The Influence Of Formulation On The Toxicity And Rate Of Uptake Of Methoxychlor In Rainbow Trout (*Salmo gairdneri* Richardson) And Some Non-Target Species Of Aquatic Insects When Used As A Black Fly (Diptera:Simuliidae) Larvicide

A Thesis
Submitted to the Faculty
of
Graduate Studies
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by
Robert John Sebastien

In Partial Fulfillment of the
Requirements for the Degree
of
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ABSTRACT

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The Influence of Formulation on the Toxicity and Rate of Uptake of Methoxychlor in Rainbow Trout (Salmo gairdneri Richardson) and some Non-target Species of Aquatic Insects when used as a Black Fly (Diptera:Simuliidae) Larvicide.

A comparison between two formulations of methoxychlor regarding toxicity and rate of uptake in rainbow trout, a species of black fly larvae, two species of stone fly nymphs and a species of chironomid larvae was made. An emulsifiable concentrate formulation (25% active ingredient) was compared with a particulate formulation consisting of celite particles the majority of which were 8-15 μm in size impregnated with methoxychlor (63% active ingredient).

Rainbow trout (Salmo gairdneri Richardson) exposed to a level of 0.3 mg/litre of the E.C. formulation of methoxychlor all exhibited signs of morbidity and after a period of 48 hrs. there was 10% mortality. Fish exposed to the particulate formulation showed no mortality after 48 hrs. and no signs of morbidity were observed during this observation period. At a concentration of 1 mg/litre the E.C. killed 98% of the fish and the particulate none after 48 hrs. At an exposure level of 5 mg/litre there was 100% mortality in fish exposed to the E.C. formulation and only 10% mortality in the particulate exposure after 48 hrs. Fish exposed to a theoretical concentration of either 0.1 or 0.3 mg/litre of the emulsifiable formulation accumulated more than five times as much methoxychlor as fish exposed to similar levels of the particulate material after a period of 1 hr. There was very

little difference between fish residues at the two application rates for the particulate formulation, but for the E.C. residues at the 0.3 mg/litre exposure level were two to three times those at the 0.1 mg/litre level.

Black fly larvae (Simulium decorum Walker) were killed by the particulate formulation as effectively as the E.C. at all concentrations and exposure times tested. Larvae of this species exposed to four concentrations of the particulate formulation (11.1, 33.0, 100.0, 300.0 ppb) accumulated significantly greater levels of methoxychlor before detaching and required much longer periods of time to detach than larvae exposed to similar concentrations of the emulsifiable concentrate formulation.

Stone fly nymphs of the species Acroneuria lycorias (Newman) after exposure for 1 and 12 hrs. to 0.3 mg/litre of the E.C. formulation accumulated at least six times as much methoxychlor as nymphs exposed to the particulate material. Stone fly nymphs of the species Pteronarcys dorsata Say after exposure for 1 and 12 hrs. to both formulations of methoxychlor at a level of 0.3 mg/litre accumulated twice as much of the E.C. formulation as the particulate formulation. After a period of 24 hrs. all nymphs of this species exposed to the E.C. formulation showed morbidity symptoms whereas only 25% morbidity was observed in nymphs exposed to the particulate formulation.

Larvae of Chironomus tentans Fabricius (4th instar) when exposed to 0.1 mg/litre of the E.C. formulation showed 99% morbidity after a period of 96 hrs. as compared to about 55% morbidity in larvae exposed

to the particulate formulation. At 0.3 mg/litre all larvae were moribund in the E.C. exposure after 96 hrs. whereas 85% morbidity was observed in the particulate exposure. Larvae exposed to the emulsifiable concentrate formulation accumulated significantly higher levels of methoxychlor than larvae exposed to the particulate formulation at both exposure concentrations (0.1 and 0.3 mg/litre) throughout the duration of the experiment.

These data indicate that particulate methoxychlor formulations should be effective in controlling black fly larvae and may reduce uptake and risk of poisoning by fish, stonefly nymphs and to a lesser extent chironomid larvae under field conditions.

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INTRODUCTION

In some regions of Canada blood-sucking black flies (Diptera: Simuliidae) are the most serious insect pests that affect both humans and livestock. Black flies can affect the health of man and animals in several ways: they are vectors of disease, their bites are injurious and because of their great abundance and habit of landing and crawling about on the head, face and other parts of the body, their presence may be at times unbearable. Large economic losses have been recorded in livestock and poultry in many parts of Canada due to uncontrolled outbreaks of black flies (Fredeen 1969, 1977; Millar and Rempel 1944; Rempel and Arnason 1947).

In an effort to prevent damaging outbreaks of black flies, chemical larvicides are continuously under test, and in the past chemicals have been the only significant means by which these pests have been controlled. Because of their highly restricted and specific breeding habits and their method of breeding in running water, black fly larvae are particularly susceptible to control by chemicals. When a chemical larvicide is applied to a stream or river, it is automatically dispersed by the current to sites below the point of treatment. Larvae may be killed by exposure to larvicides in three ways: 1) by ingestion of toxic particles, 2) by exposure of the cuticle to contact insecticides, or 3) indirectly by starvation or anoxia if forced to release from their site of attachment by the insecticide and are carried downstream into pools (Jamnback 1976). Most of the larvicides used for control purposes are emulsions or oil formulations and although very effective in eliminating black fly larvae, are also detrimental to other members of

the stream fauna (Flannagan et al. 1979; Fredeen 1974, 1975; Wallace et al. 1973, 1975).

Black fly larvae strain particulate material unselectively from the rapidly flowing waters they breed in whereas most other stream fauna tend to be a little more selective in their method of feeding (i.e. predators or foragers). Insecticide formulations as water-insoluble particles of a size range similar to that ingested by simuliid larvae, may be ingested specifically by them and not by most other stream fauna (Helson 1972).

Research concerned with particulate formulations was initiated in the early 1950's when several researchers concluded that DDT adsorbed onto inorganic matter (Fredeen et al. 1953a; Fredeen et al. 1953b; Noel-Buxton 1956) and DDT formulated as fine water-insoluble particles (Kershaw et al. 1965; Kershaw et al. 1968; Helson 1970) were effective black fly larvicides and may have been less harmful to non-target organisms. Field tests with a particulate formulation of methoxychlor also indicated a good effectiveness on black fly larvae and a reduced impact on many non-target groups of organisms (Helson 1970, 1972; Helson and West 1978).

In the present study, a particulate formulation of methoxychlor identical to Helson's formulation (Helson and West 1978) was compared with an emulsifiable concentrate formulation for efficacy against black fly larvae and detrimental effects on fish and non-target groups of aquatic fauna. The toxicity and rate of uptake of each formulation was observed in rainbow trout (Salmo gairdneri Richardson), a species of black fly (Simulium decorum Walker), two species of stoneflies (Acroneuria

lycorias (Newman) and Pteronarcys dorsata Say) and a chironomid species (Chironomus tentans Fabricius) in order to assess the potential of particulate formulations as a possible alternative to the emulsifiable formulations presently in use to control black fly larvae.

CHAPTER I

LITERATURE REVIEW

A. Economic Importance of Black Flies

In the hilly and mountainous areas of Canada which are traversed by numerous fast-flowing streams and rivers, black flies are the most serious insect pests that affect humans. Several species have been observed to feed on humans in Canada including Simulium venustum Say, Prosimulium fuscum/mixtum Syme and Davies, Prosimulium fulvum (Coquillett), Simulium decorum Walker, Simulium tuberosum (Lundstrom), Simulium parnassum Mallock, Simulium griseum Coquillett, and Simulium vittatum Zetterstedt. In the pulpwood cutting areas in Canada, black flies have caused much economic loss resulting from low efficiency and high rates of labour turnover in the peak biting periods of spring and early summer (Fredeen 1977). Similarly reductions in tourism during this period in the northern regions can probably be attributed to reluctance on the part of visitors not previously exposed to such outbreaks to "offer themselves as black fly bait". It is difficult to assess the effects of black flies on the activities of man, since individuals vary so much in their tolerances and in their abilities to understand the problems and to protect themselves. It would be very difficult to estimate unrealized tourist trade and impossible to estimate effects on resident family members whose normal outdoor and recreational activities are curtailed, not only by black flies but other biting flies as well (Fredeen 1977).

Toxins injected during an extended severe attack, can cause a

general illness sometimes called "black fly fever", characterized by headache, fever, nausea and swollen, painful neck glands. Transmission of disease organisms or parasites to humans by black flies is unknown in Canada (Fredeen 1977).

A number of species including Simulium arcticum Mallock, Simulium luggeri Nicholson and Mickel, Simulium venustum, Simulium vittatum and Prosimulium mixtum/fuscum are serious pests of livestock in Canada. The most prominent species of black fly affecting livestock in Canada is Simulium arcticum whose most severe outbreaks have occurred in the strong rapids in the lower sections of the north and south branches of the Saskatchewan River, in a portion of the North Saskatchewan in Alberta, and from large rapids in the mid-sections of the Athabasca River (Fredeen 1977). On the western Canadian prairies, and perhaps in all of Canada, Simulium arcticum is the only species known to cause livestock fatalities by direct poisoning. This is accomplished when the adult females are blood-feeding on the livestock and inject salivary components into the animals. Reactions to massive injections of the toxin are swift, and the animals can become fatally ill within a few hours after the black flies commence their attack (Millar and Rempel 1944). The number of losses in livestock attributed to black fly outbreaks in the prairie provinces has been very large over the years and in addition to cattle, losses have also been recorded in horses, sheep, swine and even wild deer (Fredeen 1969, 1977). Declines in the production of milk and beef are frequently observed by livestock owners, but losses are difficult to measure. Reports from owners such as: "black flies kept cattle in shed all day," "cows' udders red with blood,"

cows difficult to milk because of black flies," "black flies drove cattle out of the pasture," "milk production down something terrible," are not uncommon during outbreaks (Fredeen 1969). A loss that is seldom recognized in an area subjected to frequent, severe black fly outbreaks is the general shift from livestock to alternate farm enterprises. A good example of this is the Athabasca region of Alberta which is an area where livestock enterprises should predominate to ensure the healthiest economic development of the region because of the relatively short frost-free season, the rough terrain with much marginal land suitable only for pasture and forage crops, and soils that require crop rotations for best productivity. Some residents have reduced their livestock operations or shifted to less productive enterprises, and certain highly skilled breeders have even emigrated to other areas where they will not be threatened by black flies (Fredeen 1969).

