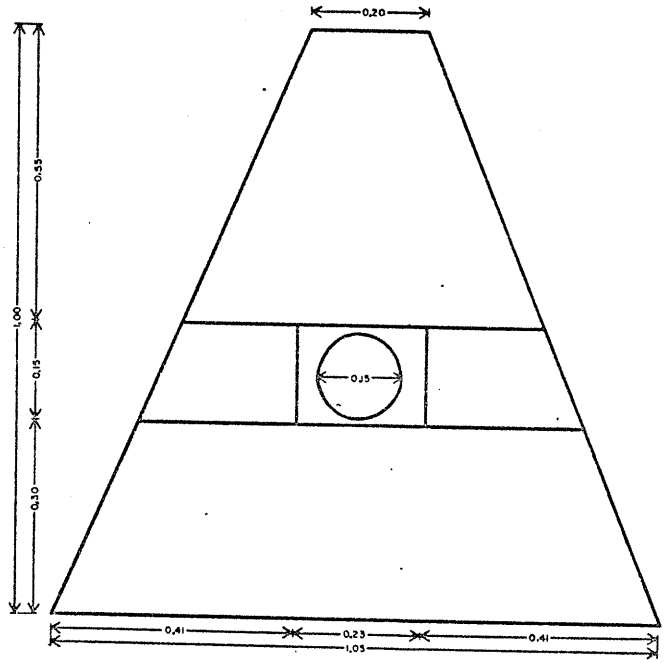
Figure 7. Dimensions of

a) West face of the emergence traps
b) East face of the emergence traps
(dimensions in meters)

a



b



end, an armhole, 12 cm in diameter (Figs. 7 and 8) closed with a cloth sleeve, was placed on the east face (the side abutting the edge of the pool) of the trap. A flexible vacuum nozzle was introduced through the armhole and all the insects in the main body of the trap were vacuumed out using a high-powered vacuum which destroyed them. Despite this, if there were any large insects present in the body of the trap that could not enter into the glass jar, e.g., large tipulids and ephemeropterans, they were removed by hand and included in that pool's emergence trap count. The traps were attached to 2, 4 x 4 cm boards which spanned the pool in an east-west orientation. The traps were located such that the south face of each trap was 75 cm from the south frame board on the east side of each pool.

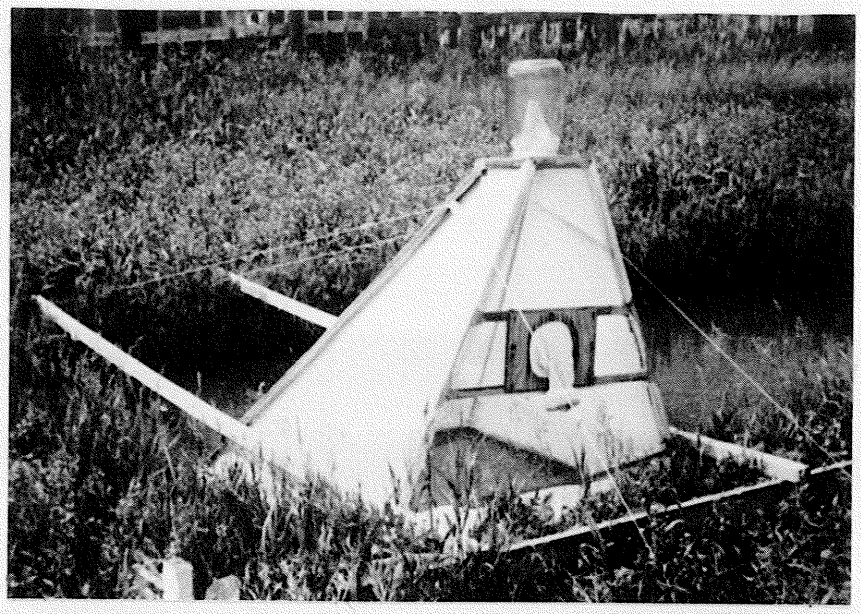
The actual sampling procedure involved first vacuuming out all of the traps, then removing the glass jars at the top of the traps containing the insects. The plastic cones were left in the jars to prevent escape of their contents. Metal lids were screwed onto the top of the jars as soon as they were removed from the trap. Immediately thereafter an empty 4 liter jar fitted with a polyethylene cone was placed on the top of the trap and left there to collect the subsequent days' emergence. The jars with insects were returned to Winnipeg and placed in a freezer at $-20^{\circ}C$ for 24 hours. The dead insects were then gently washed out of the jars onto a 100 μ mesh screen. They were then washed with 95 percent ethanol into labelled 15 ml scintillation vials for storage. The insects were subsequently identified to as low a taxa as was practical and counted in the same manner as described for the VDB samples.

Emergence traps were sampled every Monday, Wednesday and Friday from June 16 to August 23, 1976 with the exception of August 2nd, 1976 between

Figure 8. Emergence trap.

a) North and west faces
b) South and east faces

a



b

1400 hr and 1600 hr.

Qualitative Observations

During the first 2 weeks of sampling, prior to the initial treatment, it became apparent that many of the larger aquatic organisms were not being consistently caught by the VDB's. Some highly mobile aquatic insects sometimes escaped. Organisms such as notonectids, dytiscids, hydrophilids and later instar culicid larvae would react to water movement or shadow and move away from its source. As it was impossible to prevent water movement and shadows during sampling, these organisms were under-represented in the VDB samples. Also, many of the aquatic insects stayed near the sides of the pools where the VDB's could not be used.

In order to circumvent this problem qualitative observations were initiated on July 2,1976 and proceeded on a more or less regular basis until August 8, 1976. Observations could not be made after that date due to a reduction in help available and the need to continue other monitoring procedures.

When qualitative notes were taken, each pool would be observed for 10 to 15 minutes, and all organisms seen in the pool would be noted. These observations were subject to variation due to differences in algal and duckweed cover as well as water turbidity. Meteorological conditions such as incident light could also cause significant variation in ability to observe organisms in the pools. Despite these limitations, it is believed that these observations are of importance, not in view of those organisms apparently absent but, in view of what was observed in each pool.

If dead organisms were found in the pools during the qualitative

observations, they were collected and preserved in 95 percent ethanol for subsequent identification.

Statistical Analysis

GLC and bioassay data are shown as the mean and standard error. Invertebrate population data from VDB's (with the exception of Daphnia sp. and copepods) and adult emergence are shown as the mean total catch for each pool in each treatment group and its standard error. Daphnia sp. and copepod population data are presented as the mean catch for each VDB in each treatment group and its standard error.

The large standard error shown by the population data as well as the low numbers of organisms caught in most taxa made it impossible to perform parametric statistical analyses of the data except those of Daphnia sp. and copepods.

Analyses of variance were performed on both Daphnia sp. and copepod data as follows:

(a) The 12 pools were ranked from lowest to highest productivity using the numbers of organisms (either Daphnia sp. or copepods) for each sampling day before the initial treatment;

(b) Rankets (Sokel 1976) were assigned to each pool according to their numerical rank, and the mean ranket determined for each pool over the pre-treatment period. (Rankets were used to equate pools in terms of populations of Daphnia sp. or copepods based on pre-treatment data. This process results in raising means that are in low productivity pools and lowering those in high productivity pools.);

(c) All population data were then $\log_{10}X+1$ transformed to decorrelate means and variances; and

(d) The transformed data for each pool on each sampling day multiplied by the mean ranket plus 10 for each pool were used as the "raw data" for the analysis of variance. Statistically different means were determined by the Student Kneuman Keul multiple range test.

RESULTS AND DISCUSSION

Disappearance

The residues and bioactivity resulting from the treatments as determined by GLC and bioassay are shown in Tables 3 and 4.

Diflubenzuron

The Diflubenzuron - GLC data for the first treatment show a gradual increase in concentration which peaks at 4 days after treatment. After the second and third treatments, when monitoring was performed within 6 hours of treatment, an initial peak was found in addition to that at 4 days. After the analyses were performed it was determined that the GLC method used, analyzed for and co-chromatographed both the parent compound and its p-chloroaniline metabolite (Worobey and Webster 1977b). As a result it was postulated that the GLC data show an initial peak of the parent compound which very quickly diminishes after which the metabolite concentration increases as hydrolytic degradation proceeds producing the peak at 4 days after treatment. This theory is supported by the work of Schaefer and Dupras (1976) who studied the degradation of Diflubenzuron in pond water. Those authors used HPLC to separate the parent compound and the metabolite and found the degradation curves shown in Fig. 9. If these two curves are added the resulting plot is very similar to the residues of this study.

The theory is also supported by the bioassay data of this study (Table 4) which show no corresponding increase in activity at 4 days

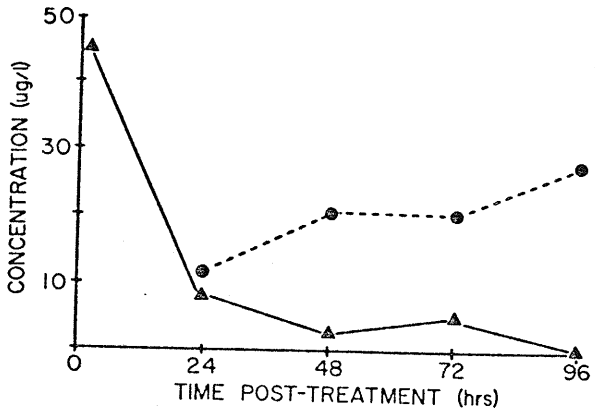


Figure 9. Residues (Diflubenzuron and p-chloroaniline) determined by HPLC resulting from treatment of pond water with Diflubenzuron (Dimilin WP-25) at ca. 0.043 mg/l (Schaefer and Dupras 1976).

Legend: Diflubenzuron ——

p-chloroaniline ------

TABLE 3. Pesticide residues resulting from Diflubenzuron and Methoprene treatment as determined by Gas Liquid Chromatography. Data are expressed as the mean residue of each treatment group in µg/ℓ with detection limits of 0.5 µg/ℓ.

	Date	30-06	03-07	04-07 (+24hr)	05-07	07-07	09-07	12-07	14-07	16-07
	Day of Yr.	(182)	(185)	(186)	(187)	(189)	(191)	(194)	(196)	(198)
Diflubenzuron		0	*	11.9	16.5	17.5	16.9	7.3	7.8	4.4
		(-)***		(1.8)	(3.4)	(2.6)	(0.8)	(1.6)	(0.9)	(0.4)
Methoprene		0	*	2.0	1.2	0	0	ND**	ND	ND
		(-)		(0.6)	(0.6)	(-)	(-)	(-)	(-)	(-)

	Date	22-07	22-07 (+1hr)	22-07 (+6hr)	23-07 (+24hr)	24-07	25-07	26-07	28-07	06-08
	Day of Yr.	(204)	(204)	(204)	(205)	(206)	(207)	(208)	(210)	(219)
Diflubenzuron		*	15.6	9.4	9.4	10.4	13.5	16.0	11.8	7.9
			(2.7)	(1.5)	(0.3)	(1.4)	(1.4)	(1.4)	(1.1)	(0.9)
Methoprene		*	39.1	3.4	0.5	0	0	ND	ND	ND
			(0.5)	(1.5)	(0.2)	(-)	(-)	(-)	(-)	(-)

	Date	10-08	10-08 (+1hr)	11-08 (+24hr)	12-08	13-08	16-08
	Day of Yr.	(223)	(223)	(224)	(225)	(226)	(229)
Diflubenzuron		*	20.3	9.7	13.3	19.3	16.2
			(3.1)	(0.6)	(1.8)	(0.7)	(4.1)
Methoprene		*	28.2	1.1	0	0	ND
			(8.0)	(0.2)	(-)	(-)	(-)

*Indicates pools treated on that date.
**No data.
***Standard error.

TABLE 4. Bioactivity resulting from pesticide treatment as determined by Aedes aegypti bioassay. Data are expressed as the mean percent mortality.

Date (Time) Day of Yr.	23-06 (175)		25-06 (177)		28-06 (180)		30-06 (182)		02-07 (184)		03-07(0hr) (185)	
Control	5 (2.9)***		29 (6.1)		11 (3.6)		1.7 (1.7)		7.0 (1.7)			
Diflubenzuron	17 (9.3)	13****	15 (9.7)	0	34 (7.5)	26	17 (3.3)	16	5.0 (2.9)	0	*	
Methoprene	25 (29)	21	11 (2.0)	0	8 (4.9)	0	0 (-)	0	5.0 (5)	0	*	
Chlorpyrifos	5 (5)	0	19 (13)	0	20 (4.2)	10	8.3 (1.7)	6.7	7.0 (1.7)	0	*	

Date (Time) Day of Yr.	03-07(+1hr) (185)		04-07(+18hr) (186)		04-07(+24hr) (186)		05-07 (187)		07-07 (189)		09-07 (191)	
Control	33 (4.4)		16 (11)		27 (8.4)		15 (7.6)		18 (4.4)		6.7 (6.7)	
Diflubenzuron	100 (0)	100	79 (8.3)	75	98 (1.6)	96	59 (3.3)	31	29 (13)	13	32 (24)	27
Methoprene	100 (0)	100	100 (0)	100	98 (1.6)	96	94 (1.3)	93	87 (8.8)	84	39 (18)	35
Chlorpyrifos	100 (0)	100	100 (0)	100	87 (4.4)	82	88 (4.4)	86	59 (13)	50	35 (7.6)	30

Date (Time) Day of Yr.	12-07 (194)		14-07 (196)		16-07 (198)		19-07 (201)		21-07 (203)		22-07 (204)	23-07 (205)	
Control	5.0 (1.6)		12 (6.7)		5 (2.9)		9.0 (4.4)		3.7 (3.7)			10 (10)	
Diflubenzuron	8.3 (1.6)	3.6	21 (8.3)	10	8.3 (3.3)	3.6	3.3 (1.7)	0	14 (1.9)	11	*	63 (16)	59
Methoprene	6.7 (1.7)	1.2	8.3 (3.3)	0	16 (9.3)	12	5.0 (3.0)	0	6.7 (6.7)	3.1	*	95 (5)	94
Chlorpyrifos	8.3 (3.3)	3.6	25 (5)	15	8.3 (6.0)	3.6	12 (4.4)	3.2	3.3 (3.3)	0		14 (2.2)	4

TABLE 4 . Cont'd.

Date (Time) Day of Yr.	26-07 (208)		28-07 (210)		30-07 (212)		04-08 (217)		06-08 (219)	09-08 (222)	10-08 (223)	10-08(+1hr) (223)	
Control	10 (2)		10 (5.0)		20 (5.0)		25 (12)		ND	ND		7 (1.2)	
Diflubenzuron	36 (18)	29	52 (6.7)	47	13 (6.7)	0	25 (5.4)	0	ND	ND	*	100 (0)	100
Methoprene	52 (24)	47	50 (10)	44	33 (33)	18	15 (9.5)	0	ND	ND	*	100 (0)	100
Chlorpyrifos	ND** (-)		15 (7.3)	6	16 (7.8)	0	35 (12)	13	ND	ND	*	100 (0)	100

Date (Time) Day of Yr.	11-08 (224)		12-08 (225)		13-08 (226)		16-08 (229)		18-08 (231)		
Control	24 (15)		87 (8.9)		12 (4.4)		70 (11)		7.0 (1.7)		
Diflubenzuron	81 (12)	75	93 (31)	46	5 (2.9)	0	41 (21)	0	15 (11)	8.6	
Methoprene	93 (3.5)	91	97 (29)	77	20 (10)	9.0	65 (30)	0	22 (2.7)	16	
Chlorpyrifos	70 (5.2)	61	80 (8.6)	0	23 (18)	13	60 (26)	0	15 (10)	8.6	

*Indicates pools treated at that date.
**No data.
***Standard error.
****Mortality as corrected for control mortality by Abbott's formula.

after the treatments. In fact, mortality decreases substantially during that time. Bioactivity is lost (significant bioactivity is arbitrarily defined as > 25% corrected mortality) from the pools from 3 to 7 days after treatment while residues, that should cause mortality if they reflect solely the Diflubenzuron concentration, are present for greater than 2 weeks after treatment.

Schaefer and Dupras (1976) showed Diflubenzuron was present for 4 days above a detectable limit of 0.001 mg/ℓ when an initial concentration of ca. 0.042 mg/ℓ was present. As the initial concentration in this experiment was 0.0156 and 0.0203 mg/ℓ for the second and third treatments respectively (very close to the theoretical concentration of 0.020 mg/ℓ) the disappearance of Diflubenzuron below 0.001 mg/ℓ could be expected within a similar or shorter time. Toxicity shown by the bioassay up to 7 days after treatment may be due to a reduced degradation rate, toxicity at very low concentrations, toxicity when adsorbed onto organic material ingested by the assay larvae, or the toxicity of a metabolite. Of these possibilities, the most probable are thought to be toxicity at low concentrations and/or toxicity when adsorbed onto organic material.

These data show that Diflubenzuron rapidly disappears from the water of this mosquito breeding habitat with, apparently, a significant degradative pathway the hydrolysis to p-chloroaniline. The residue data also show a relatively slow disappearance rate of the GLC response, again presumably the metabolite though it results in no significant toxicity to mosquito larvae.

Methoprene

GLC and bioassay data for Methoprene also show a rapid disappearance of residues and bioactivity. The GLC data show a reduction of

residues below detectable limits (0.0005 mg/ℓ) after all treatments within 2 to 4 days of treatment. The residues after the second and third treatments (when monitoring was performed within 6 hours of treatment) show a high concentration of Methoprene immediately after treatment in comparison to the expected residues, i.e. 0.0391 and 0.0282 mg/ℓ, vs the expected 0.020 mg/ℓ. This was probably a result of the Altosid SR-10 forming a slick on the surface of the pools visible for 1 to 2 hours after treatment, similar to that described by Schaefer and Dupras (1973). The GLC sampling procedure required the beaker to pass through this slick on the way in and out of the pool, and consequently some of the slick was probably collected in the GLC sample causing the high residues.