In Canada, diseases are transmitted by black flies only to domestic and wild birds and none have as yet been documented in domestic mammals or humans. However in Alberta and British Columbia there have been scattered reports by veterinarians of onchocerciasis in horses. The horses involved were treated for chronic skin conditions but were not disabled by the disease (Fredeen 1977). Leg worms, Onchocerca cervipedis in moose and elk have been reported in the prairie provinces. Certain species of black flies have been shown to transmit this parasite however the vectors have not been proven to be totally restricted to the Simuliidae (Pledger 1978). The main simuliid-transmitted parasites in Canada are the various species of Leucocytozoon. All are parasitic only in birds. In the western mountains of Canada about 45% of all birds

examined carried this haematozoon in the blood, and in the eastern Appalachian and Laurentian mountains about 23% (Greiner et al. 1975). Savage and Isa (1945) reported that leucocytozoan infections killed 5,000 turkeys out of a flock of 8,000 located near the Assiniboine River in Manitoba. Laird and Bennett (1970) observed that leucocytozoan infections were responsible for massive losses of domestic geese at Fort Chimo on Ungava Bay. A number of species of black flies have been implicated as vectors of Leucocytozoon in Canada including Simulium rugglesi Nicholson and Mickel, Simulium meridionale Riley, Simulium aureum Fries, Simulium latipes (Meigen) and Simulium venustum. Many of these same species have also been observed as vectors of microfilariae and trypanosomes in Canada (Greiner et al. 1975).

It is evident that black flies do cause a substantial amount of economic loss in Canada and demand considerable attention from a control standpoint.

B. Present Chemicals Used to Control Black Fly Larvae and Their Effects on Non-Target Stream Insects

In 1969 DDT was banned for general use in Canada with the discovery of its significant effect on non-target organisms, its long-term stability in the environment and its biomagnification in the food chain. Methoxychlor, a chlorinated hydrocarbon similar to DDT, and Abate, an organophosphorous insecticide were two of the larvicides to be tested as a possible replacement for DDT, and have been the two most commonly used chemicals for the control of black fly larvae in recent years.

1) Methoxychlor

In the past emulsifiable concentrate formulations of methoxychlor applied from the ground at a single injection point and aerial applications of methoxychlor with surfactants have demonstrated good control of black fly larvae for long distances below the point of application (Fredeen 1974, 1975). However these treatments have not been entirely selective for black fly larvae and there exists some disagreement among investigators concerning the effects of methoxychlor on non-target arthropods. G.E. Burdick et al. (1968) could detect no trace of methoxychlor in the food chain after 36 days indicating that it was not likely to accumulate in the ecosystem. A large number of insects.were observed drifting through the area after treatment but no evidence was found that all or most of these insects later died. From this work it was concluded that "methoxychlor as a black fly larvicide seemed unlikely to produce residues of the magnitude and persistence known to occur when DDT was used."

Wallace et al. (1973) noted a heavy post-treatment drift of invertebrates following treatment levels of .075 ppm/15 minutes methoxychlor. Post-treatment samples indicated that the non-target organisms studied were not completely eradicated from the streams. In further studies Wallace and Hynes (1975) recorded reductions of about 75% in stream fauna which included Trichoptera, Plecoptera, and Ephemeroptera after applications of 15% methoxychlor at a rate of 1 gallon per flight mile. This heavy mortality was attributed to the fact that the stream was sprayed over its entire length instead of the usual 1/4 mile in-

tervals. Ground application tests using methoxychlor solution (with 0.5% surfactant) applied at a rate of 0.075 ppm/15 minutes produced a large arthropod drift and reduced insect populations by about 83%.

In the Saskatchewan River, single 15-minute injections of 0.18 to 0.24 ppm methoxychlor (emulsifiable concentrate) and 7.5-minute ground applications of 0.6 ppm methoxychlor reduced populations of Plecoptera, Ephemeroptera, Trichoptera, and Chironomidae, however numbers of these groups of insects were completely restored within 1 to 7 weeks. It was also observed that species existing in sand and loose gravel were not affected (Fredeen 1974, 1975).

In contrast to this Flannagan et al. (1979) observed reductions in populations of Plecoptera, Ephemeroptera, and Trichoptera to a distance of 400 km downstream from the application point after a single 0.3 ppm/15 minute injection of methoxychlor into the Athabasca River. Little recolonization of these orders was recorded within 4 weeks after treatment. It was also observed that all non-target invertebrates, regardless of trophic level, appeared to be affected at about the same time. The similarity in reaction time among animals of differing trophic levels exhibiting different feeding mechanisms e.g. predaceous Plecoptera, detritus-feeding Ephemeroptera and filter-feeding Simuliidae, suggested that methoxychlor when applied as an emulsifiable concentrate was not necessarily an internal poison but may kill or disable on contact. This refuted the suggestion of Fredeen (1974, 1975), that methoxychlor (emulsifiable concentrate) adsorbed onto suspended particles carried in the water and was thus selective to filter feeding animals, acting as an internal poison after ingestion of the particles

with the chemical adsorbed on them.

An eleven year study was conducted in the Adirondack Mountains of New York State by Burdick et al. (1974). The spray program was done by aircraft using the swath technique which was repeated two to three times annually over an area of 150 square miles. The area was originally sprayed with DDT for three years, then left untreated for two years followed by six years of treatment with methoxychlor. Stream sampling indicated that there was a slight decline in diversity and productivity annually associated with the methoxychlor and DDT spraying, but that recovery occurred by the next season if not sooner. Stream fauna was reduced by 40.1% for DDT and 21.3% for methoxychlor when compared to control streams.

2) Abate

Abate is an organophosphorus insecticide which has been applied in a wide range of different formulations and has also demonstrated a good ability to control black flies. In the provinces of Quebec and Labrador Abate is the only larvicide approved for the ground treatment of black fly larvae in streams (Wallace et al. 1973). Effective control has been obtained using emulsions at concentrations ranging from 0.05 ppm/10 minutes to 0.54 ppm/30 minutes (Swabey et al. 1967; Wallace et al. 1973; Escaffre et al. 1974; Elouard et al. 1974).

Despite its ability to control black fly larvae, Abate has also been observed to have a definite effect on non-target organisms inhabiting the streams where it was applied. Wallace et al. (1973) observed a 20% reduction in non-target organisms 24 hours after treatment

with Abate emulsion ground application at the rate of 0.1 ppm/15 minutes. The insects affected were mayflies, stoneflies, caddisflies, and midges. However, despite the loss, all the non-targets continued to be present after treatment in all streams tested.

In West Africa reductions of 88% of invertebrate rock fauna were observed after treatment with 0.5 ppm/10 minutes Abate (Lauzanne and Dejoux 1973).

Treatments of 0.1 ppm/10 minutes of Abate were applied once a week over a period of four months to a stream in West Africa. Samples of stream arthropods taken regularly from this treated stream and adjacent untreated streams showed a 31% population reduction of stream fauna in the treated stream as compared to a slight population increase in the control stream. It was concluded that a season's treatment had some effect on the non-target organisms but that treatment of a larger area would produce a much greater reduction because recolonization from surrounding untreated streams would be severely limited (Dejoux and Troubat 1974).

Newfoundland streams treated with Abate wettable powder suspensions at rates of 0.091 ppm/22 minutes resulted in mortality of gastropods, chironomids, baetid mayflies and some caddisflies and recovery required more than three weeks. It was noted that two treatments two weeks apart did not seem to produce an additive or synergistic effect (Wilson and Snow 1972).

In tests with 2% celatom granules of Abate applied at a rate of 0.2 lbs/acre in South Carolina non-target species inhabiting the treated streams were observed to suffer no adverse effects (Kissam

et al. 1973).

C. Effects on Fish of Present Black Fly Larvicides and Possible Protection by Particulate Formulations

It has been shown that fish are able to metabolize methoxychlor to more polar non-toxic metabolites which are then eliminated. There is no evidence of the persistent concentrating effect found with DDT in fish fatty tissues. This suggests that fish surviving a dose of methoxychlor can eliminate the residues fairly rapidly. Biodegradability is irrelevant, however, if the fish receive a lethal dose of chemical (Gardner and Bailey 1975). Field tests using either methoxychlor or Abate at recommended levels for black fly control in the past have indicated little injury or adverse effects on fish subsequent to the exposure (Fredeen 1974, 1975; Lauzanne and Dejoux 1973; Wilson and Snow 1972; Wallace et al. 1973). The only documented case of a fish kill after exposure to a black fly larvicide was recorded by Philippon et al. (1973) who observed a definite lethal effect on fish 24 hours after aerial application of methoxychlor at a rate of 0.3 ppm/10 minutes. It has also been suggested that methoxychlor may not kill fish by direct poisoning but could adversely affect the population by reducing available food supplies (Lockhart 1977; Wallace 1973).

A recent study by Lockhart et al. (1977) showed that morbidity in fish was more directly related to the residue levels in tissue such as liver and kidney as opposed to muscle residues. Muscle residues (which have been used by the majority of investigators), were apparently not a suitable indicator of toxicity to rainbow trout and their value in monitoring may well be restricted to judging the quality of fish as

human food. It was also observed that caged suckers and chub contained much less liver methoxychlor than did wild suckers and chub caught in hoopnets. Thus cage bioassays used to monitor the effect of the larvicides on fish by most previous researchers had systematically underestimated risk of fish poisoning.

While there is little evidence for acute fish poisoning by black fly larvicides, few experiments have examined the effects of sublethal chemical concentrations over long periods of time. Merna et al. (1972) and Merna and Eisele (1973) exposed perch (Perca flavescens Mitchell) to a concentration of 0.625 µg/l for 6½ months and observed reduced growth when compared to controls. Long-term exposure of perch to 5 µg/l methoxychlor showed an abnormally high oxygen demand when tested in a respirometer against a water velocity of 0.6 ft/sec. Eggs from fathead minnows (Pimephales promelas Rafinesque) exposed to a concentration of 0.125 µg/l showed decreased hatchability. Clearly more field studies are required to assess the effect of sublethal concentrations of black fly larvicides on fish over long periods of time.

While it has long been known that fish may acquire chemical residues by feeding on contaminated aquatic insect drift as a result of black fly larviciding many researchers have suggested that uptake of DDT and related insecticides occurs, primarily by way of the gills. This was confirmed by Lockhart et al. (1977) who observed that the uptake of methoxychlor by rainbow trout was remarkably rapid, especially into the liver. Such a rapid uptake into deep tissues like liver and kidney by non-feeding fish implied distribution by the vascular system after uptake at some site, presumably the gills.

Lockhart (1980) found that river water containing 75 mg/litre of suspended solids decreased fish uptake of methoxychlor to about 50% of that in laboratory water containing low suspended solids.

Zitko (1974), after observing that chlorinated paraffins were much less, if at all accumulated by juvenile Atlantic Salmon when the fish were exposed to the chemical adsorbed on silica or fed contaminated food, surmized that uptake from silt particles although possible, may be less efficient than uptake of dissolved material.

Helson (1972) found that no trout in his fine screened cages died and none contained methoxychlor residues following treatment with a particulate formulation of methoxychlor whereas a slight mortality occurred in his fine screened cages several days after treatment with methoxychlor in oil solution. He concluded that particulate methoxychlor may not be absorbed directly by trout, whereas methoxychlor in oil solution was. This suggests a possible protection to fish by particulate formulations of black fly larvicides.