Bioassay data show the presence of significant toxicity for up to 8 days after treatment. The longer residual life indicated by the bioassay is probably the result of the factors discussed for the same phenomenon with Diflubenzuron.

Chlorpyrifos

Bioassay data concerning Chlorpyrifos show the presence of toxicity for ca. 6 days after treatment, similar to that reported for Diflubenzuron and Methoprene. However, this is misleading as an indicator of bioactivity as fourth instar larvae are not as sensitive to Chlorpyrifos as they are to the growth regulators. Earlier larval instars are much more sensitive and as a result residues and bioactivity should be expected for longer than the 6 days indicated by bioassay data (cf. Efficacy).

Rawn (1977) using GLC, showed the presence of Chlorpyrifos for 7 days after treatment (detectable limit 0.0005 mg/ℓ) when he applied the

2.5G formulation to pools very similar to those used here (cf. Literature Review). The relatively low residues resulting from Rawn's treatment in comparison to the theoretical application rate (0.004 mg/ℓ vs 0.010 mg/ℓ) were a result of the use of the granular formulation which caused slow release of the active compound and to adsorption of Chlorpyrifos onto organic material. Despite the above data the death of first instar Culex tarsalis larvae in the pools for 2 to 3 weeks after treatment and a lack of adult emergence for 22 days post-treatment, would suggest the presence of Chlorpyrifos in the pools for at least 2.5 weeks after the treatment either at very low concentrations or adsorbed onto organic matter in a toxic form (cf. Efficacy).

From the above data it is apparent that Methoprene and Diflubenzuron disappeared from the study habitat rapidly, within 1 week of treatment while Chlorpyrifos was probably present for up to 2.5 weeks after treatment. Despite the longer life of Chlorpyrifos, none of the insecticides should cause long term residue problems with parent compounds if used properly within registered application rates.

The bioassay data, although not specific for a given chemical, were more easily obtained both in terms of cost and time, and more sensitive than the GLC data, however, GLC data showed less variance than the bioassay data.

Efficacy

Data concerning the ability of the insecticides to control the natural breeding population of Culex tarsalis in the pools are shown in Tables 5 to 7. In general, a substantial population of mosquito larvae and a subsequent large emergence of adults occurred in late June and early July after which numbers of larvae and, to a lesser degree,

TABLE 5. Qualitative observations concerning the effects of the pesticide treatments on immature culicids.

	Date Day of Yr.	02-07 (184)	03-07 (185)	04-07 (186)	06-07 (188)	09-07 (191)	12-07 (194)	15-07 (197)	18-07 (200)
	Pool No.								
Control	4	+		+P	+P	+ER	+	+ER	+
	8	+		+	/	/	+	+	/
	9	+		+	+	+	+	+	/
Diflubenzuron	7	+	*	+P	+ER	/Ⓟ	+ER	+	+
	10	+	*	+	/	+	+ER	+ER	+
	12	+	*	+	+Ⓟ	+	/ER	+	+PER
Methoprene	2	+	*	+P	+Ⓟ	+Ⓟ	+Ⓟ	+Ⓟ ER	+ER
	5	/	*	+	+Ⓟ	+Ⓟ	+P	+	+ER
	11	+	*	+	/	/	+ER	+	+
Chlorpyrifos	3	+	*	-P	/	/	+ER	/ER	/ER
	4	/	*	/ER	/ER	/	+ER	/ER	+ER
	6	/	*	/	/ER	/ER	/ER	/ER	/ER

TABLE 5. Cont'd.

	Date Day of Yr.	22-07 (204)	23-07 (205)	26-07 (208)	29-07 (211)	01-08 (214)	04-08 (217)	10-08 (223)	12-08 (225)	14-08 (227)
	Pool No.									
Control	1		+P	ND	+	/	/		/	/
	8		/	ND	P	/	P		/	/
	9		/	ND	ND	/	/		/	/
Diflubenzuron	7	*	+⊕	ND	+	+	+	*	+	+
	10	*	+ER	+	/	+	+	*	/	ND
	12	*	+	/	/	/	/	*	/	/
Methoprene	2	*	+	/	+	+	+P	*	/ER	ER
	5	*	+Ⓟ	/	+ER	+	+	*	+ER	ND
	11	*	/ER	/	/	P	/	*	/	ND
Chlorpyrifos	3		/	/	/ER	/	/	*	/	/
	4		/	ND	/	/	/	*	/	ND
	6		/	ND	/	/	/	*	/	ND

Observations are indicated by: + Larvae Present Alive, Normal; ⊕ Larvae Present Alive, Abnormal; / No Larvae or Pupae Observed; ND No Data; P Pupae Present Normal Alive, Ⓟ Pupae Present Abnormal Alive; ER Egg Rafts Present.

*Indicates pools treated at that date.

TABLE 6. The effect of the pesticide treatments on populations of culicid larvae as determined by Van Dorne bottle sampling. Data are expressed as the mean catch per pool for each treatment group.

Date	16-06	18-06	21-06	23-06	25-06	28-06	30-06	02-07	03-07	05-07	07-07	09-07
Day of Yr.	(168)	(170)	(173)	(175)	(177)	(180)	(182)	(184)	(185)	(187)	(189)	(191)
Control	ND**	0	0	1.7	0	0.3	3	2.3		9	2.3	2.3
		(-)***	(-)	(0.3)	(-)	(0.3)	(2.5)	(1.5)		(5.7)	(0.9)	(0.3)
Diflubenzuron	ND	0.3	0	5.3	0	1	1.7	3	*	1.7	0	1
		(0.3)	(-)	(3.4)	(-)	(0)	(1.7)	(3)		(0.9)	(-)	(0.6)
Methoprene	ND	0	0.6	1.3	0.3	1	1.3	6.7	*	12	7.3	2.7
		(-)	(0.6)	(0.6)	(0.3)	(1)	(0.9)	(5.7)		(9.2)	(5.4)	(2.2)
Chlorpyrifos	ND	0.3	0.3	1.7	0.3	0.6	1.3	2.3	*	0	0	0
		(0.3)	(0.3)	(1.2)	(0.3)	(0.6)	(0.9)	(0.9)		(-)	(-)	(-)

Date	12-07	14-07	16-07	19-07	21-07	22-07	23-07	26-07	28-07	30-07	04-08	06-08
Day of Yr.	(194)	(196)	(198)	(201)	(203)	(204)	(205)	(208)	(210)	(212)	(217)	(219)
Control	1.3	0	0	0	0		0	0	0.3	0	0	0
	(0.3)	(-)	(-)	(-)	(-)		(-)	(-)	(0.3)	(-)	(-)	(-)
Diflubenzuron	0.6	3	0	0	0.3	*	0	0.3	0	0.3	0.3	0
	(0.3)	(1.5)	(-)	(-)	(0.3)		(-)	(0.3)	(-)	(0.3)	(0.3)	(-)
Methoprene	2.3	2	0.3	1	0.3	*	1	0	0	0.3	0	0
	(0.7)	(1.5)	(0.3)	(1)	(0.3)		(1)	(-)	(-)	(0.3)	(-)	(-)
Chlorpyrifos	0	0	0	0	0		0	0	0	0	0	0
	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(-)	(-)	(-)	(-)

Date	09-08	10-08	11-08	13-08	16-08	18-08	20-08	23-08
Day of Yr.	(222)	(223)	(224)	(226)	(229)	(231)	(233)	(236)
Control	0		0	0	0	0	ND	0
	(-)		(-)	(-)	(-)	(-)		(-)
Diflubenzuron	0	*	0	0	0	0	ND	0
	(-)		(-)	(-)	(-)	(-)		(-)
Methoprene	0.3	*	0.3	0.3	0	0.6	ND	0
	(0.3)		(0.3)	(0.3)	(-)	(0.3)		(-)
Chlorpyrifos	0	*	0	0	0	0	ND	0
	(-)		(-)	(-)	(-)	(-)		(-)

*Indicates pools treated at that date. *** Standard error.
**No data.

TABLE 7. The effect of the pesticide treatments on adult emergence of culicids as determined by emergence trap sampling. Data are expressed as the mean catch per pool for each treatment group.

Date	16-06	18-06	21-06	23-06	25-06	28-06	30-06	02-07	03-07	05-07	07-07	09-07
Day of Yr.	(168)	(170)	(173)	(175)	(177)	(180)	(182)	(184)	(185)	(187)	(189)	(191)
Control	0	0.6	0	0	0	1.1	9.3	13		34	30	53
	(-)**	(0.6)	(-)	(-)	(-)	(0.7)	(7.3)	(10.6)		(15.7)	(23)	(13)
Diflubenzuron	0.6	0.6	1.3	1	2	63.3	176	54.7	*	235	29	21
	(0.3)	(0.6)	(0.9)	(1)	(1.2)	(60.3)	(151)	(41.7)		(269)	(21)	(15)
Methoprene	1.6	1.3	0.6	0	0	2	9.3	11.7	*	35	37	8.0
	(1.6)	(1.3)	(0.3)	(-)	(-)	(0.6)	(5.5)	(9.2)		(21)	(19)	(4.4)
Chlorpyrifos	0	2	0.2	0.3	0.3	0.3	6.3	2.6	*	7.7	1.3	0
	(-)	(1.2)	(0.3)	(0.3)	(0.3)	(0.3)	(2)	(1.5)		(5.0)	(0.9)	(-)

Date	12-07	14-07	16-07	19-07	21-07	22-07	23-07	26-07	28-07	30-07	04-08	06-08
Day of Yr.	(194)	(196)	(198)	(201)	(203)	(204)	(205)	(208)	(210)	(212)	(217)	(219)
Control	197	25	3.7	5	2.3		1.3	3.2	3.0	2.3	7.7	0.6
	(87)	(18)	(1.2)	(2.5)	(1.2)		(0.9)	(1.6)	(2.1)	(1.2)	(7.2)	(0.6)
Diflubenzuron	3.7	0	0	5.7	13.3	*	5.7	26	2	1.2	0	0
	(3.7)	(-)	(-)	(1.7)	(2.0)		(2.9)	(13)	(1)	(1.2)	(-)	(-)
Methoprene	1.0	0	0	1.7	1.3	*	3.3	16.7	3.3	1	3.7	3.3
	(0.6)	(-)	(-)	(0.9)	(1.3)		(1.8)	(5.9)	(1.9)	(1)	(2.3)	(1.5)
Chlorpyrifos	0	0	0	0	0		0	0	0	0	0.6	0.3
	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(-)	(-)	(0.6)	(0.3)

Date	09-08	10-08	11-08	13-08	16-08	18-08	20-08	23-08
Day of Yr.	(222)	(223)	(224)	(226)	(229)	(231)	(233)	(236)
Control	0.6		0	0.3	0	0.6	0.3	4.6
	(0.3)		(-)	(0.3)	(-)	(0.6)	(0.3)	(1.9)
Diflubenzuron	15	*	4.7	2	10	0.3	0	0
	(13)		(4.2)	(1.5)	(6.1)	(0.3)	(-)	(-)
Methoprene	16	*	15	5	0.3	0.3	1.6	1.6
	(8.5)		(9.2)	(3.6)	(0.3)	(0.3)	(1.6)	(1.6)
Chlorpyrifos	0	*	0	0	0	0	0	0
	(-)		(-)	(-)	(-)	(-)	(-)	(-)

*Indicates pools treated on that date.
**Standard error.

adult emergence dropped in both control and treated pools. As a result it is difficult to discern treatment effects on larvae after the second and third treatments although adult emergence was still useful. As a result of the low efficiency of VDB's (low catch and a relatively large amount of time required to obtain the data) and mode of action of the growth regulators emergence monitoring was considered the most effective manner of determining efficacy. VDB data did however show how the larvae/pupae were affected (change in numbers or composition of immature stages).

Diflubenzuron

It is apparent from VDB and qualitative data that Diflubenzuron showed no dramatic effect on numbers of larvae present even though abnormal larvae were found after treatment. There was an alternation of composition of the larvae present after 2 days post-treatment. Before this time, larvae in the pools ranged from first to fourth instar, but from 2 to 6 days post-treatment only first instar larvae were apparent. After 6 days post-treatment later larval stages were found to be present. This is expected because (a) Diflubenzuron caused the death (after molting) of all the larvae present in the pools at the time of treatment, and (b) eggs hatching to first instar larvae that for several days were also killed when they attempted to molt. After 5 to 6 days the first instars could develop normally as the Diflubenzuron had disappeared (cf. Disappearance).

No effect was shown on adult emergence for 3 or 4 days after each treatment with any of the chemicals studied. This is due to the low toxicity of these compounds to pupae as discussed in the literature review. As it takes 3 or 4 days for all of the pupae in the pools at

the time of treatment to complete development and emerge as adults, treatment effects are not apparent until 4 or more days post-treatment. After this time emergence would be expected to immediately drop to zero.

Emergence was reduced in Diflubenzuron pools but it took up to 10 days post-treatment to drop to zero. The reason for this gradual drop may be due to a sublethal effect of Diflubenzuron causing an increase in development time of pupae such that it would take as long as 9 days for pupal development. Increase in development time has been reported after Diflubenzuron treatment but 9 days is a very long development period, especially for the resulting adult to be able to successfully emerge and fly to the top of the trap. Another possible explanation is that all of the adults in the main body of the trap were not removed by vacuuming, resulting in "carry-over" to the next sampling day. This is unlikely due to the care with which the vacuuming was done and the fact that this gradual drop in emergence is not seen with Chlorpyrifos pools (cf. infra). Recovery to control emergence rates (first and second treatments) occurred at 16 and 18 days after treatment respectively. Recovery after the third treatment did not occur before the end of the experiment 13 days after the treatment. This recovery rate corroborated the residue data by showing a loss of toxic material from the pools within 6 to 8 days of treatment with a subsequent immature development time of ca. 10 days.

The duration of time required to lower the emergence rate to zero, and the relatively short time emergence was reduced to zero (6 to 8 days) indicate a relatively low degree of efficacy for Diflubenzuron against Culex tarsalis. Efficacy under operational control conditions would be higher as emergence of adults would not be allowed

to occur prior to the initiation of treatment and recovery to normal emergence rates would then take up to 18 days after treatment.

Methoprene

The efficacy of Methoprene is similar to that of Diflubenzuron. No effects were apparent on numbers or composition of larvae as monitored by VDB and qualitative observations although abnormal pupae were found from 2 to 9 days after treatment.

As mentioned above, no effect was shown on adult emergence for 3 to 4 days after treatment (cf. supra). It also took up to 10 days post-treatment for emergence to drop to zero and it was only reduced to zero after the first treatment. The slow drop may be explained by the same factors mentioned for Diflubenzuron or in addition, may be due to the low efficacy shown by Methoprene against Culex sp. Recovery to control emergence rates occurred from 10 to 16 days after treatment supporting the GLC and bioassay data indicating rapid disappearance of Methoprene (within 2 to 6 days of treatment, with an immature development period of 8 to 10 days). The slightly shorter recovery time shown here in comparison to Diflubenzuron, and the fact that emergence did not drop to zero after the second and third treatments may be due to the specificity of Methoprene to the later larval stages. First or second instar larvae present in the pools at the time of treatment may successfully develop to emerge as adults, resulting in a shorter period of control.

The time required to reduce emergence to zero, the fact that it was reduced to zero after only one treatment, and the fast recovery to normal emergence rates all combine to indicate a low efficacy of Methoprene to Culex tarsalis. As mentioned with Diflubenzuron, efficacy would be higher in an actual operational control program, as the initial

adult emergence would not be allowed and as a result reduced emergence could be expected for up to 16 days after treatment.

A further problem with Methoprene, and to a lesser extent with Diflubenzuron, from an operational control point of view, is the fact that one cannot be certain that control has been achieved until it is too late to prevent adult emergence (cf. infra).

Chlorpyrifos

VDB data concerning Chlorpyrifos shows no larvae present after the initial treatment. Qualitative notes indicate the presence of some larvae after the treatment although these were not present in sufficient numbers to be caught in the VDB's. No larvae were seen before 7 days post-treatment and all those seen from 7 to 16 days after treatment were first instar larvae. Thereafter later stage larvae were observed. This would indicate Chlorpyrifos acted by killing all the larvae present in the pools at the time of treatment and showed residual ovicidal activity for up to 7 days after treatment. Between 7 and 16 days the residues had dropped as to allow hatching of eggs, but caused death of first instar larvae. After 16 days residues had dropped such that larvae could survive and emerge.

Emergence data show no effect due to treatment for 3 to 4 days after treatment due to resistant pupae (cf. supra). Thereafter emergence immediately dropped to zero until 22 days after treatment indicating the presence of Chlorpyrifos in the pools for between 12 and 14 days with a ca. 10 day immature developmental period.

In an actual control program reduced emergence would be expected for up to 22 days after treatment which is 4 to 6 days longer than Diflubenzuron and 6 to 12 days longer than Methoprene. Thus Chlorpy-

rifos is the most efficient of the three for the control of Culex tarsalis followed by Diflubenzuron and Methoprene respectively. It is also more practical to use Chlorpyrifos as efficacy can be determined simply by returning to a sprayed area one day after application to see if live larvae are present.