D. Control of Black Fly Larvae Using DDT in Association with Particulate Material

Fredeen et al. (1953a) observed after applications of .09 ppm 10% DDT in methylated naphthalene and kerosene for 16 minutes that in waters containing a suspended solids content of 551 ppm, larvae were eliminated for a distance of 115 miles whereas in waters having a suspended solids content of 183 ppm, larvae were removed for a distance of less than 80 miles from the treatment site. When the river was turbid, black fly larvae were destroyed, but other aquatic insects were not affected whereas when the river was less turbid, aquatic insects were affected

to a lesser degree than were simuliid larvae. In further tests it was indicated that DDT applied to clear water streams showed no selective action and was effective for only a few hundred yards to six miles. The long distances of effectiveness of the DDT applied to the Saskatchewan River was thought to be due to the adsorption of the chemical by the suspended solids in the water. The noted greater mortality of black fly larvae than of other aquatic insects was believed to be due to the direct consumption of the suspended particles in the river by the black fly larvae whereas most of the other aquatic fauna were more selective in their mode of feeding and normally did not consume small particles suspended in water. It was concluded from these experiments that effective control could be obtained in other fast flowing turbid rivers by similar treatments and in clear-water streams and rivers improved control could be attained by the addition of finely divided inorganic material with marked DDT adsorptive properties, or by treating with such material with the DDT already incorporated into it (Fredeen et al. 1953b).

This suggestion was taken by Noel-Buxton (1956) in work concerning the control of black flies on the Gold Coast of Africa. The initial test was conducted on the Kamba River in which turbidities were low with suspended solids content not exceeding 120 ppm. The insecticide was a conventional DDT solution to which was added heavily clayed water with hard soap as an emulsifying agent. An application rate of 0.1 ppm/20 minutes produced complete destruction of larvae over a distance of three miles, but was not entirely successful at 6. Throughout the duration of the application no destruction of fish or non-target insects

was observed. Further treatments using dosages exceeding 0.1 ppm proved dangerous to fish, while those below 0.07 ppm failed to yield complete destruction. In subsequent tests the DDT solution in a kerosene: clay: soap mixture was heated thoroughly to ensure a more complete adsorption of the DDT onto the clay particles than had been obtained in the previous trials. In 1954 this formulation was introduced into the Kamba River at a rate of 0.03 ppm for 23 minutes and in 1955 into the Black Volta River in Ghana at 0.044 ppm for 30 minutes. Simulium larvae were eliminated for 24 miles in the Kamba River and 40 miles in the Black Volta River. In both these trials fish and stream invertebrates were not injured (Noel-Buxton 1956).

In Alberta experiments were performed using several different formulations of DDT and heptachlor tested as black fly larvicides in irrigation canals. Oil solutions and emulsions of these insecticides applied at dosages of 0.1 and 0.2 ppm/15 minutes almost completely eliminated larvae from three canals when the water contained 521 ppm suspended solids. Larvae were only partially eliminated with a suspended solid content of 322 ppm and no effect was observed until the dosage was doubled with a suspended solid content of 25 ppm. Improved results were achieved by the use of formulations containing finely-divided diatomaceous earth in addition to heptachlor. Fifteen minute applications at 0.1 ppm of a commercial dusting powder (2.5% on celite) applied either dry or as a water slurry virtually eliminated larvae from 23 miles of one 41 mile canal, and from the entire length of a 10-mile canal. In clear waters it was believed that the celite particles were responsible for carrying the heptachlor in useful amounts for more

than 20 miles. These results confirmed that there was a direct relationship between effectiveness of the oil solution of DDT and water turbidity (Fredeen 1962).

E. Control of Black Fly Larvae with Particulate DDT

Williams et al. (1961) observed that the size of particles ingested by a British species of Simulium was about 10-12 µm. In order to test the selectivity of an insecticide having a particle size of the same order of magnitude, water-insoluble particles of DDT with a size range of 4-15 µm were tested in streams in North Wales. A dosage of 0.5 ppm for 30 minutes produced complete elimination of all larvae from polyethene tapes placed 150 yards below the application point for a period of one month, and no effect on other stream fauna was observed (Kershaw et al. 1965). In further examinations a rate of .04 ppm/30 minutes reduced the numbers of larvae, but did not remove them entirely and no change occurred among the bottom fauna. A dosage of 0.2 ppm for 30 minutes caused simuliid larvae to be removed for 150 yards with no detrimental effects on non-target organisms. An application rate of 0.4 ppm/120 minutes produced complete elimination of larvae for 1,100 yards, but also killed mayfly nymphs of the genus Baetis and the fresh water shrimp (Gammarus) population was temporarily shifted downstream. No changes in the population of benthic stonefly naiads or caddisfly larvae were noticed which along with fish are natural predators of black fly larvae. Thus, with all dosages tested, no predators of the black fly larvae were affected which prevented the recovery populations of Simulium from rising above normal as can happen when soluble

DDT formulations are used to remove simuliid larvae and also predators (Davies 1950). These results illustrate that DDT used as a particulate insecticide of a size appropriate for ingestion by Simulium larvae and having a slow rate of sedimentation which allows it to be carried in suspension in a fast-running stream, will selectively remove black fly larvae (Kershaw et al. 1968).

Travis and Wilton (1965) observed that solid formulations (e.g. suspensions and wettable powders) were more effective than the emulsions in killing black fly larvae and concluded the reason was because of the method of feeding.

More recently (Helson 1970) investigated the efficacy of particulate DDT and its selectivity for Simuliidae larvae in a number of streams in North Eastern Quebec. A dosage of 0.1 ppm/15 minutes particulate DDT controlled black fly larvae very effectively. In some streams stonefly naiads and chironomid larvae were not seriously harmed whereas the mayfly nymph of genus Baetis and a family of caddisflies (Philopotamidae) were adversely affected (Helson 1970).

F. Control of Black Fly Larvae with Particulate Abate and Methoxychlor and the Effect on Non-Target Stream Insects

Wallace (1971) investigated the effects of methoxychlor in oil solution and emulsions of Abate compared to particulate formulations of methoxychlor and Abate on invertebrate stream fauna. Ground applications of 0.075 ppm methoxychlor for 15 minutes and 0.1 ppm Abate for 15 minutes were administered. It was observed that all major orders of immature stream insects were injured after treatment with methoxychlor in oil solution. The ranges of numbers of three orders caught in post-

treatment drift nets from three streams were: 675-7,906 Ephemeroptera, 8,271-36,000 Plecoptera, and 928-3,247 Trichoptera. In comparison, ranges of 55-2,511 Ephemeroptera, 275-1,026 Plecoptera and 55-5,335 Trichoptera were obtained from three streams treated with particulate methoxychlor.

The drift of Ephemeroptera naiads was generally smaller in the streams treated with particulate methoxychlor than in the streams treated with methoxychlor in oil solutions. Baetidae naiads were the most numerous mayfly naiads in the drift nets of the "particulate" streams.

Fewer Plecoptera naiads were collected after treatment with particulate methoxychlor than after treatment with methoxychlor in oil solution. Of those collected, only Leuctridae naiads were present in high numbers in the drift net samples in the "particulate" streams whereas Perlodidae and Nemouridae naiads were most abundant in the "oil" streams.

The overall numbers of drifting Trichoptera larvae was comparable between the two formulations, however only the numbers of Philopotamidae larvae increased after application with particulate methoxychlor, whereas several families of caddisflies were numerous in the driftnets following treatment with methoxychlor in oil solution.

All major orders of immature stream insects were injured by emulsions of Abate (Wallace 1971). Ranges of 9,429-66,240 Ephemeroptera, 1,079-10,792 Plecoptera and 1,660-19,154 Trichoptera were obtained in post-treatment drift of three streams treated with an emulsion of Abate. In comparison, ranges of 605-2,035 Ephemeroptera, 1,603-4,620 Plecoptera,

and 788-94,622 Trichoptera were found in the post-treatment drift of two streams treated with particulate Abate.

A greater number of drifting mayfly naiads were collected following treatment with Abate emulsions than after treatment with particulate Abate.

Virtually every family of Ephemeroptera was harmed in the "emulsion" streams whereas only Heptageniidae were altered in the "particulate" streams.

Helson (1970, 1972) treated several streams in the vicinity of Baie Comeau, Quebec with particulate formulations of methoxychlor and Abate which were metered into streams at a dosage of 0.1 ppm for 15 minutes.

The particulate formulation of methoxychlor removed 94-100% of the larvae for a distance of 600 yards downstream of the treatment point. Results indicated that effects on Chironomidae larvae varied among streams. Very heavy drift occurred in some streams, however chironomid pupae and other Diptera larvae and pupae apparently were not affected appreciably by particulate methoxychlor.

Particulate methoxychlor did not harm most types of mayfly naiads severely, although Baetidae nymphs were affected consistently with mortalities of 15-20% being observed.

Several types of stonefly naiads apparently were not harmed by particulate methoxychlor although Leuctridae naiads were slightly affected and represented the most abundant family of stoneflies in these streams.

Trichoptera larvae were not affected following treatment with

particulate methoxychlor although one family (Philopotamidae) was harmed considerably.

Coleoptera larvae and adults were not harmed by particulate methoxychlor.

The applications of particulate Abate removed 92-100% of the simuliid larvae in the majority of streams tested. Black fly pupae did not appear susceptible to particulate Abate.

Chironomidae larvae were harmed in two streams treated with particulate Abate, however chironomid pupae and other Diptera larvae and pupae appeared to exhibit no measurable effects to particulate Abate. Particulate Abate had no significant effect on the family Ephemerellidae of the Ephemeroptera but did harm small Heptageniidae naiads. The families Nemouridae and Leuctridae were affected by the formulation however other Plecoptera groups were not affected to any great extent. Most Trichoptera larvae were not altered, however Philopotamidae larvae were harmed severely in two of the streams tested. The families Elmidae and Dytiscidae of the Coleoptera may have been affected slightly by the particulate Abate formulation.

Philopotamidae and Chironomidae larvae in some streams were harmed severely by both particulate methoxychlor and Abate. These groups are filter feeding organisms and collect particles of a size range corresponding to the particulate insecticides and thus may have ingested these chemicals inadvertently. Organisms such as Baetidae, Heptageniidae, Leuctridae and Nemouridae naiads, Elmidae larvae and adults and Dytiscidae larvae were also slightly affected by the particulate formulations. Most of these insects excluding Dytiscidae larvae are reported to be

herbivorous detritus foragers that feed on material of small size. The particulate insecticides used had a specific gravity heavier than water, hence some of the material settled out on the stream bottom in areas of sedimentation of detritus and silt (Egglishaw 1964). Subsequently some of these organisms may have ingested enough particulate insecticide to be harmed and swept downstream.

Comparison of the effects of particulate formulations of methoxychlor and Abate and the corresponding liquid formulations on non-target stream fauna indicate clearly that particulate formulations are less harmful to several kinds of immature insects. However it seems that Philopotamidae and Chironomidae larvae are harmed at least as severely by particulate larvicides as by liquid formulations.

Recently, Wallace et al. (1976) in lab experiments illustrated that black fly larvae exposed to particulate formulations versus ethanol solutions of methoxychlor concentrated the particulate preparation in much greater amounts. The larvae concentrated the ethanol formulation of methoxychlor to levels ranging from 82 to 688 µg/kg, and much higher levels of the particulate formulation (1556-2310 µg/kg). Accumulations in Trichoptera were greater with the ethanol formulation (1453-1563 µg/kg) than with the particulate one (615-782 µg/kg). This may have resulted following adsorption of the ethanol formulation through the large gill surface area of the Trichoptera larvae while the majority of the particulate formulation was screened out. Again these results confirm that particulate formulations are selective for black fly larvae and less harmful to non-targets.

G. Feeding Habits of Lotic Aquatic Invertebrates

The lotic aquatic invertebrates can be divided into two general categories based on their method of feeding: the passively feeding organisms, and the actively feeding organisms. Cummins (1973) classified aquatic insect trophic relations based on feeding mechanisms. The passively feeding organisms were termed "Collectors", whereas the actively feeding organisms were divided into a number of categories based on feeding mechanisms including "Shredders, Scrapers, Piercers, Engulfers (Predators) and Parasites".

1) Passively Feeding Organisms

Most black fly larvae have cephalic head fans which trap particles from the water flowing through them. The particles are then transferred to the mouth parts and eventually enter the cibarium for retention until a bolus is formed and subsequently swallowed (Chance 1970; Craig 1974, 1977; Maitland and Penney 1967). It is believed that black fly larvae are probably indiscriminate in the type of food they trap and ingest and have been observed ingesting diatoms, algae, detritus (organic debris), sand grains, silt, clay particles, bacteria, pollen and fungal structures (Abdelnur 1968; Peterson 1959; Anderson and Dicke 1960; Carlsson 1967; Davies and Syme 1958; Fredeen 1960, 1964).

Black fly larvae appear to ingest certain sizes of particulate matter selectively. Chance (1970) has shown that several Canadian species including Simulium decorum, Simulium venustum, and Simulium vittatum, ingest certain sizes of particles the majority of which are 10 to 100 microns in diameter. The maximum dimension of

natural food ingested was 300 microns and of Sephadex beads 345 microns; the minimum size of natural food ingested was less than 0.5 microns. It was also observed that the range of ingested particle sizes varied with the age and the species of larvae. Chance (1969) recommended that an insecticide with a particulate formulation in the larger half of the ingested size distribution (100 to 250 microns) would be more readily ingested by black fly larvae than by other species in the stream fauna.

Several British species of black fly larvae were observed to ingest particles of the following size ranges: longest axis, 11.3±1.8 to 15.1 ±2.0 microns; shortest axis, 6.0±1.7 to 8.0±1.8 microns (Williams et al. 1961).

Wotton (1976) recorded some British species of black fly larvae as ingesting particles of colloidal size (as small as 0.091 µm in diameter). It was concluded from this study that an insecticide preparation consisting of particles smaller than 10 µm in diameter would reduce sedimentation and allow the treatment to be even more selective than particulate insecticides of a 10 µm diameter. In further studies it was observed that the mean length of particles ingested by moorland stream black fly larvae ranged from 1.4±1.4 µm to 2.6±10.6 µm with an overall range of 0.5 µm to 258 µm. In all larval guts over 50% of particles were less than 1 µm long (Wotton 1977).

Helson (1970) also performed some research on the size of particles ingested by a number of Canadian species of black flies including mature larvae of Simulium venustum and Simulium decorum. These two species ingested similar sizes of particles and the size range most frequently ingested was: longest axis 1 to 35 microns; shortest axis

1 to 15 microns.

Other members of the stream benthos also exhibit filter-feeding mechanisms. Trichoptera larvae of the families Hydropsychidae, Philopotamidae, and Psychomyiidae spin silken nets that trap food material (Ross 1944; Betten 1934; Edington 1965; Philipson 1953). Most Hydropsychidae are polyphagous feeding on a variety of food items. Some small hydropsychids mainly trap and ingest algae and diatoms (Chutter 1968; Jones 1949). Philopotamidae larvae live in loose silken passageways or in sac-shaped nets in which food material is trapped and is subsequently cleaned off by the larvae with their mouth parts (Lloyd 1921). Diatoms and detritus comprise the major food items of these larvae. Although Psychomyiidae larvae spin nets in which they catch food, the family is mainly carnivorous.

Several stream-inhabiting species of Chironomidae larvae are also passive feeders (Pennak 1953). Diatoms and detritus appear to be the principle food items for chironomids in streams, however a fair amount of sand may also be consumed (Jones 1949; Percival and Whitehead 1929).

Some Ephemeroptera naiads such as Isonychia use long fringes of hairs on their forelegs as nets to trap food particles (Hynes 1970).

2) Actively Feeding Organisms

Most stream insects use their mouth parts to actively gather food from the substrate and are thus classified as actively feeding organisms.

Ephemeroptera naiads forage on a great variety of aquatic vegetation although a few rare members are partly or entirely predaceous (Edmunds

1957).

Stream dwelling Coleoptera adults and larvae have a wide range of feeding habits; e.g. Dytiscidae larvae are predaceous whereas Elmidae adults and larvae are herbivorous.

Families of Trichoptera larvae and Plecoptera naiads are either carnivorous, herbivorous, or omnivorous.

These predaceous and grazing groups are probably more selective in the type of food they consume than are the passive feeders and would probably ingest minute insecticide particles only by accident. Thus the feeding habits of the various groups of aquatic fauna seem to indicate that particulate insecticides, if formulated in the right size range may be selective for black fly larvae (Chance 1969; Fredeen et al. 1953a; Fredeen et al. 1953b; Helson 1972, 1978; Kershaw et al. 1965; Kershaw et al. 1968).

CHAPTER II

MATERIALS AND METHODS

A. Formulations of Methoxychlor

A particulate formulation of methoxychlor 2,2-bis (p-methoxyphenyl)-1,1,1-trichloroethane was supplied by Dr. A.S. West (Newfoundland-Labrador Hydro). It was originally produced by Johns-Manville Research and Engineering Centre, Manville, New Jersey, as arranged by Dupont of Canada Limited. This particulate formulation was reported to consist of 70% technical methoxychlor oil concentrate (90%), 25% micro-cel-E, 1.5% igepon T-77 and 3.5% polyfon T. The insecticide particles 8 to 15 microns in size, were produced by micropulverization to yield particles with an average specific gravity of approximately 1.5, and a methoxychlor content of 63% by weight (Fig. 1) (Helson 1972; Helson and West 1978).

An emulsifiable concentrate formulation (E.C.) of methoxychlor was obtained from a drum of commercial material being used by the City of Winnipeg. It was 25% methoxychlor supplied by Sanex Chemicals, Mississauga, Ontario.

Both formulations were analyzed a number of times by gas-liquid chromatography as described by Solomon and Lockhart (1977). The resulting assay values of 60% methoxychlor for the particulate and 21% for the E.C. were used in calculating desired doses in all experiments.

B. Preparation of ^{14}C Labelled Methoxychlor Formulations

Methoxychlor was synthesized from ^{14}C-anisole using the procedure of Schneller and Smith (1949) scaled down to milligram quantities.

This product was kindly supplied by Mr. D.A. Metner, Freshwater Institute, Winnipeg, Manitoba.

1) Particulate Formulation

The ^{14}C labelled methoxychlor was purified on a silica gel thin layer chromatography plate developed with hexane:chloroform:methanol 3:2:1. An autoradiogram was made of the plate (1 week exposure Kodak x-ray film), and the silica gel containing the purified ^{14}C-methoxychlor was scraped off and transferred to a glass tube. The methoxychlor was eluted off the silica gel with 4x3 ml washings of acetone. The washings were then combined in a 50-ml beaker and 200 mg of the particulate methoxychlor formulation was added. The acetone was slowely evaporated off with stirring for about two hours. The residue was dried at $40^{o}C$ for 1 hr and then transferred to a petri dish and pulverized with a glass pestle. The total methoxychlor was determined by gas liquid chromatography and the activity was determined by counting an aliquot of a hexane extract of a known weight of particles on a Packard Tri-Carb 3310 Scintillation Counter and by directly combusting a sample of the particulate on a Packard Tri-Carb Sample Oxidizer (model 306) followed by counting. The total amount of methoxychlor in the particulate formulation (active ingredient) was not altered by this radiolabelling process and remained at 60%. The specific activity was determined to be 1.85×10^{5} D.P.M./mg methoxychlor.

2) Emulsifiable Concentrate Formulation

The ^{14}C-methoxychlor was purified and eluted as described earlier. Sufficient activity was added to a scintillation vial containing acetone.

The acetone was evaporated off and 98.0 mg of the emulsifiable concentrate formulation was added. A further 10 mls of water was added and the vial was capped and shaken to form an emulsion. The total methoxychlor content was determined by gas liquid chromatography and the radioactivity by counting an aliquot of a hexane extract on a Packard Tri-Carb 3310 Scintillation Counter and by directly combusting a sample of the emulsifiable concentrate on a Packard Tri-Carb Sample Oxidizer (model 306) and then counting it. The amount of methoxychlor in the emulsifiable concentrate formulation (active ingredient) was not altered by this labelling process and remained at 21%. The specific activity was determined to be 1.95×10^5 D.P.M./mg methoxychlor. It was necessary to prepare a new amount of ^{14}C labelled emulsifiable concentrate methoxychlor for experiments conducted on stonefly nymphs because of a shortage of the original preparation. The same method was used except in this case the specific activity of the formulation was determined to be 2.17×10^5 D.P.M./mg methoxychlor. This value was used in calculating all uptake residue levels in stonefly nymphs.

C. Fish Studies

1) Toxicity of Each Formulation

Rainbow trout fingerlings (Salmo gairdneri) were acclimated for several weeks at 17°C prior to all experiments. Four glass aquaria were used for these experiments, each filled with 30 litres of dechlorinated Winnipeg tap water and maintained at 17°C with aeration. Quantities of the appropriate formulation of methoxychlor were added to yield theoretical concentrations of 0.3, 1, and 5 mg/litre in three

separate experiments. Each experiment was performed twice, so that emulsifiable concentrate and particulate formulations could be compared at each concentration using 4 aquaria. Glass rods were used to mix the chemical in the aquaria. After mixing, 15 rainbow trout fingerlings were added to each aquarium. A 5 ml water sample was taken at the beginning of each experiment after mixing for later analysis by gas liquid chromatography as described by Solomon and Lockhart (1977). Similar water samples were taken after 4 hrs, 8 hrs and 24 hrs in the 5 mg/litre exposure experiment. Frequent observations were made to note abnormal behaviour, onset of morbidity, and time of death. Fish were counted as dead when they were in an inverted position with no observable respiratory movement. The experiment was terminated after a period of 48 hrs.

2) Rate of Uptake of Each Formulation

Four glass aquaria were used as described above (Toxicity of Each Formulation). The desired quantity of each formulation was added to each aquarium to yield a theoretical concentration of either 0.1 or 0.3 mg/litre methoxychlor. Ten rainbow trout fingerlings were placed in each aquarium for 1 hr. Water samples (5 ml) were taken at the beginning of each experiment. After the 1 hr exposure period the fish were removed from the water, weighed, wrapped in foil, and frozen until analyzed. The entire experiment using both exposure concentrations was performed twice.