Effects on Non-Target Invertebrate Fauna

Chironomidae

Data concerning treatment effects on chironomid larval populations, and adult emergence are shown in Tables 8 to 10. The data shown in Table 9 obtained from VDB samples should be considered more qualitatively than quantitatively as the number of chironomid larvae present in a sample was to some degree dependent on how the sample was taken; i.e., if the VDB touched the bottom of the pool during sampling more chironomid larvae were caught than usual. Monitoring adult emergence was therefore considered the more efficient means of determining effects on this group.

Diflubenzuron caused no detectable effect on chironomid larvae in terms of numbers or composition though dead larvae were noted after treatment.

It is difficult to determine if there was a significant reduction in numbers of chironomid larvae present in the pools, though the facts that (a) Diflubenzuron causes death of chironomid larvae at the application rate used in the experiment (Miura and Takahashi 1975), (b) dead larvae were found, and (c) adult emergence was substantially reduced (Table 10) would suggest that a significant reduction may have occurred.

Adult emergence was not reduced until 4 days after the initial

TABLE 8. Qualitative observations concerning the effect of the pesticide treatments on immature chironomids.

	Date Day of Yr.	02-07 (184)	03-07 (185)	04-07 (186)	06-07 (188)	09-07 (191)	12-07 (194)	15-07 (197)	18-07 (200)
	Pool No.								
Control	4	/		/	+	/	/	/	+P
	8	/		/	/	/	/	/	/
	9	/		/	/	/	/	/	+P
Diflubenzuron	7	+	*	+	+	/	/	/	/
	10	/	*	/	/	/	+	/	/
	12	/	*	/	P-	/	/	/	/
Methoprene	2	/	*	/	+	/	-	+	/
	5	/	*	/	+Ⓟ	Ⓟ	-	/	/
	11	/	*	/	/	/	+	/	+
Chlorpyrifos	3	+	*	-P-	-P-	-	-	/	/
	4	P	*	-P-	-P-	-	-	+	/
	6	P	*	-P-	-P-	/	/	/	+

TABLE 8. Cont'd.

	Date Day of Yr.	22-07 (204)	23-07 (205)	26-07 (208)	29-07 (211)	01-08 (214)	04-08 (217)	10-08 (223)	12-08 (225)	14-08 (227)
	Pool No.									
Control	1		+P	ND	+P	/	/		P	/
	8		/	ND	P	/	/		P	/
	9		/	ND	ND	/	/		+	/
Diflubenzuron	7	*	/	ND	/	/	/	*	-+	-
	10	*	/	/	/	/	/	*	/	ND
	12	*	/	/	/	/	/	*	/	/
Methoprene	2	*	/	/	/	/	/	*	P	ND
	5	*	/	-	-Ⓟ	-	+	*	-+P	ND
	11	*	/	-	/	/	/	*	-	-
Chlorpyrifos	3		/	/	/	P	P	*	P	/
	4		/	ND	/	/	P	*	P	ND
	6		/	ND	/	P	P	*	/	ND

Observations are indicated by: + Larvae Present Alive, - Larvae Present Dead, / No Larvae or Pupae Observed, ND No Data, P Pupae Present Normal Alive, Ⓟ Pupae Present Abnormal Alive, P- Pupae Present Dead.
*Indicates pools treated at that date.

TABLE 9. The effect of the pesticide treatments on populations of chironomid larvae as determined by Van Dorne bottle sampling. Data are expressed as the mean catch per pool for each treatment group.

Date	16-06	18-06	21-06	23-06	25-06	28-06	30-06	02-07	03-07	05-07	07-07	09-07
Day of Yr.	(168)	(170)	(173)	(175)	(177)	(180)	(182)	(184)	(185)	(187)	(189)	(191)
Control	ND**	2.3	0.3	1.6	0.7	0.7	19	4		13	7.7	3
		(2.3)***	(0.3)	(0.9)	(0.3)	(0.7)	(15)	(0.6)		(5.5)	(5.1)	(1.5)
Diflubenzuron	ND	0.3	4.3	2	0	0.3	1.7	2.3	*	8.7	9.3	11.7
		(0.3)	(2.3)	(0.6)	(-)	(0.3)	(1.2)	(0.8)		(4.3)	(4.1)	(0.3)
Methoprene	ND	1	0	1	0.3	0.3	0.3	3.7	*	1.3	5	6.7
		(1)	(-)	(1)	(0.3)	(0.3)	(0.3)	(2.2)		(0.9)	(4.1)	(6.7)
Chlorpyrifos	ND	0	0.7	2	0.3	1.3	0.6	0.7	*	0	1.3	0.6
		(-)	(0.3)	(1.5)	(0.3)	(0.9)	(0.6)	(0.3)		(-)	(0.3)	(0.3)

Date	12-07	14-07	16-07	19-07	21-07	22-07	23-07	26-07	28-07	30-07	04-08	06-08
Day of Yr.	(194)	(196)	(198)	(201)	(203)	(204)	(205)	(208)	(210)	(212)	(217)	(219)
Control	8	4.7	3	2.3	4.3		2.6	1.5	2.7	2	3.3	5.3
	(3)	(1.9)	(2.5)	(1.5)	(1.8)		(2.6)	(0.6)	(2.7)	(1.5)	(1.9)	(4.8)
Diflubenzuron	5.3	10	9.7	6.7	6.1	*	7.7	2.8	14	10	11	9
	(0.7)	(5.3)	(6.1)	(4.7)	(1.7)		(7.7)	(1.1)	(4.9)	(5.3)	(7)	(2.3)
Methoprene	0	14.3	2	2.7	12.3	*	2.8	4	7.3	5.7	0	4.7
	(-)	(14.3)	(2)	(0.9)	(10.9)		(1.5)	(2.3)	(7.3)	(4.2)	(-)	(2.4)
Chlorpyrifos	1.3	7.3	5.7	0.3	0.3		1.3	1	1	0.7	8.7	13
	(0.7)	(6.4)	(5.2)	(0.3)	(0.3)		(1.3)	(0)	(0.6)	(0.3)	(7.2)	(7.6)

Date	09-08	10-08	11-08	13-08	16-08	18-08	20-08	23-08
Day of Yr.	(222)	(223)	(224)	(226)	(229)	(231)	(233)	(236)
Control	2.3		4	4	3	7.6	ND	1.7
	(1.9)		(2.7)	(1.5)	(3)	(5.2)		(0.7)
Diflubenzuron	11	*	11.7	9	5	13	ND	2
	(5.1)		(7.7)	(6.0)	(5)	(2.9)		(1.2)
Methoprene	3.7	*	6.7	5	4	8.3	ND	2.3
	(0.9)		(4.1)	(3.1)	(3.1)	(4.1)		(2.3)
Chlorpyrifos	4.7	*	1.7	0.3	0.7	1.3	ND	1.7
	(3.2)		(1.2)	(0.3)	(0.3)	(0.9)		(1.7)

*Indicates pools treated at that date.
**No data.
***Standard error.

TABLE 10. The effect of the pesticide treatments on adult emergence of chironomids as determined by emergence trap sampling. Data are expressed as the mean catch per pool for each treatment group.

Date	16-06	18-06	21-06	23-06	25-06	28-06	30-06	02-07	03-07	05-07	07-07	09-07
Day of Yr.	(168)	(170)	(173)	(175)	(177)	(180)	(182)	(184)	(185)	(187)	(189)	(191)
Control	234	345	520	856	272	273	308	257		201	42	22
	(109)**	(135)	(127)	(429)	(79)	(47)	(76)	(14)		(17)	(9)	(7)
Diflubenzuron	277	424	907	802	438	363	179	120	*	105	2	2.6
	(85)	(128)	(252)	(296)	(163)	(175)	(69)	(90)		(71)	(2)	(1.2)
Methoprene	34	59	140	131	58	82	72	51	*	55	7	1.6
	(20)	(47)	(113)	(111)	(46)	(48)	(63)	(41)		(42)	(3)	(1.2)
Chlorpyrifos	67	148	413	303	193	97	114	114	*	45	1.3	1
	(30)	(99)	(200)	(107)	(49)	(63)	(28)	(28)		(16)	(0.6)	(0.6)

Date	12-07	14-07	16-07	19-07	21-07	22-07	23-07	26-07	28-07	30-07	04-08	06-08
Day of Yr.	(194)	(196)	(198)	(201)	(203)	(204)	(205)	(208)	(210)	(212)	(217)	(219)
Control	6	6.6	3	16.3	6.7		12	39.6	26	15	117	54
	(2.6)	(1.8)	(0.6)	(2.3)	(1.3)		(2)	(4.6)	(10)	(6.7)	(68)	(29)
Diflubenzuron	2	1.3	2.3	4.6	1.3	*	2.3	2	1.6	6	26.3	12
	(2)	(0.7)	(1.2)	(0.7)	(0.8)		(1.9)	(1.2)	(0.7)	(1)	(8.0)	(2)
Methoprene	0.3	1	2	14	6.3	*	10.5	65	46	72.3	242	26.6
	(0.3)	(1)	(2)	(13)	(4.4)		(9.5)	(64)	(43)	(70)	(239)	(23)
Chlorpyrifos	0.3	1.3	0	4.3	1		0.3	5	2.3	4	17	5
	(0.3)	(0.9)	(-)	(2.3)	(1)		(0.3)	(4)	(0.3)	(1.5)	(4.6)	(2.0)

Date	09-08	10-08	11-08	13-08	16-08	18-08	20-08	23-08
Day of Yr.	(222)	(223)	(224)	(226)	(229)	(231)	(233)	(236)
Control	167		46.3	41.3	75	112	119	229
	(55)		(5.5)	(20)	(49.1)	(48)	(36)	(75)
Diflubenzuron	20.3	*	5	3.3	0.7	0.3	1	2.3
	(2.6)		(1.5)	(0.8)	(0.7)	(0.3)	(1)	(0.3)
Methoprene	47.6	*	10	4	0.3	4.3	2	7
	(33)		(3.6)	(2.5)	(0.3)	(0.9)	(1.5)	(4.5)
Chlorpyrifos	18	*	11	4.3	4	1.7	2	1.7
	(5.7)		(4.2)	(1.2)	(3.5)	(1.2)	(2)	(1.7)

*Indicates pools treated at that date.
**Standard error.

treatment due to the low sensitivity shown by pupae (cf. Efficacy). Thereafter adult emergence was reduced in comparison to the controls for the rest of the experiment. This may have been caused by the slower development rate of chironomids and/or a higher sensitivity to Diflubenzuron in comparison to culicids; i.e., if a large proportion of all chironomid larval instars is affected with each treatment and the developmental rate is slow, the effect on the adult emergence rate is a prolonged reduction. The fact that emergence was not reduced to zero may indicate a lower sensitivity than mosquitoes or a lack of penetration of Diflubenzuron throughout the substrate to all of the chironomid larvae.

Methoprene also showed no detectable effect on numbers or composition of chironomid larvae caught though dead larvae were observed after treatment. Abnormal pupae were observed from 3 to 7 days after treatment. Again it is difficult to determine treatment effects on numbers of chironomid larvae though the following arguments suggest that any effects were small: relatively few dead larvae were observed (in comparison to Diflubenzuron or Chlorpyrifos); no effect was noted on adult emergence after the second treatment; and there is little evidence in the literature to indicate reductions in numbers of chironomid larvae at the application rate used in this study. Adult emergence was reduced from 4 up to 13 days after the first and third treatments though no effect is apparent after the second treatment. The delay in emergence reduction of 4 days is expected due to low sensitivity of pupae while the resumption of normal emergence within 13 days of treatment with Methoprene but not with Diflubenzuron may be due to Methoprene's specificity to the later larval instars. The

fact that emergence was not deleteriously affected and in fact was substantially greater than the control emergence after the second treatment may be due to genus/species-specific resistance to Methoprene similar to that described by Mulla et al. (1974). Identification of chironomid adults below family was impractical though most chironomids emerging from July 19 to August 6 were visibly different (smaller and different colour) than those emerging before or after that date. The fact that emergence was greater at this time than in the controls may not be a treatment effect though Steelman et al. (1975) has reported an increase in chironomid larval numbers after Methoprene treatment which they attribute to a reduction in predators. No detectable reduction in predators is shown by the data presented herein (cf. infra) and as a result it is impossible to definitely attribute this apparent increase to Methoprene treatment.

Chlorpyrifos did cause a reduction in numbers of larvae caught in the pools for up to 32 days after treatment though larvae were almost always caught. Dead larvae were observed for up to 9 days after treatment. Adult emergence was reduced from 4 days after initial treatment (due to resistant pupae) until the end of the experiment. Emergence did not return to control levels but did recover to some extent 32 days after the first treatment though it was reduced again by the second treatment.

These data show a major reduction in numbers of larvae and adult emergence though, with the exception of 1 day, neither parameter was reduced to zero. This latter observation may be due to resistance of some individuals to Chlorpyrifos or lack of penetration of the insecticide throughout the substrate to all of the chironomid larvae.

Chlorpyrifos caused the most significant reduction in numbers of larvae and adult emergence of the three insecticides. Diflubenzuron may have caused a reduction in larval numbers but did not cause a reduction of adult emergence compared to that of Chlorpyrifos. Methoprene may have caused a slight reduction in larval numbers and caused the least reduction in adult emergence.

Chaoboridae

Data concerning the effects of the treatments on chaoborid larval populations and adult emergence are shown in Tables 11 and 12. General trends in populations show a steady increase in numbers of larvae throughout the experiment although adult emergence was generally low throughout the experiment. Emergence and larval monitoring were of equal value in determining the effects on chaoborids though low adult emergence in controls may have masked treatment effects.

Diflubenzuron caused no detectable reduction in numbers of larvae in comparison to the controls though dead chaoborid larvae were apparent from 4 to 6 days after treatment. A change in composition of larvae was present similar to that reported for culicids, i.e. older instars were not present from 4 to 8 days after treatment (cf. Efficacy).

Six days after the initial treatment emergence dropped to zero in both control and Diflubenzuron-treated pools. This would be the same time that a treatment-related decrease in emergence would be expected. Similar reductions in emergence in both control and Diflubenzuron pools are evident after the other 2 treatments and as a result treatment-related reductions may be present, shown by the larval composition change and the observation of dead larvae after

TABLE 11. The effect of the pesticide treatments on populations of chaoborid larvae as determined by Van Dorne bottle sampling. Data are expressed as the mean catch per pool for each treatment group.

Date	16-06	18-06	21-06	23-06	25-06	28-06	30-06	02-07	03-07	05-07	07-07	09-07
Day of Yr.	(168)	(170)	(173)	(175)	(177)	(180)	(182)	(184)	(185)	(187)	(189)	(191)
Control	ND**	2	1.6	2.3	1.7	5	5	2.7		4.6	1	1.3
		(1.2)***	(0.9)	(0.7)	(1.2)	(0.6)	(4.5)	(0.9)		(2.7)	(1)	(0.3)
Diflubenzuron	ND	5.7	11	22	3.3	5.3	7.3	6.3	*	4	1.7	1
		(5.2)	(10.5)	(14.8)	(2.4)	(4.3)	(7.3)	(3.8)		(3)	(1.2)	(0.6)
Methoprene	ND	0.3	1	1.7	2.6	3	2.7	5.3	*	3.3	4	4
		(0.3)	(1)	(1.2)	(2.2)	(0.6)	(1.7)	(3.4)		(1.9)	(2)	(3)
Chlorpyrifos	ND	1.3	1.3	12.7	11	7.3	5.7	10.7	*	1.3	0	0
		(1.3)	(1.3)	(7.5)	(11)	(3.5)	(4.7)	(5.5)		(0.9)	(-)	(-)

Date	12-07	14-07	16-07	19-07	21-07	22-07	23-07	26-07	28-07	30-07	04-08	06-08
Day of Yr.	(194)	(196)	(198)	(201)	(203)	(204)	(205)	(208)	(210)	(212)	(217)	(219)
Control	0.6	2	2.3	4.3	4.7		2	2.7	4.3	4.6	4.3	4
	(0.6)	(1.2)	(0.9)	(2.3)	(3.7)		(2)	(1.4)	(2.8)	(2.0)	(1.8)	(1.5)
Diflubenzuron	0.6	1.3	2.7	2.7	8	*	0	0.7	6.3	6.1	10.3	36
	(0.6)	(0.6)	(1.5)	(0.7)	(4)		(-)	(0.7)	(4.5)	(3.2)	(7.4)	(11)
Methoprene	2	2.7	3	7.3	6.7	*	3	6	7.3	8.5	20	39.7
	(2)	(1.5)	(1)	(3.8)	(3.3)		(1.7)	(3.9)	(2.4)	(3.4)	(11.2)	(28.7)
Chlorpyrifos	0.3	0.3	0.7	1.3	0.3		0.3	9.3	1.7	1.3	42.7	29
	(0.3)	(0.3)	(0.3)	(0.9)	(0.3)		(0.3)	(5.9)	(0.7)	(0.3)	(21.4)	(10.1)

Date	09-08	10-08	11-08	13-08	16-08	18-08	20-08	23-08
Day of Yr.	(222)	(223)	(224)	(226)	(229)	(231)	(233)	(236)
Control	5.7		6	4.7	10.7	11.7	ND	7
	(1.3)		(0.6)	(2.0)	(3.2)	(4.2)		(3.1)
Diflubenzuron	7.3	*	3.8	13.3	11.7	1.3	ND	2
	(4.4)		(2.2)	(10.3)	(6.8)	(0.9)		(2)
Methoprene	23	*	28.7	58.3	64.7	54.3	ND	34.7
	(15)		(18.0)	(47.9)	(57.1)	(46.6)		(24.7)
Chlorpyrifos	62.3	*	2	0	0	0.6	ND	0.6
	(32.7)		(2)	(-)	(-)	(0.3)		(0.6)

*Indicates pools treated at that date.
**No data.
***Standard error.

TABLE 12. The effect of the pesticide treatments on adult emergence of chaoborids as determined by emergence trap sampling. Data are expressed as the mean catch per pool for each treatment group.

Date	16-06	18-06	21-06	23-06	25-06	28-06	30-06	02-07	03-07	05-07	07-07	09-07
Day of Yr.	(168)	(170)	(173)	(175)	(177)	(180)	(182)	(184)	(185)	(187)	(189)	(191)
Control	0	0	0	1	4	3.6	2.3	3.3		4	1	0
	(-)**	(-)	(-)	(1)	(3.1)	(1.9)	(2.3)	(1.6)		(3)	(0.6)	(-)
Diflubenzuron	0	0	0	3.7	14	40.7	41	21.6	*	43.3	2.3	0
	(-)	(-)	(-)	(2.7)	(13)	(39.7)	(41)	(21.2)		(42.8)	(2.3)	(-)
Methoprene	0	0	0	0	0	0	1	1.3	*	1	2	0
	(-)	(-)	(-)	(-)	(-)	(-)	(1)	(1.3)		(0.6)	(2)	(-)
Chlorpyrifos	0	0	0	0	0.3	3	2.3	1.7	*	2.3	0	0
	(-)	(-)	(-)	(-)	(0.3)	(2.5)	(1.9)	(1.7)		(1.9)	(-)	(-)

Date	12-07	14-07	16-07	19-07	21-07	22-07	23-07	26-07	28-07	30-07	09-08	06-08
Day of Yr.	(194)	(196)	(198)	(201)	(203)	(204)	(205)	(208)	(210)	(212)	(217)	(219)
Control	0	0.6	1	0.6	1.3		1	0.3	0.3	0	7	3
	(-)	(0.6)	(0.6)	(0.3)	(1.3)		(0.6)	(0.3)	(0.3)	(0)	(5.5)	(0.6)
Diflubenzuron	0	0	0	0	0	*	0	0.6	0	0	0.6	0
	(-)	(-)	(-)	(-)	(-)		(-)	(0.6)	(-)	(0)	(0.6)	(-)
Methoprene	0	0	0	0	0.3	*	0	3.6	0.6	0	4.7	10.7
	(-)	(-)	(-)	(-)	(0.3)		(-)	(1.9)	(0.3)	(0)	(3.3)	(4.7)
Chlorpyrifos	0	0	0	0	0		0	0	0	0	1.3	0
	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(-)	(0)	(1.3)	(-)

Date	09-08	10-08	11-08	13-08	16-08	18-08	20-08	23-08
Day of Yr.	(222)	(223)	(224)	(226)	(229)	(231)	(233)	(236)
Control	11.3		4.3	2	1.3	1	1.7	0.6
	(7.0)		(2.4)	(0)	(1.3)	(0.6)	(0.9)	(0.3)
Diflubenzuron	0.6	*	0	0.3	0.3	0	0	0
	(0.6)		(-)	(0.3)	(0.3)	(-)	(-)	(-)
Methoprene	24.3	*	12.3	4	1.2	1	1.3	1.3
	(4.7)		(4.5)	(2.3)	(0.9)	(1)	(1.3)	(1.3)
Chlorpyrifos	0	*	0.6	0.3	1.3	0	0	0
	(-)		(0.6)	(0.3)	(0.7)	(-)	(-)	(-)

*Indicates pools treated at that date.
**Standard error.

treatment but are not evident in emergence data. Adult emergence was generally lower in Diflubenzuron pools than in controls after the initial treatment indicating a possible treatment effect though the reduction is not dramatic.

Methoprene treatment caused no detectable effect on numbers or composition of chaoborid larvae and in fact more larvae were caught in Methoprene in comparison to control pools, especially near the end of the experiment. It is difficult to determine whether this increase is biologically significant and does indicate a treatment-related effect. If this increase was due to treatment it would have to have been due to either an increase in food for this predacious family, or a decrease in predatory pressure on chaoborid larvae. Neither of these factors is apparent from the data presented herein.

Adult emergence fluctuations generally follow and are comparable or greater in numbers to those of the control pools. Emergence was reduced in both control and Methoprene pools when treatment related reductions would be expected (cf. supra) and as a result effects on emergence if present were masked by normal emergence reductions.

Chlorpyrifos caused a reduction in numbers of larvae for at least 20 days after the first treatment. Dead larvae were found from 2 to 9 days after treatment and adult emergence reduced from 2 to 32 days after the initial treatment. It took 6 days to reduce emergence to zero after the second treatment but it did not recover before the end of the experiment. These data show resistance of pupae to Chlorpyrifos and a sensitivity of larvae comparable to that of culicids.

Chlorpyrifos caused the greatest effect on larval numbers and adult emergence. Diflubenzuron did cause a larval composition change

and perhaps a reduction in adult emergence while Methoprene had no apparent effect on chaoborids.

Tipulidae

Data concerning the emergence of tipulids are shown in Table 13. As the larval tipulids in the pools lived in the substrate, they were not monitored except by adult emergence. A minor emergence occurred on June 16 and July 28 followed by a gradual increase in emergence as the experiment progressed.

No effect was shown by any of the chemicals on numbers of emerging adults. This may be due to a high resistance of this Family to the insecticides or, more likely, the fact that tipulid larvae breeding in the experimental pools live in the substrate and may not have been exposed to the insecticides. All 3 compounds have a low water solubility and a high affinity for organic material, therefore the penetration of the insecticides through the detritus on the substrate surface may have been limited.

Hurlbert et al. (1970) reported deleterious effects on populations of tipulid larvae in pools treated with Chlorpyrifos though these appear to have been pelagic rather than benthic in habitat.

Ephemeroptera

Effects of the treatments on Ephemeroptera nymphs and adult emergence are shown in Tables 14 and 15. Numbers of nymphs and emerging adults generally increased as the experiment progressed. Despite the low numbers of organisms caught some treatment effects are apparent in both emergence and VDB data.

As numbers of nymphs caught were low during the first and second

TABLE 13. The effect of the pesticide treatments on adult emergence of tipulids as determined by emergence trap sampling. Data are expressed as the mean catch per pool for each treatment group.

Date	16-06	18-06	21-06	23-06	25-06	28-06	30-06	02-07	03-07	05-07	07-07	09-07
Day of Yr.	(168)	(170)	(173)	(175)	(177)	(180)	(182)	(184)	(185)	(187)	(189)	(191)
Control	0.6	0	0	0.3	0.3	0.6	0	0		0	1	1
	(0.6)**	(-)	(-)	(0.3)	(0.3)	(0.6)	(-)	(-)		(-)	(0.6)	(0.6)
Diflubenzuron	0	0	0	1	0.7	0	0	0	*	0	0.6	5.7
	(-)	(-)	(-)	(1)	(0.3)	(-)	(-)	(-)		(-)	(0.6)	(1.3)
Methoprene	0	0.3	0.3	0.3	0.7	0	0	0	*	0	0.3	1.7
	(-)	(0.3)	(0.3)	(0.3)	(0.7)	(-)	(-)	(-)		(-)	(0.3)	(1.2)
Chlorpyrifos	0	0	0.3	0.3	0	0	0	0	*	0.3	0	1.3
	(-)	(-)	(0.3)	(0.3)	(0)	(-)	(-)	(-)		(0.3)	(-)	(0.9)

Date	12-07	14-07	16-07	19-07	21-07	22-07	23-07	26-07	28-07	30-07	04-08	06-08
Day of Yr.	(194)	(196)	(198)	(201)	(203)	(204)	(205)	(208)	(210)	(212)	(217)	(219)
Control	4.3	3.7	1.7	8.3	4.3		3.7	3	5.3	6.7	8.3	1.3
	(4.3)	(3.7)	(0.7)	(4.9)	(2.9)		(3.7)	(0.6)	(1.9)	(3.5)	(4.3)	(1.3)
Diflubenzuron	18	16	4	19.7	8	*	4	3.6	5	3.7	7.7	0.3
	(4.7)	(5)	(2.5)	(5.8)	(2.1)		(2.3)	(0.9)	(2.1)	(2.0)	(1.2)	(0.3)
Methoprene	4.3	2	2	5	4.3	*	3.7	2.7	2	1.7	3.7	1.3
	(3.0)	(1.2)	(1.2)	(3.6)	(3.4)		(2.7)	(1.3)	(0.6)	(0.9)	(3.7)	(0.9)
Chlorpyrifos	5.3	6	3	6.7	6		4.3	4	3	3.3	0.3	1
	(5.3)	(5.5)	(3)	(5.7)	(5.0)		(4.3)	(4)	(2)	(2.0)	(0.3)	(1)

Date	09-08	10-08	11-08	13-08	16-08	18-08	20-08	23-08
Day of Yr.	(222)	(223)	(224)	(226)	(229)	(231)	(233)	(236)
Control	6.3		3.7	1.3	3	2.6	1	8
	(4.3)		(2.2)	(1.3)	(2.5)	(1.5)	(0.6)	(2.1)
Diflubenzuron	2.7	*	4.3	11.3	2.7	12.3	13	34
	(0.9)		(0.9)	(8.9)	(1.3)	(4.7)	(5.1)	(23.7)
Methoprene	2.7	*	2.3	1	0	1	0.3	0.3
	(2.2)		(1.9)	(0.6)	(-)	(0.6)	(0.3)	(0.3)
Chlorpyrifos	0.3	*	0.3	0.6	0.3	0.3	1	0
	(0.3)		(0.3)	(0.6)	(0.3)	(0.3)	(0.6)	(-)

*Indicates pools treated at that date.

**Standard error.

TABLE 14. The effect of the pesticide treatments on populations of ephemeropteran nymphs as determined by Van Dorne bottle sampling. Data are expressed as the mean catch per pool for each treatment group.

Date	16-06	18-06	21-06	23-06	25-06	28-06	30-06	02-07	03-07	05-07	07-07	09-07
Day of Yr.	(168)	(170)	(173)	(175)	(177)	(180)	(182)	(184)	(185)	(187)	(189)	(191)
Control	ND**	0	0	0	0	0	0	0		0	0	0.3
		(-)***	(-)	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(0.3)
Diflubenzuron	ND	0	0	0	0	0	0	0.7	*	0	1.3	0.3
		(-)	(-)	(-)	(-)	(-)	(-)	(0.3)		(-)	(1.3)	(0.3)
Methoprene	ND	0	0	0	0	0	0	0	*	0.3	1.3	0.6
		(-)	(-)	(-)	(-)	(-)	(-)	(-)		(0.3)	(0.9)	(0.6)
Chlorpyrifos	ND	0	0	0	0	0	0	0	*	0	0.7	1
		(-)	(-)	(-)	(-)	(-)	(-)	(-)		(-)	(0.7)	(1)

Date	12-07	14-07	16-07	19-07	21-07	22-07	23-07	26-07	28-07	30-07	04-08	06-08
Day of Yr.	(194)	(196)	(198)	(201)	(203)	(204)	(205)	(208)	(210)	(212)	(217)	(219)
Control	0	0.3	0	0	0		0	3.7	0.3	0	6	1
	(-)	(0.3)	(-)	(-)	(-)		(-)	(3.7)	(0.3)	(-)	(5)	(0.6)
Diflubenzuron	0	0.3	0.3	0	0	*	0	0	0	0	0.3	0.3
	(-)	(0.3)	(0.3)	(-)	(-)		(-)	(-)	(-)	(-)	(0.3)	(0.3)
Methoprene	0.7	0	0	0.3	0	*	0.3	0.6	0	0.3	2.8	1.3
	(0.7)	(-)	(-)	(0.3)	(-)		(0.3)	(0.6)	(-)	(0.3)	(1.6)	(1.3)
Chlorpyrifos	0	0.6	0	0	0		0	0	0	0	2	0
	(-)	(0.6)	(-)	(-)	(-)		(-)	(-)	(-)	(-)	(0.6)	(-)

Date	09-08	10-08	11-08	13-08	16-08	18-08	20-08	23-08
Day of Yr.	(222)	(223)	(224)	(226)	(229)	(231)	(233)	(236)
Control	0		1.3	1	0.3	4.3	ND	2
	(-)		(1.3)	(1)	(0.3)	(4.3)		(1.5)
Diflubenzuron	0.3	*	0	0.3	0	0	ND	0.3
	(0.3)		(-)	(0.3)	(-)	(-)		(0.3)
Methoprene	8.3	*	4.3	4.7	8	3.3	ND	0.3
	(7.8)		(4.3)	(4.7)	(8)	(3.3)		(0.3)
Chlorpyrifos	0	*	0	0.6	0	0	ND	0
	(-)		(-)	(0.6)	(-)	(-)		(-)

*Indicates pools treated at that date.
**No data.
***Standard error.

TABLE 15. The effect of the pesticide treatments on adult emergence of ephemeropteran adults as determined by emergence trap sampling. Data are expressed as the mean catch per pool for each treatment group.

Date	16-06	18-06	21-06	23-06	25-06	28-06	30-06	02-07	03-07	05-07	07-07	09-07
Day of Yr.	(168)	(170)	(173)	(175)	(177)	(180)	(182)	(184)	(185)	(187)	(189)	(191)
Control	0	0	0	0	0	0	1.3	0		0	0	0
	(-)**	(-)	(-)	(-)	(-)	(-)	(1.3)	(-)		(-)	(-)	(-)
Diflubenzuron	0	0	0	0	0	0	0	0	*	0	0	0
	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(-)
Methoprene	0	0	0	0	0	0	0.7	0	*	0	0	0
	(-)	(-)	(-)	(-)	(-)	(-)	(0.7)	(-)		(-)	(-)	(-)
Chlorpyrifos	0	0	0	0	0	0	0	0	*	0	0	0.3
	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(0.3)

Date	12-07	14-07	16-07	19-07	21-07	22-07	23-07	26-07	28-07	30-07	04-08	06-08
Day of Yr.	(194)	(196)	(198)	(201)	(203)	(204)	(205)	(208)	(210)	(212)	(217)	(219)
Control	0	0	0	0	0.3		1	2	0.3	0	3.3	3.3
	(-)	(-)	(-)	(-)	(0.3)		(1)	(1.2)	(0.3)	(-)	(2.0)	(2.0)
Diflubenzuron	0	0	0	0	0	*	0	0	0	0	0	0
	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(-)	(-)	(-)	(-)
Methoprene	0	0	0	0	0	*	0	2	2.7	0.7	0.3	0.6
	(-)	(-)	(-)	(-)	(-)		(-)	(1.2)	(1.3)	(0.7)	(0.3)	(0.6)
Chlorpyrifos	0	0	0	0	0		0	0	0	0	0	0
	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(-)	(-)	(-)	(-)

Date	09-08	10-08	11-08	13-08	16-08	18-08	20-08	23-08
Day of Yr.	(222)	(223)	(224)	(226)	(229)	(231)	(233)	(236)
Control	4.7		0.7	2	9	4	2	0
	(1.7)		(0.7)	(0)	(6.0)	(2.7)	(2)	(-)
Diflubenzuron	0	*	0	0	0	0	0	0
	(-)		(-)	(-)	(-)	(-)	(-)	(-)
Methoprene	1.7	*	1	3.7	1	1.7	2.7	1.7
	(1.2)		(0.6)	(3.7)	(1)	(0.3)	(2.7)	(1.7)
Chlorpyrifos	0	*	0	0	0	0	0	0
	(-)		(-)	(-)	(-)	(-)	(-)	(-)

*Indicates pools treated at that date.
**Standard error.

treatments, no effect on nymphs was apparent due to Diflubenzuron treatment. After the third Diflubenzuron treatment a reduction in nymphs was evident. All nymphs caught after the initial treatment were very small indicating recent hatch. This apparent lack of development beyond the early nymphal stages was confirmed by the lack of ephemeropteran emergence from Diflubenzuron pools. This effect on Ephemeroptera has also been reported by Miura and Takahashi (1974).

Methoprene caused no decrease in nymphs and in fact Methoprene pools often showed higher numbers than control pools. The nymphs caught were of a wide range of instars in contrast to those in the Diflubenzuron pools. This is not in agreement with the results of Norland (1973) and Norland and Mulla (1975) who showed a high sensitivity of Ephemeroptera nymphs to Methoprene. This difference may be due to the lower application rate used in this study or genus/species-specific sensitivity. Adult emergence was consistent with that of the control pools. These data, and the observation that all adults caught were true adults, indicate no effect on the nymph-subimago or subimago-imago molt.

Chlorpyrifos caused similar effects to those reported for Diflubenzuron. Nymphal populations were reduced in comparison to those of control pools, and most nymphs caught were of the early developmental instars. The one adult that was caught on July 9 may indicate a low sensitivity of the late nymphal instars. The lack of adult emergence and reduction in nymphs indicates a severe effect on this taxon.

Methoprene had no deleterious effect on Ephemeroptera, while

Diflubenzuron and Chlorpyrifos reduced nymphal populations and adult emergence.

Corixidae

Effects of the treatments on corixid populations are shown in Tables 16 and 17. Adults and nymphs are grouped together as there were no differences in effects on these 2 groups. Overall numbers did increase as the experiment progressed with nymphs dominating the population until June 30 after which time the adults became predominant.

Neither Diflubenzuron nor Methoprene caused detectable effects on populations of corixids.

Chlorpyrifos did cause a reduction in both nymphs and adults in comparison to control pools. Dead and moribund nymphs and adults were seen for up to 6 days after treatment. This is not in agreement with Hurlbert et al. (1970) who showed that Chlorpyrifos caused a reduction in numbers of corixid nymphs in treated pools, but no effect on corixid adults. This apparent contradiction in results may be due to species-specific sensitivity.