3) Methoxychlor Residue Analysis

Methoxychlor in whole fish was measured by gas liquid chromatography

using a Tracor MT-220 instrument equipped with an electron-capture detector (Solomon and Lockhart 1977). Fish were cut into pieces and extracted with n-hexane in a ball-mill. Samples were "cleaned-up" on small Florisil columns and column effluents were concentrated with the glass filament concentrator described by Solomon and Muir (1978). Concentrated extracts were analyzed by injection into the gas chromatograph and peak areas were compared with those resulting from injection of known quantities of methoxychlor.

D. Black Fly Larval Studies

1) Comparative Larval Mortality at Different Concentrations and Exposure Times of the Particulate and Emulsifiable Concentrate Formulations

Black fly larvae were collected from trailing vegetation and a small dam face at La Barriere Park in Winnipeg. The larvae were placed in a plastic bucket between two pieces of damp paper towels for transport to the laboratory. All black fly larvae used in these tests were identified as Simulium decorum. In the laboratory 30 larvae were placed in each of four 2000 ml pyrex beakers containing 1800 ml of dechlorinated Winnipeg tap water. The beakers were placed on magnetic stirrers and aerated. Three glass rods were placed in each beaker and the temperature for the entire experiment was maintained at 19°C (Fig. 2). Larvae were allowed about 1 hr to attach to the glass rods in areas of maximum current and aeration created by the magnetic stirrers and air stones. At the end of this period the desired quantity of the appropriate formulation of methoxychlor was added to 3 beakers to yield a theoretical concentration of either 0.3, 0.09 or 0.03 mg/

litre methoxychlor. The 4th beaker was used as a control in each test and 1 ml of acetone was added in place of the chemical. Each of these three initial concentration levels of methoxychlor was tested at three different exposure times (7.5 min, 30 min, and 120 min) for both the particulate and emulsifiable formulations to observe comparative % mortality of the black fly larvae. In each test the chemical was added at 10 minute intervals to the three test beakers in order to prevent congestion of work at the end of the exposure period. After introduction of the methoxychlor, a 5 ml water sample was taken from the test beakers and from the control beaker to be analyzed later by gas liquid chromatography as described by Solomon and Lockhart (1977). When the desired exposure time had expired for the concentration of methoxychlor formulation being tested (e.g. .3 mg/litre/30 min, particulate), the contents of the beakers were poured off through a piece of fine mesh netting. Larvae that had become detached during the exposure period were thus caught by the netting, and they were then rinsed with river water (from La Barriere Park) back into the beaker. River water was added until the beakers contained 1800 ml. The larvae were left in the river water with continuous stirring and aeration for approximately 20 hours, when they were removed and examined in a dissecting tray.

To determine larval mortality rates, each larva in turn was pressed down firmly with the edge of a needle and its reaction observed. If the larva reacted immediately by bending double and continued this sort of motion for at least several seconds, it was classed as "alive". If the larva did not respond at all when thus irritated, or if only the head capsule of a decomposed larva was found, it was classed as "dead".

If the larva flexed its body incompletely and only once, before returning to a straightened position with the mouth parts quivering, it was considered moribund and classed also as dead (Thompson 1975).

The numbers of live and dead larvae for the three test replicates at each concentration and exposure time for each formulation were summed, and the mortality of the test group relative to the control mortality for each concentration and exposure time of the methoxychlor formulations was calculated using Abbot's formula:

$$\text{Corrected test mortality} = \frac{\%\ \text{test mortality} - \%\ \text{control mortality}}{100\% - \%\ \text{control mortality}} \times 100\%$$

2) Methoxychlor Residues Causing Single Black Fly Larvae to Release and Drift Using ^{14}C Labelled Methoxychlor

a) Emulsifiable Concentrate Formulation

Black fly larvae were collected from the site previously mentioned at La Barrier Park in Winnipeg. Larvae were placed in a plastic bucket between two pieces of damp paper towels and then transported to the laboratory. All black fly larvae used in these experiments were identified as Simulium decorum. In the laboratory 20 larvae were placed in each of four 2000 ml pyrex beakers containing 1800 ml of dechlorinated Winnipeg tap water. The experimental apparatus was the same as described in part 1 of this section (Fig. 2). Larvae were allowed about 1 hr to attach to the glass rods in areas of maximum current and aeration created by the magnetic stirrers and air stones. After this period the ^{14}C labelled emulsifiable concentrate methoxychlor (activity level - 1.95×10^5 D.P.M./mg methoxychlor) was added to yield theoretical concentrations of 11.1, 33.0, 100.0, and 300.0 p.p.b. to

the four beakers. The chemical was added at 1 hr time intervals to the four test beakers in order to prevent congestion of work when the larvae began to release and drift. A 5 ml water sample was taken after introduction of the methoxychlor in each beaker for subsequent analysis by gas chromatography. Larvae were now observed for abnormal behaviour and when they released from their attachment sites on the glass rods and began drifting (Fig. 3), the time of drift of each individual larva was recorded, and larvae were removed from the beaker using a small fine-meshed net. Each larva was then rinsed thoroughly with distilled water and dried on a paper towel, then wrapped in a small piece of filter paper and frozen for later analysis. When the experiment had terminated the individual larvae were "thawed" for about 10 minutes and then combusted on a Packard Tri-Carb Sample Oxidizer (model 306) and subsequently counted on a Packard Tri-Carb 3310 Scintillation Counter. The entire experiment was performed twice.

b) Particulate Formulation

The same experimental procedure was followed using the ^{14}C labelled particulate formulation (activity level - 1.85×10^5 D.P.M./mg methoxychlor) as was previously described using the ^{14}C labelled emulsifiable concentrate formulation except for a few minor changes. Individual drifting black fly larvae were placed in small cellulose cups which were weighed both before and after the larvae were placed in them so that the weight of each drifting simuliid larvae was recorded. Also after combustion on the Packard Tri-Carb Sample Oxidizer (model 306), individual larvae were counted on a Beckman LS-7500 Scintillation

Counter.

E. Stonefly Nymphal Studies

1) Toxicity of Each Formulation

Nymphs of the stone fly species Pteronarcys dorsata were collected from the Roseau River in Manitoba and transported back to the laboratory in plastic bags filled with river water. The bags were kept cool by placing them in a cooler on top of ice packs. A re-circulating flow-thru system was used to perform the experiment in the laboratory. The system consisted of four plexi-glass troughs 60x11.5x17.5 cm. Two small 115 volt, 60 cycle pumps were used to pump the water through the troughs. Two aquaria, each containing 28 litres of dechlorinated Winnipeg tap water, served as reservoirs into which the pumps were placed to circulate the water out through p.v.c. plastic pipes, into the troughs, and then back to the aquaria (Fig. 4). Stones collected from the Roseau River were placed in the troughs to serve as substrate for the stonefly nymphs. Twenty-five nymphs of Pteronarcys dorsata were placed in each of the four troughs and allowed 12 hrs before introduction of the methoxychlor formulations. The temperature was maintained at 17°C throughout the experiment (temp. of water in Roseau River). A theoretical concentration of 0.3 mg/litre of the particulate formulation was applied to one aquarium, and 0.3 mg/litre of the emulsifiable concentrate formulation was applied to the second aquarium. Separate glass rods were used to mix the chemical into each of the aquaria. After mixing, frequent observations were made to note

abnormal behaviour and onset of morbidity. Nymphs were considered moribund if they showed total loss of equilibrium and failed to right themselves and seek shelter when probed. Water samples (5 ml) were taken immediately after mixing of the chemical, and at 1 hr, 4 hrs, and 24 hrs after introduction of the methoxychlor formulations. The experiment was terminated after 24 hr.

2) Rate of Uptake of Each Formulation Using ^{14}C Labelled Methoxychlor

Stonefly nymphs of the species Acroneuria lycorias and Pteronarcys dorsata (Fig. 5) were collected from the Roseau River Manitoba and transported back to the laboratory in ice-cooled plastic bags containing river water. The same re-circulating flow-thru system was used to perform this set of experiments as was used previously in the toxicity experiment (Fig. 4). Eight nymphs of Acroneuria lycorias and eight nymphs of Pteronarcys dorsata were placed in each of the four troughs 12 hrs before the methoxychlor formulations were applied. The temperature was maintained at $17^{o}C$ throughout the experiment. A theoretical concentration of 0.3 mg/litre of the ^{14}C labelled particulate formulation (activity level - 1.85×10^{5} D.P.M./mg methoxychlor) was applied to one aquarium, and 0.3 mg/litre of the ^{14}C labelled emulsifiable concentrate formulation (activity level - 2.17×10^{5} D.P.M./mg methoxychlor) was applied to the second aquarium. The chemical was mixed into each aquarium using separate glass rods. After a period of 1 hr, all nymphs were removed from the troughs, rinsed thoroughly (individually) with distilled water, dried on clean paper towels, and placed in pre-weighed cellulose cups (Fig. 6). The cups

containing the individual nymphs were then re-weighed so the weight of each individual stonefly nymph could be calculated. These samples were then combusted on a Packard Tri-Carb Sample Oxidizer (model 306) (Fig. 7) and subsequently counted on a Beckman LS-7500 Scintillation Counter. Five ml water samples were taken in each trough immediately after introduction of the methoxychlor formulations, and 1 hr after introduction of the chemical when the nymphs were removed. A second similar experiment was performed except that nymphs were removed 12 hrs after exposure to the methoxychlor formulations instead of at 1 hr. In this experiment water samples were taken immediately after mixing of the chemical, 6 hrs after mixing, and 12 hrs after introduction of the methoxychlor formulations when the stonefly nymphs were removed.

F. Chironomid Larval Studies

1) Toxicity of Each Formulation

A colony of Chironomus tentans was initiated in the laboratory as described by Townsend (1980). The laboratory culture apparatus consisted of an aquarium with an air-stone at one end separated from the rest of the aquarium by a screen partition. Silica sand was placed in the large area on the opposite side of the partition from the air-stone to serve as substrate and the tank was filled with dechlorinated Winnipeg tap water (Fig. 8). Chironomus tentans reproduces under these conditions, has a short life cycle (21-34 days at 20°C) and readily feeds on commercially available fish food, such as Tetramin. The large, bright red, fourth and final instar larvae emerge from the third molt about 14-20 days after introduction of an

egg mass (Townsend 1980).

Silica sand was placed in five 2000 ml pyrex beakers until the 200 ml level was reached in each beaker with sand. Dechlorinated Winnipeg tap water was introduced into each beaker until the water reached the 1200 ml level. Thirty-five Chironomus tentans fourth instar larvae were placed in each beaker with a small amount of Tetramin fish food and allowed 12 hrs to burrow into the substrate before the methoxychlor formulations were introduced. A theoretical concentration of 0.1 mg/litre of the particulate formulation of methoxychlor was applied to two of the beakers and 0.1 mg/litre of the emulsifiable concentrate formulation was added to two of the beakers. The final beaker served as a control and 1 ml of acetone was added in place of the methoxychlor formulations. The chemical was gently mixed into each beaker using separate glass rods. After mixing a 5 ml water sample was taken from each beaker for later analysis by gas liquid chromatography as described by Solomon and Lockhart (1977). The experiment was conducted at 20°C. Frequent observations were made to note signs of morbidity characterized by the chironomid larvae leaving their burrows in the sediment and exhibiting a continuous wiggling motion (Fig. 9). After a period of 48 hrs the water in each beaker was siphoned off and replaced by fresh dechlorinated Winnipeg tap water and a small amount of Tetramin fish food. The larvae were then further observed for signs of morbidity for a period extending to 96 hrs. After this period, the number of dead chironomid larvae in each beaker was also recorded.