The only compound to deleteriously affect this taxon is Chlorpyrifos.

Notonectidae

Data concerning the effect of the treatments on notonectids are shown in Tables 18 and 19. Adults and nymphs are combined for the same reasons corixids were. All notonectids caught before August 9 were nymphs while those caught after that date were adults. Numbers of notonectids caught are very low due to their low populations in the pools and their high degree of mobility (avoidance of the VDB). As a

TABLE 16. Qualitative observations concerning the effect of the pesticide treatments on corixids.

	Date Day of Yr.	02-07 (184)	03-07 (185)	04-07 (186)	06-07 (188)	09-07 (191)	12-07 (194)	15-07 (197)	18-07 (200)
	Pool No.								
Control	1	+		+	+	+	+	+	+
	8	/		+	+	+	+	+	+
	9	/		+	+	+	+	+	+
Diflubenzuron	7	/	*	+	+	+	+	+	+
	10	+	*	+	+	+	+	+	+
	12	/	*	+	+	+	/	+	+
Methoprene	2	+	*	/	+	/	+	+	+
	5	/	*	/	+	+	/	+	+
	11	+	*	+	+	+	+	+	+
Chlorpyrifos	3	+	*	-+	-+	-	/	+	/
	4	+	*	-	-	-+	+	+	+
	6	+	*	-	-	+	+	+	+

TABLE 16. Cont'd.

	Date Day of Yr.	22-07 (204)	23-07 (205)	26-07 (208)	29-07 (211)	01-08 (214)	04-08 (217)	10-08 (223)	12-08 (225)	14-08 (227)
	Pool No.									
Control	1		+	ND	+	+	+		+	ND
	8		+	ND	+	+	+		+	ND
	9		+	ND	ND	+	+		+	+
Diflubenzuron	7	*	+	ND	/	+	+	*	+	+
	10	*	+	+	/	+	+	*	+	+
	12	*	+	+	+	+	+	*	+	+
Methoprene	2	*	+	+	+	+	+	*	+	ND
	5	*	+	+	+	+	+	*	+	+
	11	*	+	/	+	+	+	*	+	+
Chlorpyrifos	3		+	ND	+	+	+	*	-	ND
	4		+	ND	+	+	+	*	-	ND
	6		+	+	+	+	+	*	-	-+

Observations are indicated by: + Present Alive, - Present Dead, / Not Observed, ND No Data.
*Indicates pools treated at that date.

TABLE 17. The effect of the pesticide treatments on populations of corixids as determined by Van Dorne bottle sampling. Data are expressed as the mean catch per pool for each treatment group.

Date Day of Yr.	16-06 (168)	18-06 (170)	21-06 (173)	23-06 (175)	25-06 (177)	28-06 (180)	30-06 (182)	02-07 (184)	03-07 (185)	05-07 (187)	07-07 (189)	09-07 (191)
Control	ND**	0.3 (0.3)***	0.3 (0.3)	0.6 (0.6)	0 (-)	0 (-)	0.6 (0.6)	0.6 (0.3)		2.3 (0.7)	2 (1)	0.3 (0.3)
Diflubenzuron	ND	0 (-)	0 (-)	0.6 (0.3)	0 (-)	0.6 (0.3)	0.3 (0.3)	1 (1)	*	2.3 (1.5)	3.3 (1.8)	0.3 (0.3)
Methoprene	ND	0 (-)	0 (-)	0.3 (0.3)	0 (-)	0 (-)	0.3 (0.3)	1 (0.6)	*	1.3 (0.9)	0.6 (0.6)	1.3 (1.3)
Chlorpyrifos	ND	0 (-)	0.3 (0.3)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	*	1 (1)	0 (-)	0 (-)

Date Day of Yr.	12-07 (194)	14-07 (196)	16-07 (198)	19-07 (201)	21-07 (203)	22-07 (204)	23-07 (205)	26-07 (208)	28-07 (210)	30-07 (212)	04-08 (217)	06-08 (219)
Control	1.3 (0.3)	1.3 (0.9)	1.3 (0.6)	0.3 (0.3)	0.3 (0.3)		0.6 (0.6)	0 (-)	1.3 (1.3)	1.3 (0.9)	2 (1.5)	5 (4)
Diflubenzuron	0 (-)	1.3 (0.9)	1.3 (0.6)	0.3 (0.3)	0.3 (0.3)	*	2.3 (1.9)	1.6 (1.6)	7.7 (5.2)	4.3 (1.3)	2 (1.2)	5 (0.6)
Methoprene	0.3 (0.3)	0.6 (0.6)	0.3 (0.3)	0.3 (0.3)	1.6 (1.6)	*	0.6 (0.6)	1 (1)	2 (2)	3.3 (2.4)	0.3 (0.3)	5 (5)
Chlorpyrifos	0 (-)	0 (-)	0.3 (0.3)	0 (-)	0 (-)		0.3 (0.3)	0 (-)	0 (-)	0 (-)	0.3 (0.3)	0.6 (0.3)

Date Day of Yr.	09-08 (222)	10-08 (223)	11-08 (224)	13-08 (226)	16-08 (229)	18-08 (231)	20-08 (233)	23-08 (236)
Control	2.3 (1.9)		5.3 (4.9)	5.6 (3.3)	1.6 (1.6)	4.3 (3.4)	ND	8 (4.7)
Diflubenzuron	1.3 (0.6)	*	5 (2.1)	1.6 (1.6)	6.7 (2.3)	4.3 (2.3)	ND	5 (3.2)
Methoprene	3 (1.2)	*	9.3 (7.4)	1.3 (0.9)	6.3 (3.2)	1 (0.6)	ND	1.3 (0.8)
Chlorpyrifos	0 (-)	*	0.6 (0.3)	1 (0.6)	0.3 (0.3)	0 (-)	ND	0.3 (0.3)

*Indicates pools treated at that date.
**No data.
***Standard error.

TABLE 18. Qualitative observations concerning the effect of the pesticide treatments on notonectids.

	Date Day of Yr.	02-07 (184)	03-07 (185)	04-07 (186)	06-07 (188)	09-07 (191)	12-07 (194)	15-07 (197)	18-07 (200)
	Pool No.								
Control	1	/		/	/	+	/	/	+
	8	/		/	/	+	+	+	+
	9	/		/	/	+	+	/	+
Diflubenzuron	7	/	*	/	/	/	/	/	/
	10	/	*	/	/	+	+	/	/
	12	/	*	/	/	/	/	+	/
Methoprene	2	/	*	/	/	/	+	/	/
	5	/	*	+	/	/	/	/	/
	11	+	*	/	+	/	/	/	+
Chlorpyrifos	3	/	*	/	/	/	/	/	/
	4	/	*	/	/	-	/	/	+
	6	/	*	/	/	/	/	/	+

TABLE 18. Cont'd.

	Date Day of Yr.	22-07 (204)	23-07 (205)	26-07 (208)	29-07 (211)	01-08 (214)	04-08 (217)	10-08 (223)	12-08 (225)	14-08 (227)
	Pool No.									
Control	1		+	ND	+	+	+		+	ND
	8		+	ND	+	+	/		+	ND
	9		+	ND	ND	+	+		+	+
Diflubenzuron	7	*	/	ND	/	/	/	*	/	/
	10	*	+	/	/	/	/	*	/	/
	12	*	/	/	/	/	/	*	/	/
Methoprene	2	*	/	/	/	/	/	*	/	ND
	5	*	/	/	+	+	+	*	+	+
	11	*	+	+	+	+	+	*	+	+
Chlorpyrifos	3		+	ND	/	/	/	*	/	ND
	4		/	ND	/	/	/	*	/	ND
	6		+	/	/	/	/	*	/	/

Observations are indicated by: + Present Alive, - Present Dead, / Not Observed, ND No Data.
*Indicates pools treated at that date.

TABLE 19. The effect of the pesticide treatments on populations of notonectids as determined by Van Dorne bottle sampling. Data are expressed as the mean catch per pool for each treatment group.

Date	16-06	18-06	21-06	23-06	25-06	28-06	30-06	02-07	03-07	05-07	07-07	09-07
Day of Yr.	(168)	(170)	(173)	(175)	(177)	(180)	(182)	(184)	(185)	(187)	(189)	(191)
Control	ND**	0	0	0.3	0	0	0	0		0.3	0	0
		(-)***	(-)	(0.3)	(-)	(-)	(-)	(-)		(0.3)	(-)	(-)
Diflubenzuron	ND	0	0	0	0	0	0	0	*	0	0	0
		(-)	(-)	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(-)
Methoprene	ND	0	0	0	0	0	0	0	*	0	0	0
		(-)	(-)	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(-)
Chlorpyrifos	ND	0	0	0	0	0	0	0	*	0	0	0
		(-)	(-)	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(-)

Date	12-07	14-07	16-07	19-07	21-07	22-07	23-07	26-07	28-07	30-07	04-08	06-08
Day of Yr.	(194)	(196)	(198)	(201)	(203)	(204)	(205)	(208)	(210)	(212)	(217)	(219)
Control	0.3	0	0.3	0.3	0		0	0	0	1	0	0
	(0.3)	(-)	(0.3)	(0.3)	(-)		(-)	(-)	(-)	(1)	(-)	(-)
Diflubenzuron	0	0	0	0	0	*	0	0	0	0	0	0
	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(-)	(-)	(-)	(-)
Methoprene	0	0	0	0.3	0	*	0	0	0	0	0	0
	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(-)	(-)	(-)	(-)
Chlorpyrifos	0	0	0	0	0		0	0	0	0	0	0
	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(-)	(-)	(-)	(-)

Date	09-08	10-08	11-08	13-08	16-08	18-08	20-08	23-08
Day of Yr.	(222)	(223)	(224)	(226)	(229)	(231)	(233)	(236)
Control	0		0.3	0	0	0.6	ND	0
	(-)		(-)	(-)	(-)	(0.6)		(-)
Diflubenzuron	0	*	0	0.3	0.3	0	ND	0
	(-)		(-)	(-)	(0.3)	(-)		(-)
Methoprene	0	*	0	0	0	0.3	ND	0.6
	(-)		(-)	(-)	(-)	(0.3)		(0.6)
Chlorpyrifos	0	*	0	0	0.3	0	ND	0
	(-)		(-)	(-)	(0.3)	(-)		(-)

*Indicates pools treated at that date.
**No data.
***Standard error.

result treatment effects, if present, were impossible to detect.

No definite treatment-related effects are apparent from the VDB data though more notonectids were caught in control pools than in those treated. Qualitative observations show the presence of dead notonectids after the first Chlorpyrifos treatment, and generally fewer observations in Diflubenzuron and Chlorpyrifos than in Methoprene and Control pools. No definite conclusions can be drawn from these data though Chlorpyrifos and perhaps Diflubenzuron may have deleteriously affected notonectids.

Gerridae

Data concerning the effects of treatment on gerrids are shown in Table 20. As this taxon lives on the water surface rather than under it the only form of data collected concerning gerrids were qualitative observations. These observations indicate no effect of Diflubenzuron or Methoprene on gerrids. Chlorpyrifos did cause some mortality immediately after treatment but populations quickly recovered probably by immigration.

Dytiscidae

Data concerning the populations of larval and adult dytiscids are shown in Tables 21 to 24. As it was impossible to distinguish between dytiscid and hydrophilid larvae when taking qualitative observations, qualitative data for these two families were combined. As seen with notonectids numbers of dytiscids collected were very low and treatment-related effects may have occurred that were not detected by the monitoring procedures used.

Diflubenzuron caused no apparent effect on larval or adult popula-

TABLE 20. Qualitative observations concerning the effect of the pesticide treatments on gerrids.

	Date Day of Yr.	02-07 (184)	03-07 (185)	04-07 (186)	06-07 (188)	09-07 (191)	12-07 (194)	15-07 (197)	18-07 (200)
	Pool No.								
Control	1	+		+	+	+	+	+	+
	8	+		+	+	+	+	+	+
	9	+		+	+	+	+	+	/
Diflubenzuron	7	+	*	+	+	+	+	+	+
	10	+	*	+	+	/	/	+	+
	12	+	*	+	+	+	+	+	/
Methoprene	2	+	*	+	+	+	+	+	+
	5	+	*	+	+	+	+	+	+
	11	+	*	+	+	+	+	+	+
Chlorpyrifos	3	+	*	+	-+	/	/	/	+
	4	+	*	+	-	+	+	+	+
	6	+	*	+	-	+	/	+	+

TABLE 20. Cont'd.

	Date Day of Yr.	22-07 (204)	23-07 (205)	26-07 (208)	29-07 (211)	01-08 (214)	04-08 (217)	10-08 (223)	12-08 (225)	14-08 (227)
	Pool No.									
Control	1		+	ND	+	+	+		+	ND
	8		+	ND	/	+	+		+	ND
	9		+	ND	ND	+	+		+	/
Diflubenzuron	7	*	+	ND	+	+		*	/	+
	10	*	+	+	+	+	+	*	/	+
	12	*	+	+	+	+	+	*	+	+
Methoprene	2	*	+	+	+	+	+	*	+	ND
	5	*	+	+	+	+	+	*	+	+
	11	*	+	+	+	+	+	*	+	+
Chlorpyrifos	3		/	ND	+	+	+	*	+	ND
	4		+	ND	+	+	+	*	+	ND
	6		+	+	+	/	+	*	+	/

Observations are indicated by: + Present Alive, - Present Dead, / Not Observed, ND No Data.
*Indicates pools treated at that date.

TABLE 21. Qualitative observations concerning the effect of the pesticide treatments on dytiscid adults.

	Date Day of Yr.	02-07 (184)	03-07 (185)	04-07 (186)	06-07 (188)	09-07 (191)	12-07 (194)	15-07 (197)	18-07 (200)
	Pool No.								
Control	1	/		/	/	/	/	/	+
	8	/		/	+	+	+	/	+
	9	+		/	/	+	/	+	/
Diflubenzuron	7	+	*	+	+	+	/	/	+
	10	/	*	/	/	+	/	/	+
	12	+	*	+	-+	+	+	+	+
Methoprene	2	/	*	/	-+	/	+	+	+
	5	/	*	/	-+	+	/	+	/
	11	+	*	+	+	+	+	/	/
Chlorpyrifos	3	/	*	-+	-	-	-	-+	+
	4	+	*	-+	-	-+	-+	-	/
	6	/	*	-+	-	/	-+	-	+

TABLE 21. Cont'd.

	Date Day of Yr.	22-07 (204)	23-07 (205)	26-07 (208)	29-07 (211)	01-08 (214)	04-08 (217)	10-08 (223)	12-08 (225)	14-08 (227)
	Pool No.									
Control	1		/	ND	/	+	/		/	ND
	8		+	ND	/	/	/		/	ND
	9		+	ND	ND	/	+		+	+
Diflubenzuron	7	*	+	ND	+	/	+	*	/	+
	10	*	/	+	+	+	+	*	+	+
	12	*	+	+	/	/	+	*	+	/
Methoprene	2	*	+	/	+	+	/	*	+	ND
	5	*	+	+	/	/	/	*	/	+
	11	*	+	/	/	/	+	*	/	+
Chlorpyrifos	3		+	ND	/	/	/	*	/	ND
	4		+	ND	/	/	/	*	/	ND
	6		+	+	/	/	+	*	/	-

Observations are indicated by: + Present Alive, - Present Dead, / Not Observed, ND No Data.
*Indicates pools treated at that date.

TABLE 22. Qualitative observations concerning the effect of the pesticide treatments on dytiscid and hydrophilid larvae.

	Date Day of Yr.	02-07 (184)	03-07 (185)	04-07 (186)	06-07 (188)	09-07 (191)	12-07 (194)	15-07 (197)	18-07 (200)
	Pool No.								
Control	1	/		+	+	+	+	+	+
	8	+		+	+	+	/	/	+
	9	/		+	+	+	+	+	+
Diflubenzuron	7	/	*	/	-+	+	+	/	+
	10	+	*	+	+	+	+	/	+
	12	+	*	/	+	+	/	/	/
Methoprene	2	/	*	+	+	+	+	+	+
	5	+	*	+	+	+	+	+	+
	11	+	*	+	+	+	+	+	+
Chlorpyrifos	3	/	*	-+	/	-+	/	+	+
	4	+	*	-+	/	-+	/	/	+
	6	/	*	/	-+	/	/	/	+

TABLE 22. Cont'd.

	Date Day of Yr.	22-07 (204)	23-07 (205)	26-07 (208)	29-07 (211)	01-08 (214)	04-08 (217)	10-08 (223)	12-08 (225)	14-08 (227)
	Pool No.									
Control	1		+	ND	/	+	/		/	ND
	8		/	ND	+	/	+		/	ND
	9		+	ND	ND	/	/		/	+
Diflubenzuron	7	*	/	ND	+	/	/	*	+	/
	10	*	/	/	+	/	+	*	+	/
	12	*	+	/	+	/	+	*	/	/
Methoprene	2	*	+	+	+	+	+	*	+	ND
	5	*	+	/	+	+	+	*	/	/
	11	*	+	+	+	+	+	*	+	/
Chlorpyrifos	3		/	ND	+	+	+	*	/	ND
	4		+	ND	+	+	+	*	+	ND
	6		+	/	+	/	+	*	/	-+

Observations are indicated by: + Present Alive, - Present Dead, / Not Observed, ND No Data.
*Indicates pools treated at that date.

TABLE 23. The effect of the pesticide treatments on populations of dytiscid larvae as determined by Van Dorne bottle sampling. Data are expressed as the mean catch per pool for each treatment group.