A second experiment was performed similar to the first except that a theoretical concentration of 0.3 mg/litre of the particulate and

emulsifiable formulations of methoxychlor was used as the initial exposure level.

2) Rate of Uptake of Each Formulation Using ^{14}C Labelled Methoxychlor

Forty Chironomus tentans fourth instar larvae were placed in each of four 2000 ml beakers containing silica sand, a small amount of Tetramin fish food and dechlorinated Winnipeg tap water as described in part 1 of this section on chironomid larval studies. The entire experiment was conducted at 20°C. The larvae were allowed 12 hrs to burrow into the substrate before the methoxychlor formulations were introduced. A theoretical concentration of 0.1 mg/litre of the ^{14}C labelled particulate formulation of methoxychlor (activity level - 1.85×10^5 D.P.M./mg methoxychlor) was applied to two beakers and 0.1 mg/litre of the ^{14}C labelled emulsifiable concentrate formulation (activity level - 1.95×10^5 D.P.M./mg methoxychlor) was added to each of the remaining two beakers. The chemical was gently mixed into each beaker using separate glass rods. A 5 ml water sample was taken after mixing from each beaker for later analysis by gas liquid chromatography as described by Solomon and Lockhart (1977). After a period of 1 hr, 5 larvae from each beaker were removed, rinsed thoroughly (individually) with distilled water, dried on clean paper towels, and placed in pre-weighed cellulose cups. The cups containing the individual chironomid larvae were then reweighed so the weight of each larva could be calculated, then placed in a freezer for later analysis. This sampling procedure was continued at periods of 2 hrs, 4 hrs, 8 hrs, 12 hrs, 24 hrs, 48 hrs, and 96 hrs after introduction of the ^{14}C labelled methoxychlor formulations. When the sampling

was completed, all samples were combusted on a Packard Tri-Carb Sample Oxidizer (model 306) and later counted on a Packard Tri-Carb 3310 Scintillation Counter. After the 96 hr sampling period a duplicate sediment sample was taken from each beaker and oxidized and counted as described above.

A second similar experiment was performed using a theoretical concentration of 0.3 mg/litre of the ^{14}C labelled particulate and emulsifiable concentrate formulations as the initial exposure level.

G. Statistical Analysis

1) Fish Studies

Data from the rate of uptake of each formulation in rainbow trout were subjected to a 2-way Analysis of Variance. An SNK multiple range test was then conducted on the uptake means at each concentration for both formulations.

2) Black Fly Larval Studies

Results from the comparative larval mortality at different concentrations and exposure times of the particulate and emulsifiable concentrate formulations were expressed as rates. A 3-way Analysis of Variance was then performed on this data.

An analysis of covariance-single classification bivariate regressions was conducted on the methoxychlor residues causing single black fly larvae to release and drift data. The adjusted group means for uptake levels in the black fly larvae when they released and drifted for both the particulate and emulsifiable formulations of methoxychlor at the

overall mean of the exposure concentrations used was also calculated.

3) Stonefly Nymphal Studies

Unpaired-t-tests were performed on uptake levels of methoxychlor recorded in stonefly nymphs after exposure to both the particulate and emulsifiable concentrate formulations of methoxychlor.

Figure 1. Particulate formulation of methoxychlor in suspension. Magnification approx. 576x.

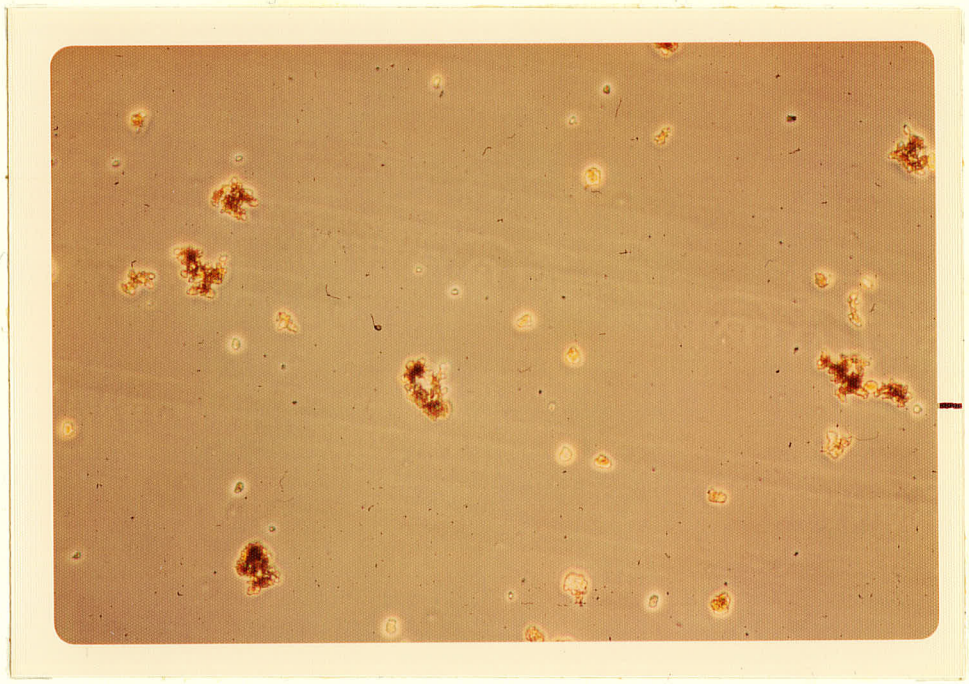
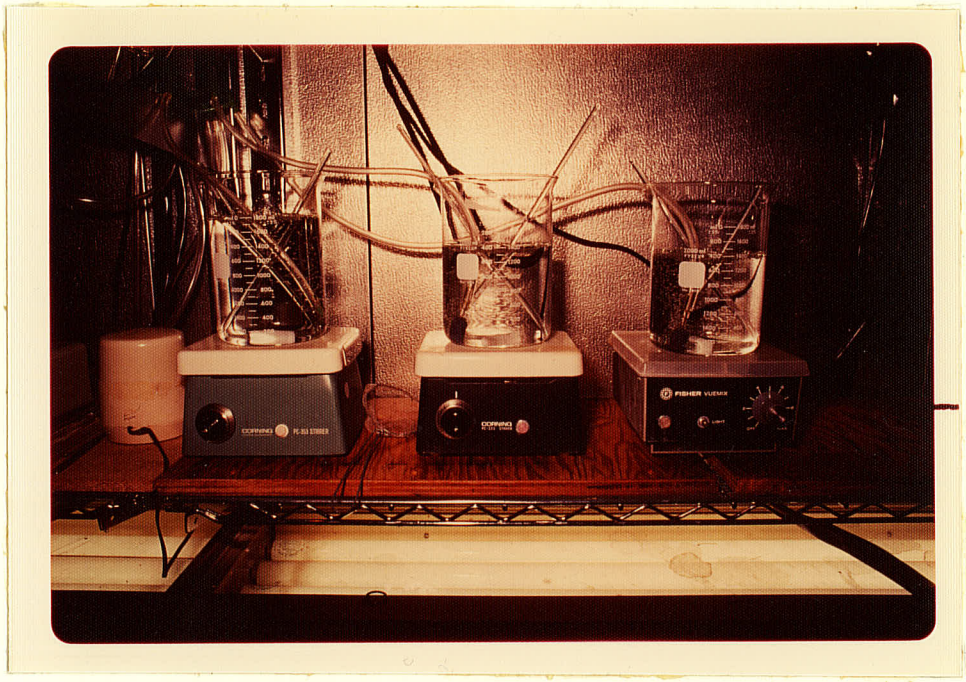


Figure 2. Laboratory apparatus used to test the particulate and emulsifiable concentrate formulations of methoxychlor for efficacy against black fly larvae.

Figure 3. Test beaker showing drifting larvae of Simulium decorum Walker after exposure to 0.3 mg/litre of the particulate formulation of methoxychlor.


CORNING
CORNING
FISHER VUEMIX


1800
1600
1400
1200
1000
800
600
400
200

Figure 4, Re-circulating flow-thru apparatus used to examine toxicity and uptake of the particulate and emulsifiable formulations of methoxychlor in stonefly nymphs.

Figure 5. Species of stonefly nymphs used in uptake experiments.
Left - Acroneuria lycorias (Newman)
Right - Pteronarcys dorsata Say