Date	16-06	18-06	21-06	23-06	25-06	28-06	30-06	02-07	03-07	05-07	07-07	09-07
Day of Yr.	(168)	(170)	(173)	(175)	(177)	(180)	(182)	(184)	(185)	(187)	(189)	(191)
Control	ND**	0 (-)***	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0.3 (0.3)		0 (-)	0 (-)	0 (-)
Diflubenzuron	ND	0.3 (0.3)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0.3 (0.3)	*	0 (-)	0 (-)	0.3 (0.3)
Methoprene	ND	0 (-)	0.3 (0.3)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	*	0 (-)	0 (-)	0 (-)
Chlorpyrifos	ND	0 (-)	0 (-)	0 (-)	0.3 (0.3)	0 (-)	0 (-)	0.3 (0.3)	*	0 (-)	0 (-)	0 (-)

Date	12-07	14-07	16-07	19-07	21-07	22-07	23-07	26-07	28-07	30-07	04-08	06-08
Day of Yr.	(194)	(196)	(198)	(201)	(203)	(204)	(205)	(208)	(210)	(212)	(217)	(219)
Control	0 (-)	0.3 (0.3)	0 (-)	0 (-)	0 (-)		0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
Diflubenzuron	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	*	0 (-)	0 (-)	0.3 (0.3)	0.3 (0.3)	0 (-)	0.3 (0.3)
Methoprene	0 (-)	0 (-)	0 (-)	0.3 (-)	0 (-)	*	0 (-)	0 (-)	0.3 (0.3)	0.3 (0.3)	0 (-)	0 (-)
Chlorpyrifos	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)		0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	1.3 (1.3)

Date	09-08	10-08	11-08	13-08	16-08	18-08	20-08	23-08
Day of Yr.	(222)	(223)	(224)	(226)	(229)	(231)	(233)	(236)
Control	0 (-)		0 (-)	0.3 (0.3)	0 (-)	0.3 (0.3)	ND	0 (-)
Diflubenzuron	0 (-)	*	0 (-)	0 (-)	0 (-)	0.6 (0.6)	ND	0 (-)
Methoprene	0 (-)	*	0.6 (0.6)	0 (-)	0.6 (0.6)	0.6 (0.6)	ND	0.3 (0.3)
Chlorpyrifos	0.6 (0.6)	*	0 (-)	0 (-)	0 (-)	0 (-)	ND	0 (-)

*Indicates pools treated at that date.
**No data.
***Standard error.

TABLE 24. The effect of the pesticide treatments on populations of dytiscid adults as determined by Van Dorne bottle sampling. Data are expressed as the mean catch per pool for each treatment group.

Date Day of Yr.	16-06 (168)	18-06 (170)	21-06 (173)	23-06 (175)	25-06 (177)	28-06 (180)	30-06 (182)	02-07 (184)	03-07 (185)	05-07 (187)	07-07 (189)	09-07 (191)
Control	ND**	1.3 (1.3)***	0.3 (0.3)	0 (-)	0 (-)	0 (-)	0 (-)	0.3 (0.3)		0 (-)	0.3 (0.3)	0 (-)
Diflubenzuron	ND	0.3 (0.3)	0.3 (0.3)	0 (-)	0 (-)	0.3 (0.3)	0 (-)	0.3 (0.3)	*	0 (-)	2.3 (1.2)	0.6 (0.6)
Methoprene	ND	0.6 (0.3)	0.3 (0.3)	0.3 (0.3)	0 (-)	0 (-)	0 (-)	0 (-)	*	0 (-)	0 (-)	0 (-)
Chlorpyrifos	ND	0.6 (0.3)	0.3 (0.3)	0 (-)	0.6 (0.3)	0 (-)	0 (-)	0.3 (0.3)	*	0.3 (0.3)	0.3 (0.3)	0 (-)

Date Day of Yr.	12-07 (194)	14-07 (196)	16-07 (198)	19-07 (201)	21-07 (203)	22-07 (204)	23-07 (205)	26-07 (208)	28-07 (210)	30-07 (212)	04-08 (217)	06-08 (219)
Control	0 (-)	0.3 (0.3)	0 (-)	0.3 (0.3)	0 (-)		0 (-)	0 (-)	0 (-)	0.3 (0.3)	0 (-)	0 (-)
Diflubenzuron	0.3 (0.3)	2 (1.2)	0.6 (0.3)	0 (-)	0.3 (0.3)	*	0 (-)	0 (-)	1.3 (0.3)	0.3 (0.3)	1 (0.6)	0.3 (0.3)
Methoprene	0.3 (0.3)	1 (1)	1.0 (0.6)	0 (-)	0.3 (0.3)	*	0 (-)	0.3 (0.3)	0 (-)	0.3 (0.3)	0 (-)	0 (-)
Chlorpyrifos	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)		0 (-)	0 (-)	0.6 (0.6)	0.3 (0.3)	0 (-)	0.3 (0.3)

Date Day of Yr.	09-08 (222)	10-08 (223)	11-08 (224)	13-08 (226)	16-08 (229)	18-08 (231)	20-08 (233)	23-08 (236)
Control	0.3 (0.3)		0.3 (0.3)	0.3 (0.3)	0 (-)	0 (-)	ND	0 (-)
Diflubenzuron	0.3 (0.3)	*	1 (1)	0 (-)	0.3 (0.3)	0.3 (0.3)	ND	0.3 (0.3)
Methoprene	0 (-)	*	0.3 (0.3)	0 (-)	0.6 (0.3)	0.3 (0.3)	ND	2.3 (1.9)
Chlorpyrifos	0 (-)	*	0 (-)	0 (-)	0 (-)	0 (-)	ND	0.3 (0.3)

*Indicates pools treated at that date.
**No data.
***Standard error.

tions as monitored by VDB's though dead larvae and adults were found for up to 3 days after treatment. The numbers of dead larvae and adults found appeared to be small in relation to those present alive. As a result no dramatic effect on the populations probably occurred during the experiment. Reports in the literature show deleterious effects on dytiscid larvae which may only be evident at the next molt or in the adult (Miura and Takahashi 1975; Steelman et al. 1975). It would appear that only some species and developmental stages are sensitive and as a result overall effects on dytiscid populations are probably not dramatic. Effects may have been present that were not apparent during the experiment due to the long development time of dytiscids.

Methoprene showed similar effects to those described for Diflubenzuron though fewer dead larvae and adults were evident after treatment. No detectable effect on adults or larvae were seen though again they may have occurred and not been apparent in VDB data, or were only in evidence after the experiment.

Despite the fact that VDB data shows no definite effect of Chlorpyrifos on dytiscids, many dead larvae and adults were observed after both treatments. Similar data reporting significant death of dytiscid larvae and adults are reported by Hurlbert et al. (1970).

Chlorpyrifos affected both larval and adult dytiscids to a more significant extent than the growth regulators. Whether Diflubenzuron and Methoprene did seriously reduce populations of dytiscids cannot be determined from these data. It is apparent that VDB's are not sufficient to monitor effects on dytiscids.

Hydrophilidae

Data concerning the effects of the treatments on hydrophilid larvae and adults are shown in Tables 22 and 25 to 27. As very few hydrophilids were caught, treatment effects may have occurred that were not apparent in the VDB data.

Diflubenzuron caused some death of hydrophilid larvae but no apparent death of adults and no effect on the overall population of larvae or adults as monitored by VDB sampling. Similar effects, death of some larvae but no observable effect on the overall population are reported by Miura and Takahashi (1975) although significant reductions of larvae are reported by Steelman et al. (1975). As a result of the low numbers caught it is impossible to definitely state whether there were significant deleterious effects on hydrophilid larvae or adults though no effect is in evidence concerning the adults. Also due to the long developmental time of the Family, effects may have occurred that were not observed during the experiment.

Methoprene had no apparent effect on hydrophilids as monitored by VDB or qualitative observations. Deleterious effects on hydrophilid adults have been reported by Steelman et al. (1975) but not until 80 days after exposure of larvae to Methoprene; any such long-term effects would not be observed in this experiment due to the relatively short post-treatment observation period.

Chlorpyrifos caused mortality in both larvae and adult hydrophilids. From the numbers of dead larvae and adults observed a significant reduction in the population would be expected but is not apparent from VDB data. Washino et al. (1972) reported deleterious effects of Chlorpyrifos on hydrophilid larvae but not on adults. The death of adults as well as

TABLE 25. Qualitative observations concerning the effect of the pesticide treatments on hydrophilid adults.

	Date Day of Yr.	02-07 (184)	03-07 (185)	04-07 (186)	06-07 (188)	09-07 (191)	12-07 (194)	15-07 (197)	18-07 (200)
	Pool No.								
Control	1	/		+	+	+	+	/	+
	8	+		+	/	+	+	+	+
	9	/		/	/	+	+	+	+
Diflubenzuron	7	+	*	+	+	+	+	+	+
	10	+	*	+	+	+	+	+	+
	12	/	*	+	+	/	+	+	+
Methoprene	2	/	*	+	+	+	+	+	+
	5	/	*	+	/	+	+	+	+
	11	/	*	+	/	+	+	+	+
Chlorpyrifos	3	/	*	-	-+	+	/	-+	/
	4	/	*	-	-	-+	-	-	/
	6	/	*	-+	-+	-+	-+	+	/

TABLE 25. Cont'd.

	Date Day of Yr.	22-07 (204)	23-07 (205)	26-07 (208)	29-07 (211)	01-08 (214)	04-08 (217)	10-08 (223)	12-08 (225)	14-08 (227)
	Pool No.									
Control	1		+	ND	+	/	+		+	ND
	8		+	ND	+	+	+		/	ND
	9		+	ND	ND	+	+		+	/
Diflubenzuron	7	*	+	ND	+	+	+	*	/	/
	10	*	+	+	/	+	+	*	+	+
	12	*	+	+	+	+	+	*	+	+
Methoprene	2	*	+	+	+	+	+	*	+	ND
	5	*	+	+	+	+	+	*	-+	+
	11	*	+	+	/	+	+	*	+	/
Chlorpyrifos	3		+	ND	+	+	+	*	/	ND
	4		+	ND	/	-	+	*	-	ND
	6		+	+	+	+	+	*	-	-

Observations are indicated by: + Present Alive, - Present Dead, / Not Observed, ND No Data.
*Indicates pools treated at that date.

TABLE 26. The effect of the pesticide treatments on populations of hydrophilid larvae as determined by Van Dorne bottle sampling. Data are expressed as the mean catch per pool for each treatment group.

Date	16-06	18-06	21-06	23-06	25-06	28-06	30-06	02-07	03-07	05-07	07-07	09-07
Day of Yr.	(168)	(170)	(173)	(175)	(177)	(180)	(182)	(184)	(185)	(187)	(189)	(191)
Control	ND**	0	0	0	0	0.3	0	0.3		1.3	0	0.6
		(-)***	(-)	(-)	(-)	(0.3)	(-)	(0.3)		(0.7)	(-)	(0.6)
Diflubenzuron	ND	0	0	0.3	2	0.3	0	0	*	0	0.6	1
		(-)	(-)	(0.3)	(2)	(0.3)	(-)	(-)		(-)	(0.3)	(0)
Methoprene	ND	0	0	0	0	0	0	0	*	0	0	0
		(-)	(-)	(-)	(-)	(-)	(-)	(-)		(-)	(-)	(-)
Chlorpyrifos	ND	0	0	0	0.3	0	0	0	*	0	0.3	0.6
		(-)	(-)	(-)	(0.3)	(-)	(-)	(-)		(-)	(0.3)	(0.6)

Date	12-07	14-07	16-07	19-07	21-07	22-07	23-07	26-07	28-07	30-07	04-08	06-08
Day of Yr.	(194)	(196)	(198)	(201)	(203)	(204)	(205)	(208)	(210)	(212)	(217)	(219)
Control	0.6	0	0.3	0.3	0		0	0	0.6	0	0.6	0.3
	(0.6)	(-)	(0.3)	(0.3)	(-)		(-)	(-)	(0.6)	(-)	(0.6)	(0.3)
Diflubenzuron	0	0.3	1	0.6	0	*	0	0	2	1.7	1.3	0
	(-)	(0.3)	(0.6)	(0.3)	(-)		(-)	(-)	(0.6)	(0.9)	(0.9)	(-)
Methoprene	0	0.3	0	0	0	*	0.6	0.3	0	0	0	0
	(-)	(0.3)	(-)	(-)	(-)		(0.3)	(0.3)	(-)	(-)	(-)	(-)
Chlorpyrifos	0	0.3	0	0	0		0	0	0	0	0	0
	(-)	(0.3)	(-)	(-)	(-)		(-)	(-)	(-)	(-)	(-)	(-)

Date	09-08	10-08	11-08	13-08	16-08	18-08	20-08	23-08
Day of Yr.	(222)	(223)	(224)	(226)	(229)	(231)	(233)	(236)
Control	0.3		0	0	0	0	ND	0
	(0.3)		(-)	(-)	(-)	(-)		(-)
Diflubenzuron	0	*	0	0.3	0	0	ND	0
	(-)		(-)	(0.3)	(-)	(-)		(-)
Methoprene	0	*	0	0	0	0	ND	0
	(-)		(-)	(-)	(-)	(-)		(-)
Chlorpyrifos	0.3	*	0	0	0	0	ND	0
	(0.3)		(-)	(-)	(-)	(-)		(-)

*Indicates pools treated at that date.
**No data.
***Standard error.

TABLE 27. The effect of the pesticide treatments on populations of hydrophilid adults as determined by Van Dorne bottle sampling. Data are expressed as the mean catch per pool for each treatment group.

Date	16-06	18-06	21-06	23-06	25-06	28-06	30-06	02-07	03-07	05-07	07-07	09-07
Day of Yr.	(168)	(170)	(173)	(175)	(177)	(180)	(182)	(184)	(185)	(187)	(189)	(191)
Control	ND**	0 (-)***	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)		1.3 (1.3)	0 (-)	0.3 (0.3)
Diflubenzuron	ND	0.6 (0.6)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	*	0 (-)	0 (-)	0 (-)
Methoprene	ND	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	*	0.3 (0.3)	0 (-)	0 (-)
Chlorpyrifos	ND	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	*	0 (-)	0.3 (0.3)	0 (-)

Date	12-07	14-07	16-07	19-07	21-07	22-07	23-07	26-07	28-07	30-07	04-08	06-08
Day of Yr.	(194)	(196)	(198)	(201)	(203)	(204)	(205)	(208)	(210)	(212)	(217)	(219)
Control	0 (-)	0 (-)	0 (-)	0 (-)	0.3 (0.3)		0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
Diflubenzuron	0 (-)	0 (-)	0.3 (0.3)	0 (-)	0 (-)	*	0 (-)	0.3 (0.3)	0 (-)	0 (-)	0.3 (0.3)	0.3 (0.3)
Methoprene	0 (-)	0 (-)	0 (-)	0 (-)	1 (1)	*	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
Chlorpyrifos	0 (-)	0.6 (0.6)	0 (-)	0 (-)	0 (-)		0 (-)	0 (-)	0.3 (0.3)	0.3 (0.3)	0.6 (0.6)	1.3 (1.3)

Date	09-08	10-08	11-08	13-08	16-08	18-08	20-08	23-08
Day of Yr.	(222)	(223)	(224)	(226)	(229)	(231)	(233)	(236)
Control	0 (-)		0 (-)	0 (-)	0 (-)	0 (-)	ND	0 (-)
Diflubenzuron	0 (-)	*	0 (-)	0 (-)	0 (-)	0 (-)	ND	0 (-)
Methoprene	0.3 (0.3)	*	0.3 (0.3)	0.3 (0.3)	0 (-)	0 (-)	ND	0 (-)
Chlorpyrifos	0 (-)	*	0.6 (0.6)	0.3 (0.3)	0 (-)	0 (-)	ND	0 (-)

*Indicates pools treated at that date.
**No data.
***Standard error.

larvae in this study may be due to species sensitivity differences and/or the differences in formulations or application rates used.

Definite conclusions concerning hydrophilids cannot be made but Chlorpyrifos caused mortality in both adults and larvae while Diflubenzuron caused some mortality in larvae and Methoprene had no apparent effect. It is also apparent that VDB's are not adequate to monitor effects on hydrophilids.

Daphnia sp.

Data describing the effect of the treatments on Daphnia sp. are shown in Table 28. Overall population trends show high numbers of Daphnia sp. at the start of monitoring which generally decreased but showed large fluctuations and variance within treatments, especially in control pools. Statistical analyses which were performed on Daphnia sp. data often suffered due to this large variance with means that appeared different, not statistically different.

Diflubenzuron caused a decrease in Daphnia sp. populations from the initial treatment until the end of the experiment. This reduction is statistically significant on most sampling dates in comparison to control populations. This effect is similar to the results of Miura and Takahashi (1974) though in this experiment the repetitive treatments prevented the recovery reported by Miura and Takahashi (1974). Despite the reduction, the Daphnia sp. was not annihilated and as a result recovery would be expected after the treatments were stopped.

Populations of Daphnia sp. in Methoprene treated pools were often lower than those of the control pools but only on August 8 statistically so. As previous studies have shown (Miura and Takahashi 1974a, 1973) no effect of Methoprene on Daphnia sp. populations, and the means here,

TABLE 28. The effect of the pesticide treatments on populations of *Daphnia* sp. as determined by Van Dorne bottle sampling. Data are expressed as the mean catch per Van Dorne bottle for each treatment group. Means followed by the same letters are not statistically different ($P \leq 0.05$).

Date	16-06	18-06	21-06	23-06	25-06	28-06	30-06	02-07	03-07	05-07	07-07	09-07
Day of Yr.	(168)	(170)	(173)	(175)	(177)	(180)	(182)	(184)	(185)	(187)	(189)	(191)
Control	ND**	175^{a} (43)***	466^{a} (241)	374^{a} (107)	264^{a} (101)	140^{a} (25)	133^{a} (59)	369^{a} (205)		221^{a} (173)	196^{a} (159)	88^{a} (65)
Diflubenzuron	ND	221^{a} (161)	179^{a} (143)	237^{a} (110)	105^{a} (26)	130^{a} (30)	116^{a} (50)	322^{a} (100)	*	13^{ab} (4.7)	1.7^{b} (1.6)	0.9^{b} (0.5)
Methoprene	ND	221^{a} (108)	404^{a} (179)	239^{a} (108)	677^{a} (316)	284^{a} (93)	168^{a} (47)	156^{a} (80)	*	97^{ab} (66)	32^{a} (17)	27^{ab} (20)
Chlorpyrifos	ND	136^{a} (57)	218^{a} (156)	91^{a} (74)	149^{a} (114)	47^{a} (5.2)	28^{a} (20)	105^{a} (81)	*	1.0^{b} (0.5)	1.0^{b} (0.6)	0.3^{b} (0.2)

Date	12-07	14-07	16-07	19-07	21-07	22-07	23-07	26-07	28-07	30-07	04-08	06-08
Day of Yr.	(194)	(196)	(198)	(201)	(203)	(204)	(205)	(208)	(210)	(212)	(217)	(219)
Control	48^{a} (27)	63^{a} (37)	58^{a} (32)	185^{a} (93)	423^{a} (388)		490^{a} (461)	248^{a} (235)	216^{a} (201)	32^{a} (16)	155^{a} (80)	169^{a} (103)
Diflubenzuron	0.3^{b} (0.3)	0.6^{b} (0.3)	0^{b} (-)	0.8^{b} (0.4)	0.5^{b} (0.3)	*	2^{b} (1)	1.1^{ab} (1.1)	2^{ab} (2)	0.1^{b} (0.1)	0.3^{b} (0.2)	1.2^{b} (1.1)
Methoprene	19^{a} (9)	15^{a} (6.7)	58^{a} (45)	118^{ab} (106)	93^{ab} (90)	*	28^{ab} (20)	52^{ab} (50)	56^{a} (48)	56^{a} (37)	91^{a} (44)	114^{a} (51)
Chlorpyrifos	0.4^{b} (0.2)	4.3^{ab} (4.3)	0^{b} (-)	1^{b} (1)	0.6^{b} (0.6)		1.7^{b} (0.9)	0.1^{b} (0.1)	0^{b} (-)	0^{b} (-)	0.1^{b} (0.1)	0^{b} (-)

Date	09-08	10-08	11-08	13-08	16-08	18-08	20-08	23-08
Day of Yr.	(222)	(223)	(224)	(226)	(229)	(231)	(233)	(236)
Control	483^{a} (241)		557^{a} (333)	42^{a} (37)	410^{a} (300)	555^{aa} (260)	ND	440^{a} (114)
Diflubenzuron	1.3^{ab} (0.7)	*	0.7^{c} (0.4)	0.4^{b} (0.1)	2.3^{b} (1.5)	0 (-)	ND	2.6^{b} (2.0)
Methoprene	129^{a} (110)	*	65^{b} (40)	126^{a} (61)	216^{a} (18)	661^{a} (190)	ND	346^{a} (200)
Chlorpyrifos	0^{b} (-)	*	0.1^{c} (0.1)	0.5^{b} (0.3)	0.4^{b} (0.4)	0.5^{b} (0.5)	ND	0.4^{b} (0.2)

*Indicates pools treated at that date.
**No data.
***Standard error.

though low, do not appear to be dependent upon the treatment dates, there is probably no effect on the Daphnia sp. populations in the pools.

Chlorpyrifos caused a reduction in Daphnia sp. numbers comparable to that of Diflubenzuron and similar to that reported by Hurlbert et al. (1972, 1970). This reduction is statistically significant on most sampling dates after the initial treatment until the end of the experiment. As mentioned, with Diflubenzuron Daphnia sp. populations were not annihilated and recovery would be expected after the treatments stopped.

Diflubenzuron and Chlorpyrifos caused severe reductions in Daphnia sp. which did not recover before the end of the experiment though recovery could be expected after the end of the experiment. Methoprene caused no detectable effect on Daphnia sp. populations.

Copepoda

Data concerning the effect of the treatments on copepod populations are shown in Table 29. There are no overall population trends as fluctuations were considerable especially with control pools. As seen with Daphnia sp. statistical analysis performed on this group suffered from this large variance and as a result treatment effects that are intuitively apparent are not statistically significant.

Diflubenzuron caused a population reduction after each treatment which was occasionally statistically significant. Recovery from this reduction was apparent within ca. 10 days of treatment though populations did not return to control levels during the post-treatment observation periods. This effect is similar to that reported by Miura and Takahashi (1974) though the repetitive treatments of this experiment delayed recovery.

TABLE 29. The effect of the pesticide treatments on populations of Copepoda as determined by Van Dorne bottle sampling. Data are expressed as the mean catch per Van Dorne bottle for each treatment group. Means followed by the same letters are not statistically different, (P≤0.05).

Date	16-06	18-06	21-06	23-06	25-06	28-06	30-06	02-07	03-07	05-07	07-07	09-07
Day of Yr.	(168)	(170)	(173)	(175)	(177)	(180)	(182)	(184)	(185)	(187)	(189)	(191)
Control	ND**	9.5^{a}	32^{a}	183^{a}	301^{a}	487^{a}	195^{a}	283^{a}		163^{a}	228^{a}	262^{a}
		(2.0)***	(12)	(82)	(184)	(160)	(134)	(72)		(64)	(166)	(208)
Diflubenzuron	ND	33^{a}	131^{a}	228^{a}	191^{a}	620^{a}	641^{a}	861^{a}	*	94^{ab}	39^{ab}	14^{ab}
		(19)	(86)	(64)	(67)	(407)	(416)	(509)		(70)	(26)	(8)
Methoprene	ND	18^{a}	44^{a}	59^{a}	112^{a}	439^{a}	103^{a}	328^{a}	*	450^{a}	476^{a}	269^{a}
		(16)	(36)	(39)	(53)	(238)	(81)	(199)		(246)	(248)	(191)
Chlorpyrifos	ND	84^{a}	127^{a}	68^{a}	96^{a}	417^{a}	323^{a}	387^{a}	*	6.1^{b}	3^{b}	4.0^{b}
		(3.8)	(22)	(15)	(8)	(161)	(104)	(113)		(2.4)	(0.8)	(1.0)

Date	12-07	14-07	16-07	19-07	21-07	22-07	23-07	26-07	28-07	30-07	04-08	06-08
Day of Yr.	(194)	(196)	(198)	(201)	(203)	(204)	(205)	(208)	(210)	(212)	(217)	(219)
Control	84^{a}	103^{ab}	151^{a}	842^{ab}	816^{a}		1380^{a}	600^{a}	802^{ab}	678^{a}	490^{ab}	424^{a}
	(12)	(44)	(68)	(226)	(766)		(1274)	(371)	(411)	(374)	(280)	(185)
Diflubenzuron	12^{b}	40^{ab}	55^{a}	136^{ab}	130^{a}	*	133^{a}	6.5^{b}	2.8^{b}	1.4^{b}	42^{b}	64^{a}
	(11)	(3.6)	(31)	(77)	(103)		(80)	(4.4)	(1.4)	(0.7)	(23)	(29)
Methoprene	141^{a}	127^{a}	163^{a}	244^{a}	173^{a}	*	253^{a}	552^{a}	621^{a}	801^{a}	727^{a}	481^{a}
	(101)	(15)	(49)	(14)	(41)		(116)	(257)	(275)	(334)	(55)	(39)
Chlorpyrifos	8.5^{b}	5.4^{b}	7.5^{a}	22^{b}	38^{a}		55^{a}	14^{b}	268^{b}	164^{ab}	271^{ab}	257^{a}
	(2.7)	(1.0)	(1.3)	(6.6)	(3.0)		(34)	(12)	(264)	(160)	(261)	(242)

Date	09-08	10-08	11-08	13-08	16-08	18-08	20-08	23-08
Day of Yr.	(222)	(223)	(224)	(226)	(229)	(231)	(233)	(236)
Control	515^{ab}		731^{b}	664^{b}	370^{ab}	1054^{a}	ND	469^{b}
	(198)		(422)	(319)	(227)	(493)		(199)
Diflubenzuron	43^{b}	*	11^{a}	15^{a}	18^{b}	28^{a}	ND	40^{a}
	(20)		(7.9)	(14)	(8)	(14)		(37)
Methoprene	890^{a}	*	714^{b}	686^{b}	551^{a}	1157^{a}	ND	493^{b}
	(309)		(262)	(358)	(216)	(394)		(309)
Chlorpyrifos	249^{ab}	*	220^{ab}	110^{ab}	123^{ab}	96^{a}	ND	24^{ab}
	(99)		(180)	(80)	(44)	(27)		(9.3)

*Indicates pools treated at that date.
**No data.
***Standard error.

Similar to results of Miura and Takahashi (1975) Methoprene caused no significant effect on copepod populations.

The first Chlorpyrifos treatment caused a significant reduction in copepods comparable to that of Diflubenzuron. A much less definite decrease is apparent after the second treatment. Hurlbert et al. (1970, 1972) reported a reduction in copepods and a recovery rate similar to that reported herein. The lesser effect of the second treatment may be due to a repopulation by the hatching of large numbers of copepod eggs present in the pools.

Diflubenzuron and the first Chlorpyrifos treatment caused comparable reductions in copepod populations while Methoprene caused no apparent effect.

Other Organisms

No detectable effect was apparent on populations of hydraacarina, Gastropoda or Anura (tadpoles) due to any of the treatments as monitored by VDB samples and/or qualitative observations as shown in Tables 30 to 33.

TABLE 30. The effect of the pesticide treatments on populations of hydraacarina as determined by Van Dorne bottle sampling. Data are expressed as the mean catch per pool for each treatment group.

Date Day of Yr.	16-06 (168)	18-06 (170)	21-06 (173)	23-06 (175)	25-06 (177)	28-06 (180)	30-06 (182)	02-07 (184)	03-07 (185)	05-07 (187)	07-07 (189)	09-07 (191)
Control	ND**	0 (-)***	0 (-)	0.3 (0.3)	0 (-)	0 (-)	0.3 (0.3)	0.3 (0.3)		0 (-)	0 (-)	0.3 (0.3)
Diflubenzuron	ND	0.3 (0.3)	0.3 (0.3)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	*	0.3 (0.3)	0.3 (0.3)	1 (0.6)
Methoprene	ND	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	*	0 (-)	0.6 (0.6)	0.3 (0.3)
Chlorpyrifos	ND	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	*	0.3 (0.3)	0.6 (0.3)	0 (-)

Date Day of Yr.	12-07 (194)	14-07 (196)	16-07 (198)	19-07 (201)	21-07 (203)	22-07 (204)	23-07 (205)	26-07 (208)	28-07 (210)	30-07 (212)	04-08 (217)	06-08 (219)
Control	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)		0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
Diflubenzuron	0.3 (0.3)	0.3 (0.3)	0.3 (0.3)	0 (-)	0.3 (0.3)	*	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
Methoprene	0.3 (0.3)	0 (-)	0.3 (0.3)	0 (-)	0 (-)	*	0.3 (0.3)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)
Chlorpyrifos	0 (-)	0 (-)	0.3 (0.3)	0 (-)	0.3 (0.3)		0 (-)	0 (-)	0 (-)	0 (-)	0 (-)	0 (-)

Date Day of Yr.	09-08 (222)	10-08 (223)	11-08 (224)	13-08 (226)	16-08 (229)	18-08 (231)	20-08 (233)	23-08 (236)
Control	0 (-)		0 (-)	0 (-)	0 (-)	0.3 (0.3)	ND	0 (-)
Diflubenzuron	0 (-)	*	0 (-)	0 (-)	0 (-)	0 (-)	ND	0 (-)
Methoprene	0 (-)	*	0 (-)	0 (-)	0 (-)	0.3 (0.3)	ND	0 (-)
Chlorpyrifos	0 (-)	*	0 (-)	0 (-)	0 (-)	0 (-)	ND	0.3 (0.3)

*Indicates pools treated at that date.
**No data.
***Standard error.

TABLE 31. Qualitative observations concerning the effect of the pesticide treatments on hydraacarina.

	Date Day of Yr.	02-07 (184)	03-07 (185)	04-07 (186)	06-07 (188)	09-07 (191)	12-07 (194)	15-07 (197)	18-07 (200)
	Pool No.								
Control	1	/		/	/	+	+	+	+
	8	/		/	/	+	/	/	/
	9	/		/	/	/	/	/	/
Diflubenzuron	7	/	*	+	+	+	+	/	/
	10	/	*	/	/	/	+	+	/
	12	/	*	+	+	/	+	+	+
Methoprene	2	+	*	/	+	+	+	/	+
	5	/	*	/	/	+	/	/	/
	11	+	*	+	+	/	+	+	+
Chlorpyrifos	3	/	*	/	/	/	/	/	/
	4	/	*	/	/	/	/	/	/
	6	/	*	/	/	/	/	/	/

TABLE 31. Cont'd.

	Date Day of Yr.	22-07 (204)	23-07 (205)	26-07 (208)	29-07 (211)	01-08 (214)	04-08 (217)	10-08 (223)	12-08 (225)	14-08 (227)
	Pool No.									
Control	1		/	ND	/	/	/		/	ND
	8		+	ND	+	/	/		/	ND
	9		/	ND	/	+	/		+	+
Diflubenzuron	7	*	+	ND	+	+	+	*	+	+
	10	*	/	+	/	/	+	*	+	/
	12	*	/	+	/	+	+	*	+	/
Methoprene	2	*	+	ND	/	/	/	*	/	ND
	5	*	/	ND	+	+	+	*	+	+
	11	*	/	/	/	/	/	*	+	+
Chlorpyrifos	3		/	/	/	/	/	*	/	ND
	4		/	/	/	+	+	*	/	ND
	6		/	+	/	/	/	*	/.	/

Observations are indicated by: + Present Alive, - Present Dead, / Not Observed, ND No Data.
*Indicates pools treated at that date.

TABLE 32. Qualitative observations concerning the effect of the pesticide treatments on anurans (tadpoles).

	Date Day of Yr.	02-07 (184)	03-07 (185)	04-07 (186)	06-07 (188)	09-07 (191)	12-07 (194)	15-07 (197)	18-07 (200)
	Pool No.								
Control	1	/		/	+	+	/	/	/
	8	/		/	/	/	/	/	+
	9	/		/	+	/	+	+	+
Diflubenzuron	7	/	*	/	+	/	+	+	+
	10	/	*	/	/	/	/	/	+
	12	/	*	+	/	/	+	/	+
Methoprene	2	/	*	/	/	+	/	/	/
	5	/	*	/	/	/	/	/	/
	11	/	*	/	/	/	/	/	+
Chlorpyrifos	3	/	*	/	/	/	/	/	/
	4	/	*	/	/	/	/	/	/
	6	+	*	/	/	/	/	/	/

TABLE 32. Cont'd.

	Date Day of Yr.	22-07 (204)	23-07 (205)	26-07 (208)	29-07 (211)	01-08 (214)	04-08 (217)	10-08 (223)	12-08 (225)	14-08 (227)
	Pool No.									
Control	1		/	ND	/	/	/		/	ND
	8		/	ND	/	/	/		/	ND
	9		/	ND	ND	/	/		/	/
Diflubenzuron	7	*	+	ND	+	/	/	*	/	/
	10	*	/	/	/	+	/	*	/	/
	12	*	/	/	/	/	/	*	/	/
Methoprene	2	*	/	/	/	/	/	*	/	ND
	5	*	/	/	/	/	/	*	/	/
	11	*	/	/	/	/	/	*	/	/
Chlorpyrifos	3		/	ND	/	/	/	*	/	ND
	4		/	ND	/	/	/	*	/	ND
	6		/	/	/	/	/	*	/	/

Observations are indicated by: + Present Alive, - Present Dead, / Not Observed, ND No Data.
*Indicates pools treated at that date.

TABLE 33. Qualitative observations concerning the effect of the pesticide treatments on gastropods.

	Date Day of Yr.	02-07 (184)	03-07 (185)	04-07 (186)	06-07 (188)	09-07 (191)	12-07 (194)	15-07 (197)	18-07 (200)
	Pool No.								
Control	1	/		+	/	+	/	+	+
	8	+		+	/	+	+	+	+
	9	+		+	+	+	+	/	+
Diflubenzuron	7	+	*	/	+	+	+	/	+
	10	+	*	/	/	+	+	/	+
	12	/	*	/	/	+	/	/	+
Methoprene	2	/	*	/	/	+	+	/	/
	5	/	*	/	/	+	+	/	/
	11	/	*	/	/	/	/	/	/
Chlorpyrifos	3	/	*	+	+	+	/	/	+
	4	/	*	+	+	+	/	/	/
	6	+	*	+	+	+	+	+	+

TABLE 33. Cont'd.