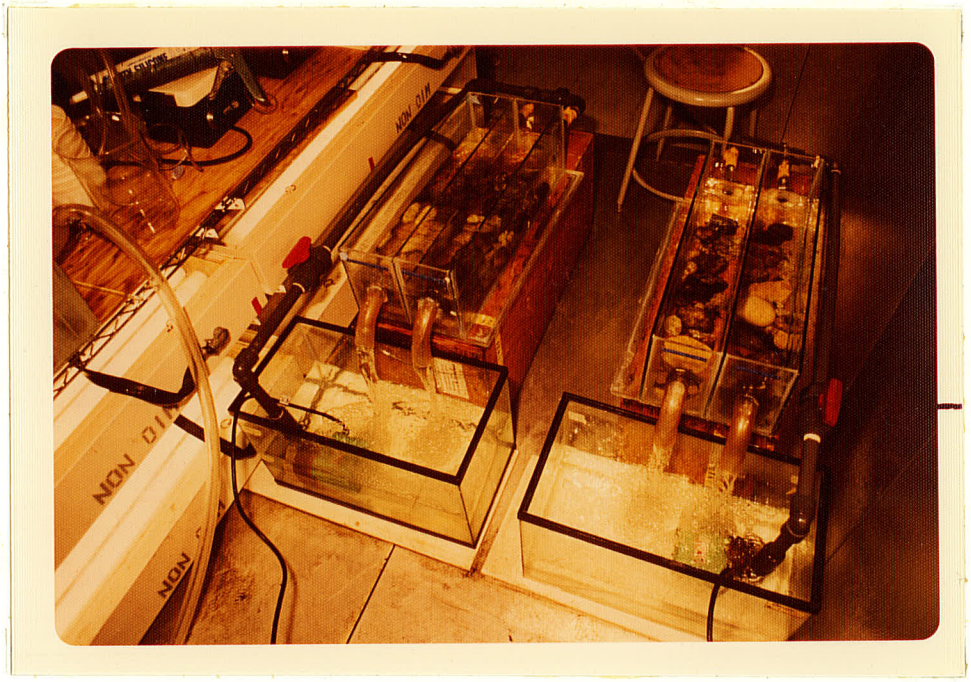
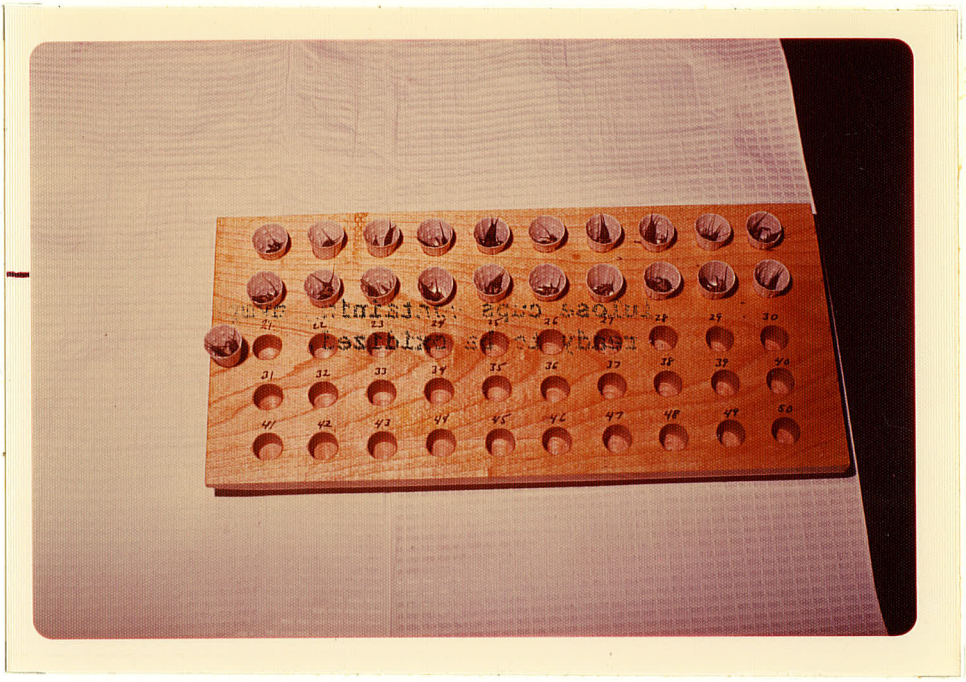


Figure 6. Tray of cellulose cups containing nymphs of Pteronarcys dorsata Say ready to be oxidized.

Figure 7. Packard Tri-Carb Sample Oxidizer (model 306) combusting a Pteronarcys dorsata Say nymph.



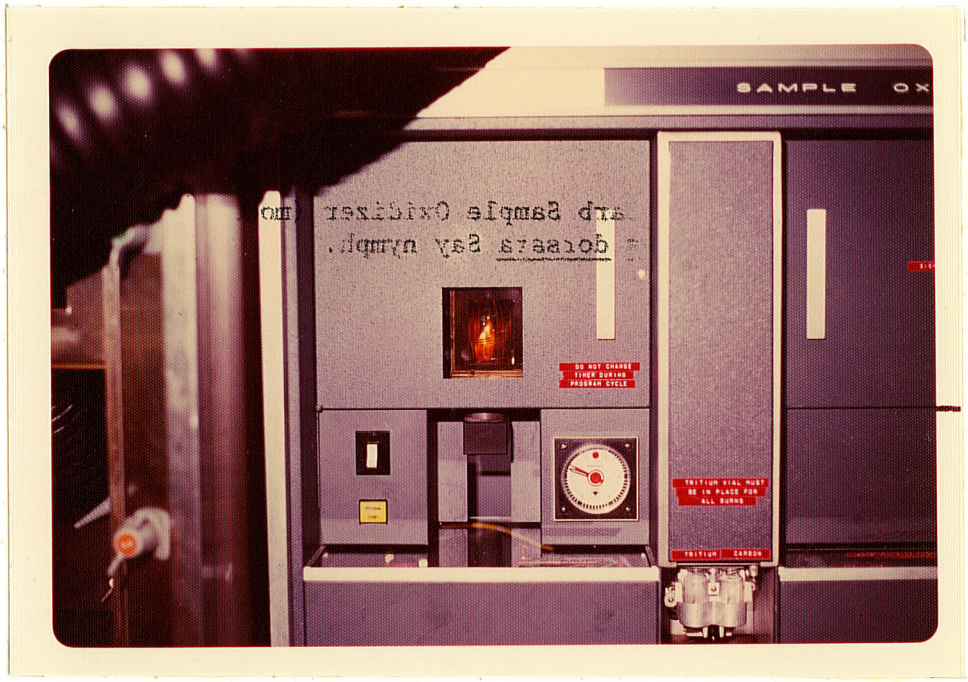
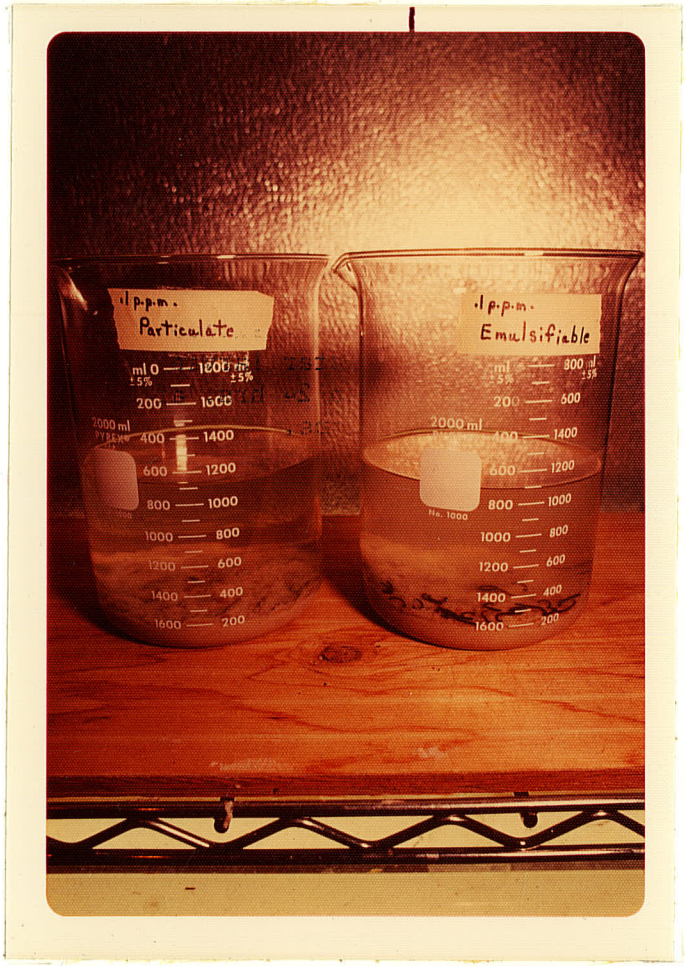

SAMPLE OX
DO NOT CHANGE TIMER DURING PROGRAM CYCLE
TRITIUM VIAL MUST BE IN PLACE FOR ALL BURNS
TRITIUM
CARBON

Figure 8. Laboratory culture of Chironomus tentans Fabricius larvae.

Figure 9. Test beakers showing the effect of a 0.1 mg/litre exposure of the particulate and emulsifiable formulations of methoxychlor on 4th instar larvae of Chironomus tentans Fabricius. Photo taken 24 hrs. after exposure to the methoxychlor formulations.


.1 p.p.m.
Particulate
.1 p.p.m.
Emulsifiable
2000 ml

CHAPTER III

RESULTS AND DISCUSSION

A. Fish Studies

1) Toxicity of Each Formulation.

When the methoxychlor formulations were added to yield a theoretical concentration of 0.3 mg/litre, rainbow trout exposed to the emulsifiable concentrate formulation all showed abnormal "tail-fanning" and "coughing" motions within half an hour. After a period of 3 hrs. some fish in the E.C. tanks were observed to apparently take air at the surface and all the fish had moved into the top half of the aquarium (Fig. 10). Five hours after introduction of the chemical a few fish in one E.C. exposure tank showed clear signs of morbidity characterized by loss of equilibrium (vertical orientation in water, bobbing for air at surface). When the experiment was terminated after 48 hrs. there was 10% mortality in rainbow trout exposed to the emulsifiable concentrate formulation but surviving fish remaining in the exposure tanks appeared normal, having apparently recovered.

Rainbow trout exposed to the particulate formulation at the same concentration (0.3 mg/litre) showed neither mortality nor any sign of morbidity or abnormal behaviour throughout the duration of the experiment (48 hours) and remained randomly distributed in the lower half of the exposure tanks (Fig. 11). Water samples taken at the beginning of this experiment were analysed for methoxychlor with the results recorded in Table 1.

At an exposure level of 1 mg/litre the emulsifiable concentrate killed 98% of the fish and the particulate none after 48 hrs (Fig. 12).

Rainbow trout fingerlings exposed to the particulate formulation appeared at least qualitatively, slightly hyperactive during the course of the experiment, but at no time showed any sign of morbidity. Water analyses from samples taken at the beginning of this experiment are shown in Table 2.

At the highest application rate of 5 mg/litre there was 100% mortality in fish exposed to the emulsifiable concentrate formulation and only 10% mortality in the particulate exposure after 48 hrs (Fig. 13). The fish remaining in the "particulate tanks" after the 48 hr observation period all appeared normal and active. Table 3 shows residues in water samples taken during this experiment.

2) Rate of Uptake of Each Formulation

Fish exposed to a theoretical concentration of either 0.1 or 0.3 mg/litre of the emulsifiable formulation accumulated more than five times as much methoxychlor as fish exposed to similar levels of the particulate material after a period of 1 hr (Table 5). This difference in methoxychlor uptake between particulate and emulsifiable exposed fish was statistically significant ($P \leq .01$). There was no significant difference ($P \geq .01$) between fish residues at the two application rates for the particulate formulation, but for the E.C. residues at the 0.3 mg/litre exposure were meaningfully (two to three times) higher than those at the 0.1 mg/litre level ($P \leq .01$). Methoxychlor residues in water samples taken at the initiation of this experiment are recorded in Table 4.

There seems little doubt that the particulate formulation acted to protect fish relative to the E.C. in these experiments. Adsorption of pesticide to particles before introduction of fish may impose an un-

favorable partition coefficient between pesticide and fish. Although vigorous aeration was maintained in all aquaria during exposures in an attempt to minimize sedimentation of particulates, some proportion of the reduced uptake and toxicity of the particulate formulation may have been due to its physical removal from the water column. However, water analyses for methoxychlor (Table 3) indicate that very little sedimentation took place. In western rivers, adsorption of methoxychlor to suspended materials probably creates a situation very like a particulate formulation. Lockhart (1980) found that river water (75 mg/litre of suspended solids) decreased fish uptake to about 50% of that in laboratory water containing low suspended solids, and yet the particulate formulation used here decreased uptake to only 20% or less. Apparently, the artificial particulate formulation was more than twice as efficient as natural suspended materials in protecting fish. The noted 10% mortality in rainbow trout exposed to 5 mg/litre of the particulate formulation may have been due to desorption of some methoxychlor from particles followed by partitioning from water directly into fish through the gills (Lockhart et al. 1977). More study is required to understand the partitioning of methoxychlor between water and suspended solids in rivers where it is applied as an emulsifiable concentrate for black fly control, and to determine the rate of methoxychlor desorption from particulate materials.