	Date Day of Yr.	22-07 (204)	23-07 (205)	26-07 (208)	29-07 (211)	01-08 (214)	04-08 (217)	10-08 (223)	12-08 (225)	14-08 (227)
	Pool No.									
Control	1		+	ND	+	+	+		+	ND
	8		+	ND	+	+	+		+	ND
	9		/	ND	ND	/	+		/	+
Diflubenzuron	7	*	/	ND	+	+	+	*	+	+
	10	*	+	/	/	/	/	*	+	+
	12	*	/	/	/	+	/	*	+	/
Methoprene	2	*	+	/	/	+	/	*	+	ND
	5	*	/	/	/	/	/	*	/	/
	11	*	/	/	/	/	/	*	/	/
Chlorpyrifos	3		+	ND	/	/	/	*	/	ND
	4		+	ND	+	+	/	*	+	ND
	6		+	+	+	+	+	*	+	/

Observations are indicated by: + Present Alive, - Present Dead, / Not Observed, ND No Data.
*Indicates pools treated at that date.

SUMMARY AND CONCLUSIONS

The disappearance from, efficacy in and effect of Diflubenzuron, Methoprene and Chlorpyrifos on a mosquito breeding habitat were studied in trial field experiments designed to determine relative effects of the three compounds and to determine whether the methods used were suitable for evaluating mosquito larvicides.

Methoprene and Diflubenzuron were found to disappear more rapidly than Chlorpyrifos from the water in the habitat as monitored by chemical analyses and/or bioassays with mosquito larvae, though none of the parent compounds would be expected to cause long term residues in the water. In view of the observed disappearance, the fate of these compounds, i.e. degradation pathways and/or adsorption to the substrate etc. warrants further study.

Chlorpyrifos was found to be the most effective compound for the control of Culex tarsalis while both Methoprene and Diflubenzuron showed lesser efficacy. Further research is warranted concerning development of slow release formulations of the growth regulators to improve their efficacy against mosquito species breeding in permanent water habitats.

Chlorpyrifos was the least environmentally desireable compound as it killed more non-target organisms in more taxa and resulted in a long non-target organism recovery period. Diflubenzuron was more selective and taxa affected recovered faster than with Chlorpyrifos while Methoprene was the most selective compound causing only brief population

reductions in specific taxa. The selectivity of compounds when applied to water is of considerable significance in view of the need to preserve non-target organisms which may, for example, serve as predators on target species, or provide prey for desireable organisms like fish.

Emergence trap sampling was considered the most efficient monitoring procedure used for the majority of organisms though this type of monitoring does not directly reflect the effects of the compounds (especially the growth regulators) on the aquatic ecosystem and does not monitor effects on solely aquatic species. Further research needs to be performed to develop sampling methods allowing detection of population trends in at least a few key taxa with a minimum of time and cost.

The ultimate goal of this type of experiment is to advise mosquito control agencies on the question of whether or not they should change their control tactics to use a new chemical. In the case of the chemicals studied here, clearly the opposing factors are proven efficacy and relatively low cost with highly undesireable side effects (Chlorpyrifos) and lesser efficacy with higher cost and minimal side effects (both growth regulators).

In western Canada Culex tarsalis is a significant vector of western equine encephalomyelitis. For the control of this species duration of control is of prime importance. In this view Chlorpyrifos (Dursban 2.5G) would be recommended when effects on non-target organisms in the habitat are of little significance. If application must occur to sites where non-target organisms are of importance, Diflubenzuron (Dimilin WP-25) would be the preferred insecticide despite the shorter period of control.

Methoprene could not be recommended due to its very low efficacy.

These recommendations refer to the above formulations only and could be altered with development of slow release formulations for the growth regulators or resistance to the insecticides.

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APPENDICES

APPENDIX A. Analysis of Soil in Pools 1 and 12, June 29/76.

Pool	pH	Conductivity (mmhos)	NO_3-N (mg/l)	Available P (mg/l)	Available K (µg/l)	Organic material (%)
1	6.9	0.8	23.8	45.4	700+	13.2
12	7.2	0.8	2.0	54.8	700+	8.2

APPENDIX B. Water temperature readings of three probes in pool 12.

	Temperature (C°)					
	Top		Middle		Bottom	
Date (Day)	Max.	Min.	Max.	Min.	Max.	Min.
28-06 (180)	24.2	16.6	20.4	16.7	18.9	16.5
29-06 (181)	26.2	17.0	22.3	17.1	20.0	16.8
30-06 (182)	ND*	18.0	ND	18.2	ND	18.0
01-07 (183)	30.0	ND	26.1	ND	23.7	ND
02-07 (184)	29.3	20.9	26.6	21.0	24.5	20.6
03-07 (185)	28.7	20.5	26.0	20.6	24.6	20.3
04-07 (186)	28.9	20.4	26.2	20.4	25.1	20.1
05-07 (187)	27.8	20.4	25.5	20.4	24.3	20.1
06-07 (188)	27.8	21.2	26.5	21.5	25.8	21.4
07-07 (189)	31.0	19.1**	26.5	20.4	25.3	20.0
08-07 (190)	29.1	16.0**	24.9	21.0	24.1	20.5
09-07 (191)	35.0	19.2**	27.9	21.1	20.9	25.8
10-07 (192)	28.3	19.1**	25.0	22.5	24.7	20.6
(Water added to all pools)						
11-07 (193)	24.6	18.6	23.5	17.6	22.7	17.3
12-07 (194)	23.3	18.1	22.5	18.5	22.1	17.9
13-07 (195)	29.5	19.4	25.4	19.4	24.2	19.0
14-07 (196)	28.0	20.6	26.3	20.6	26.9	20.1
15-07 (197)	22.7	17.2	22.7	17.7	22.2	16.8
16-07 (198)	23.2	15.1	21.3	15.9	21.7	14.9
17-07 (199)	29.8	16.0	24.0	17.0	21.7	16.1
18-07 (200)	28.0	18.1	24.0	19.2	21.8	18.8
19-07 (201)	25.4	19.1	23.2	20.2	22.1	20.1
20-07 (202)	25.5	19.1	23.1	18.2	21.6	17.7
(Water added to all pools)						
21-07 (203)	25.9	18.0	23.6	18.2	22.2	17.8
22-07 (204)	26.5	18.8	23.5	19.2	22.2	18.4
23-07 (205)	25.4	18.8	23.3	19.1	22.8	18.5
24-07 (206)	28.8	18.2	24.5	18.4	22.7	18.2
25-07 (207)	26.2	19.8	23.7	20.0	23.2	19.6
26-07 (208)	30.5	17.0**	24.7	20.1	23.8	19.5
27-07 (209)	32.3	11.2**	24.4	19.5	22.8	18.5
28-07 (210)	29.7	10.0**	23.8	19.3	22.7	18.5
(Water added to all pools)						
29-07 (211)	21.2	16.3	20.6	18.7	20.6	17.5
30-07 (212)	26.2	18.2	23.6	17.9	21.6	17.6
31-07 (213)	25.0	18.0	24.7	18.5	22.7	18.0
01-08 (214)	25.0	18.1	22.1	18.7	20.4	18.1
02-08 (215)	25.4	18.2	21.4	18.7	19.8	18.1
03-08 (216)	28.4	18.7	25.6	19.3	23.1	19.2
04-08 (217)	25.3	18.6	24.9	19.2	22.9	19.2
05-08 (218)	21.0	18.0	20.0	19.0	20.0	18.6
06-08 (219)	26.2	18.1	24.6	19.0	22.6	18.4
07-08 (220)	30.2	17.9	26.1	19.1	22.4	18.3
(Water added to all pools)						

APPENDIX B. Cont'd.

Date (Day)	Temperature (C°)					
	Top		Middle		Bottom	
	Max.	Min.	Max.	Min.	Max.	Min.
08-08 (221)	24.7	17.4	23.6	18.4	21.6	17.6
09-08 (222)	22.6	18.0	22.4	18.6	21.4	18.1
10-08 (223)	27.8	17.6	22.6	18.3	20.6	17.6
11-08 (224)	25.0	16.0	23.2	17.9	20.0	17.4
12-08 (225)	20.0	16.8	20.1	17.8	19.2	17.1
13-08 (226)	21.1	17.8	20.4	18.4	19.4	17.4
14-08 (227)	ND	16.9	ND	18.0	ND	17.2
15-08 (228)	24.4	ND	20.5	ND	19.6	ND
16-08 (229)	29.0	11.4**	19.8	16.2	18.8	15.4
17-08 (230)	31.0	16.6**	22.8	17.7	20.7	17.5
18-08 (231)	32.0	17.8**	22.6	19.8	21.6	19.4
(Water added to all pools)						
19-08 (232)	27.3	21.2	23.6	21.0	22.9	20.9
20-08 (233)	27.0	21.1	24.8	21.1	22.9	20.9
21-08 (234)	25.3	20.2	24.0	20.3	21.9	19.7
22-08 (235)	27.0	18.3	22.0	18.5	21.0	18.4
23-08 (236)	ND	17.0	ND	18.3	ND	17.8

*No data.
**Probes above water level.

APPENDIX C. Glenlea, Manitoba Meteorological Data.

		Daily air temp. (C^{o}) Max.	Min.	Rainfall (cm)	Sunshine (hrs)
June/1976	1	27.8	17.8	-	11.6
	2	30.0	18.9	-	10.4
	3	30.5	18.3	-	7.1
	4	31.1	18.3	-	5.3
	5	30.5	17.2	0.56	3.5
	6	28.9	13.3	3.43	6.0
	7	22.2	13.3	0.10	1.0
	8	23.8	13.3	0.31	6.2
	9	27.8	15.0	3.25	1.5
	10	27.2	13.8	-	13.5
	11	28.3	16.1	2.06	6.7
	12	25.0	13.3	1.49	2.3
	13	17.7	12.2	0.64	0.1
	14	16.7	9.4	0.08	0
	15	12.8	3.3	-	3.1
	16	20.5	10.0	0.58	6.3
	17	14.4	6.7	-	3.9
	18	20.0	8.9	-	15.1
	19	25.6	12.7	-	15.5
	20	32.2	14.4	0.03	11.3
	21	23.8	11.6	0.05	9.1
	22	24.4	17.2	-	8.9
	23	28.8	17.2	-	14.4
	24	22.7	12.8	3.48	4.4
	25	20.0	11.7	0.25	5.0
	26	18.8	10.0	0.86	2.5
	27	22.7	8.3	-	10.8
	28	21.1	10.0	0.36	5.9
	29	22.7	10.0	-	14.8
	30	25.0	12.2	-	13.8
July/76	1	26.7	12.7	-	14.6
	2	27.7	15.0	-	14.5
	3	26.7	14.4	-	13.2
	4	28.3	17.2	-	14.9
	5	30.0	14.4	0.13	5.8
	6	27.2	12.2	-	12.1
	7	27.7	16.6	-	14.1
	8	27.2	18.3	0.79	4.4
	9	30.0	17.2	-	13.5
	10	23.8	10.6	-	3.6
	11	20.5	10.0	-	10.2
	12	23.9	13.9	0.03	5.3
	13	29.4	14.4	-	11.2
	14	27.7	10.6	0.05	15.1
	15	16.7	7.2	0.15	3.0

APPENDIX C. Cont'd.

		Daily air temp. (C^{o}) Max.	Min.	Rainfall (cm)	Sunshine (hrs)
July/76	16	23.8	7.8	-	12.8
	17	27.7	13.8	-	14.5
	18	31.1	17.7	0.13	7.9
	19	27.7	6.7	0.10	2.9
	20	26.1	8.3	-	13.6
	21	26.1	16.7	-	14.6
	22	29.4	9.4	-	11.0
	23	27.2	7.2	-	14.7
	24	28.8	15.6	0.43	13.4
	25	26.7	13.3	-	7.3
	26	27.7	8.8	-	13.5
	27	30.6	7.7	0.03	7.9
	28	25.0	15.0	-	13.9
	29	22.2	10.0	0.23	2.8
	30	21.2	5.6	-	7.0
	31	24.4	7.2	-	13.2
August/76	1	26.1	7.2	-	13.6
	2	27.2	11.7	-	13.1
	3	32.2	16.1	-	9.9
	4	25.6	8.3	-	4.5
	5	21.1	6.1	-	14.0
	6	26.7	10.0	-	13.8
	7	31.1	15.5	-	12.9
	8	25.6	12.2	0.05	2.4
	9	22.8	13.3	0.76	2.8
	10	29.4	12.2	-	11.1
	11	25.0	16.1	1.14	7.4
	12	18.3	14.4	-	0
	13	23.9	14.4	-	6.2
	14	25.0	9.4	-	11.1
	15	24.4	11.7	-	13.5
	16	26.1	15.0	-	9.8
	17	28.9	15.6	-	11.3
	18	32.2	18.9	0.99	5.4
	19	31.1	17.8	0.48	6.4
	20	33.3	12.2	-	9.6
	21	27.8	9.4	-	13.3
	22	28.9	12.7	-	13.3
	23	31.7	18.3	-	12.8
	24	36.7	14.4	-	12.2
	25	28.9	14.4	0.15	7.8
	26	28.9	13.8	0.41	7.0
	27	18.9	5.0	0.03	4.9
	28	12.7	3.9	-	5.7
	29	23.3	8.3	-	12.1
	30	27.8	7.2	-	9.0
	31	18.3	2.8	-	8.9

APPENDIX D. Water Quality Analysis.

June 29/76

181 Pool #	NH_3-N µg/l	TDN µg/l	PO_4-P µg/l	TDP µg/l	Susp P µg/l	CO_2 µmole/l	DOC µmole/l	Susp C µg/l	Cl mg/l	Na mg/l
1	170	4440	1310	1570	633	6650	2640	2600	46.5	31.1
2	1090	5840	2180	2480	766	7730	2490	3380	53.0	30.1
3	240	4860	1300	1530	628	7690	2640	2640	58.5	32.1
4	310	5320	1750	2020	904	7530	2650	3890	47.0	30.9
5	1390	5820	2620	2910	930	7570	2150	3110	54.0	30.7
6	40	4280	1120	1320	1583	8310	2280	20750	64.5	70.8
7	110	4440	940	1130	813	7500	2330	3930	70.5	33.1
8	140	4130	880	1050	781	6900	2230	4780	55.0	31.1
9	320	5210	690	890	887	8170	2290	3820	73.5	34.1
10	70	3820	870	1065	687	8320	1935	4170	73.0	34.5
11	30	4020	1360	1620	575	8630	2300	6660	59.0	27.5
12	30	4420	650	910	920	8790	2210	14050	85.5	29.7

	K mg/l	Mg mg/l	Ca mg/l	Mn mg/l	pH	TDS mg/l	Chloro-a µg/l	TSS mg/l	Fe mg/l
1	14.7	50.2	60.7	4.15	8.03	550	15.0	9	1.76
2	19.1	47.0	74.7	4.56	7.86	640	29.8	12	2.98
3	18.8	48.0	75.2	4.04	7.91	630	15.1	14	2.37
4	17.8	45.9	73.0	3.54	7.83	630	40.5	16	2.37
5	19.7	44.8	71.4	3.38	7.78	660	17.3	16	2.20
6	22.8	51.2	80.6	3.79	7.64	690	152	140	1.66
7	19.7	49.1	73.0	3.27	8.04	660	21.0	17	1.36
8	16.3	44.8	65.5	3.32	7.84	580	21.6	19	1.02
9	20.0	53.4	82.7	4.67	7.91	720	21.8	19	1.42
10	19.4	52.3	87.8	2.94	7.94	700	25.4	17	1.22
11	22.5	54.4	85.4	2.61	7.87	680	17.1	23	0.78
12	22.5	58.7	98.3	4.51	7.75	820	106	39	0.78

APPENDIX E. Pool water pH readings prior to treatment.

Pool	Date Day of Yr.	30-06 (182)	22-07 (204)	20-08 (233)
1		7.8	7.7	8.3
2		7.7	7.9	7.3
3		7.8	7.8	7.8
4		7.6	7.8	8.1
5		7.8	8.0	7.6
6		7.6	7.7	7.4
7		7.8	7.8	7.5
8		7.8	8.0	8.2
9		7.7	8.1	7.6
10		7.6	8.1	7.6
11		7.7	7.9	7.9
12		7.8	7.8	7.5

APPENDIX F. Storage Study - Diflubenzuron.

This study was undertaken to determine the stability of Diflubenzuron when stored in the dark for 180 days as were the Diflubenzuron-GLC samples discussed in this thesis.

Pond water was obtained through the ice from the large pond to the east of the experimental pools (the water source for the experimental pools) on January 3, 1977. One hundred ml of this water was quantitatively delivered into each of 50, 250 ml bottles on January 3, sealed (cf. Methods), frozen, and stored in the dark at -20°C. On January 4, and every 30 days thereafter for 180 days, 8 bottles were removed from the freezer and thawed. Four of these samples were spiked at 10 µg/ℓ and 4 at 40 µg/ℓ with analytical grade Diflubenzuron. Ten ml of pesticide grade ethyl acetate were added to 2 samples of each concentration after which the samples were sealed and hand shaken for 2 mins. All 8 samples were then returned to the freezer until the end of 180 days.

After 180 days the samples spiked on days 1, 30 and 180 were analyzed by GLC (cf. Methods) to determine the concentration of Diflubenzuron present.

The results, corrected for recovery (cf. Worobey and Webster 1977a) are as follows:

Theoretical Conc. (µg/ℓ)	Mean Measured Conc. (µg/ℓ) Day 1	Day 30	Day 180
10	9.7 (97**) (0.2)*	12.1 (121) (0.4)	9.4 (94) (0.6)
40	43.3 (108) (4.6)	41.6 (104) (3.2)	39.4 (99) (3.7)

*Standard error.
**Percent recovery.

As these results indicated no appreciable decrease in concentration the samples between Day 30 and 180 were not analyzed.

Degradation may have occurred though as the analyses used co-chromatographs of the parent compound and its primary metabolite. The results shown above as well as those discussed in the thesis would indicate that degradation did not occur and that the samples were stable as stored.