B. Black Fly Larval Studies

1) Comparative Larval Mortality at Different Concentrations and Exposure Times of the Particulate and Emulsifiable Concentrate Formulations.

A comparison of the two formulations at three different concentrations (.3, .09, and .03 mg/litre) and three different exposure times (120, 30 and 7.5 min.) in terms of mortality of black fly larvae of the species Simulium decorum is shown in Table 6. Statistical analysis (3-way Analysis of Variance) indicated that for these concentrations and exposure times there was no significant difference at a probability of 0.01 between the particulate and emulsifiable concentrate formulations for mortality in black fly larvae. Similarly there were no differences in mortality attributable to the three concentrations used in this test (.3, .09, .03 mg/litre). Larvae were killed with comparable effectiveness by all three exposure concentrations. There was a significant difference ($P \leq .01$) among the three exposure times used in this experiment. Both formulations applied for the lowest exposure time (7.5 min.) proved less effective at killing black fly larvae than at the two higher ones (30, 120 min.) at the concentrations tested. It should be noted that variances were not homogeneous (Bartlett's test $P = .002$) and so these results may be questionable. Because of this further statistical comparisons between concentration levels and exposure times for both formulations on % larval mortality were not made. It can be concluded that under these laboratory test conditions the particulate formulation was as effective in killing black fly larvae as the emulsifiable concentrate formulation at the concentrations and exposure times used. This effectiveness of the particulate material agrees with field tests by Helson

(1972) and Helson and West (1978). These authors treated several streams near Baie Comeau, Quebec at a dosage of 0.1 ppm for 15 min. with this same particulate formulation of methoxychlor and 96-100% of the black fly larvae were removed from the sampling cones in all but one stream for a distance 915 m below the treatment site (furthest sample site). Water content of methoxychlor during this experiment is recorded in Table 7; concentrations used for statistical calculations were the theoretical values of 0.3, 0.09, 0.03 mg/litre.

2) Methoxychlor Residues Causing Single Black Fly Larvae to Release and Drift Using ^{14}C-Labelled Methoxychlor.

The cumulative % larval detachment versus time for larvae of Simulium decorum when exposed to four concentrations, (11.1, 33.0, 100.0 and 300.0 ppb) of ^{14}C-labelled emulsifiable concentrate are plotted in Fig. 14. Similarly Fig. 15 shows cumulative % larval detachment versus time for larvae of this species when exposed to the same four concentrations presented as the particulate formulation. At the lowest exposure concentration (11.1 ppb) no larvae drifted in one replicate and only 26% drifted in the second experimental replicate after the observation period of 100 min. when exposed to the E.C. formulation (Fig. 14a). When the methoxychlor was presented as a particulate at this concentration the average time between the two replicates required for all larvae to detach and drift was 220 min. (Fig. 15a). Larvae exposed to 33.0 ppb of the E.C. formulation showed 100% detachment in one replicate and 91% detachment in the second replicate after the observation period of 100 min. (Fig. 14b). The average time between the two replicates required to induce 100% detachment of larvae when the methoxychlor was presented as a

particulate at 33.0 ppb was 135 min. (Fig. 15b). Larvae exposed to a concentration of 100.0 ppb of the E.C. formulation all drifted after an average time of about 27 min. between the two replicates (Fig. 14c) while it required an average time of 105 min. between the two replicates to obtain the same result with larvae exposed to the particulate formulation at this concentration (Fig. 15c). At this exposure level (100.0 ppb) it required almost four times the length of time to achieve complete drift among black fly larvae in the particulate formulation as compared with those in the emulsifiable concentrate. An average time of about 16 min. between the two replicates was needed to induce all larvae to detach when exposed to the E.C. formulation at a level of 300.0 ppb (Fig. 14d), but it required an average time between the two replicates of 105 min. for all larvae to drift when exposed to the particulate formulation at this same concentration (Fig. 15d). Thus the particulate formulation took about seven times as long as the E.C. formulation to cause 100% of the exposed black fly larvae to release and drift at the 300.0 ppb concentration. At each exposure level examined, except the 11.1 ppb concentration where not all larvae exposed to the E.C. formulation drifted, larvae exposed to the particulate formulation required a much greater time to release and drift than did larvae exposed to the emulsifiable concentrate formulation. Also as the exposure concentration was increased from 11.1 to 300.0 ppb the time required to cause larvae to detach was significantly decreased for both formulations except for one case when the particulate concentration was increased from 100.0 to 300.0 ppb and the time to induce drift remained constant.

The results of the statistical analysis performed on the residues of

either formulation of methoxychlor causing the black fly larvae to release and drift (Analysis of Covariance - single classification, Bivariate Regressions) are tabulated in Table 8. The mean uptake residues of both formulations of methoxychlor at each exposure level (11.1, 33.0, 100.0, and 300.0 ppb) causing single black fly larvae to release and drift and their respective regressions are diagramed in Fig. 16. Black fly larvae exposed to the particulate formulation accumulated 68 times more methoxychlor than larvae exposed to the emulsifiable concentrate formulation before drifting at the overall average exposure level of 49.0 ppb (Table 8). This difference was highly significant ($P>.9995$). As the concentration was increased from 11.1 to 300.0 ppb, black fly larvae exposed to the particulate accumulated significantly higher levels of methoxychlor ($P>.9995$) at each succeeding level than black fly larvae exposed to the E.C. formulation. The slope of the particulate regression was close to 1, indicating near proportionality between concentration and uptake, however, the slope for the E.C. formulation was far less than 1 (Fig. 16). This indicates very little relationship between concentration and uptake in larvae exposed to the E.C. Formulation, at the exposure concentrations tested. Increasing the concentration from 11.1 ppb to 300.0 ppb did not result in a highly significant increase in uptake of methoxychlor in exposed larvae. Water samples taken during this experiment are listed with measured methoxychlor concentrations in Table 9.

The greater amount of time required for black fly larvae exposed to the particulate formulation to detach as compared with larvae exposed to the E.C. formulation at similar concentrations would be expected if larvae had to ingest the particulate for it to be effective; the E.C.

might act as a contact insecticide inducing detachment more quickly. The greater quantities of methoxychlor accumulated by black fly larvae exposed to the particulate before detaching as compared to the E.C. at similar concentrations can also be explained by this proposed difference in modes of action of the formulations. Following ingestion the particulate formulation probably required a certain amount of time for the methoxychlor to be absorbed and exert its effect to produce a response in the black fly larvae, i.e. detachment from the substrate. Black fly larvae feeding continually on particulate material would ingest more particles of the particulate methoxychlor before the initial ones consumed caused detachment. At a higher concentration of the formulation, there would be more particles exposed to the larvae and more would probably be consumed before the methoxychlor in the gut induced the larvae to detach, hence the greater uptake in larvae exposed to the particulate formulation at succeedingly higher concentrations. The emulsifiable concentrate caused the black fly larvae to respond very rapidly by detaching from the substrate. This might be expected if the E.C. formulation is taken up through respiratory surfaces and distributed to various organs with no lag time required for digestion as for the particulate. This rapid mode of action resulted in low levels of methoxychlor accumulation in larvae exposed to the E.C. formulation at the time of drift. Wallace _et al_. (1976) supported these results by illustrating in lab experiments that black fly larvae exposed to particulate formulations versus ethanol solutions of methoxychlor concentrated the particulate preparation in much greater amounts. Because the particulate formulation must have to be ingested to be effective and

because large quantities are ingested, larvae affected by this formulation would definitely die, whereas larvae induced to detach by exposure to the E.C. formulation contain comparatively little methoxychlor and may under favourable conditions re-attach, if a lethal dose was not originally received. The behavioural response preceeds the lethal one and it may protect larvae from accumulating lethal doses of the E.C.

C. Stonefly Nymphal Studies

1) Toxicity of Each Formulation

After exposure to 0.3 mg/litre of both formulations of methoxychlor stonefly nymphs of the species Pteronarcys dorsata showed 100% morbidity symptoms when exposed to the E.C. formulation as compared with 25% morbidity when exposed to the particulate formulation after the observation period of 24 hrs (Fig. 17). The emulsifiable concentrate formulation initiated an immediate response in exposed stonefly nymphs and after a period of 1 hr the majority of nymphs were moribund (Fig. 18). Nymphs exposed to the particulate formulation showed no sign of morbidity until at least 10 hrs after exposure began, and at the end of the 24 hr observation period 25% were moribund as mentioned previously; some appeared slightly agitated and were induced to walk on top of the rocks but were responsive and showed no loss of equilibrium. Water samples analyzed for methoxychlor during the course of this experiment gave the results in Table 10. From these data it should be noted that initial exposure levels of the methoxychlor were twice as high in the particulate exposure as in the E.C. exposure making the results all the more striking.

The exposure concentration of the particulate formulation used in these experiments was therefore at least five times greater than levels used by Helson (1972) and Helson and West (1978) in field trials of this same particulate formulation which proved very effective at controlling black fly larvae.

2) Rate of Uptake of Each Formulation Using ^{14}C-Labelled Methoxychlor.

After exposure for 1 and 12 hrs to the E.C. formulation at a concentration of 0.3 mg/litre stonefly nymphs of the species Acroneuria lycorias accumulated at least six times as much methoxychlor as nymphs exposed to the particulate material for the same time periods (Table 11). This difference in methoxychlor uptake between the two formulations was significant ($P \leq .01$). It should be noted that an uptake level of 133.3 ppm in one nymph exposed to the E.C. formulation for 1 hr was so atypical that it was not included with these data. Similarly a level of 25.2 ppm recorded in one nymph of this species exposed to the particulate formulation for 12 hrs was not included. These two values were abnormally high when compared to the other uptake levels calculated for their respective groups, and the reason for their large difference from the other values is unknown.

Stonefly nymphs of the species Pteronarcys dorsata after exposure for 1 and 12 hrs to both formulations of methoxychlor at a concentration of 0.3 mg/litre accumulated twice as much of the E.C. formulation as the particulate formulation (Table 12). This difference in methoxychlor uptake between the two formulations was also significant ($P \leq .01$) in all cases except in the second replicate of the 1 hr exposure

which was slightly less significant at ($P \leq 0.2$). An uptake level of 238.4 ppm in ony nymph of this species exposed to the particulate formulation for 12 hrs was not included with these data for reasons mentioned above.

Throughout the course of this experiment, no signs of abnormal behaviour or morbidity were noted in stonefly nymphs of either species exposed to the particulate formulation, whereas nymphs of both Acroneuria lycorias and Pteronarcys dorsata responded almost immediately to exposure to the E.C. formulation, and after a period of 1 hr most were exhibiting signs of morbidity. Residues in water samples are recorded in Table 13.

The particulate formulation protected stonefly nymphs in these experiments relative to the E.C. The results were most striking with the species Acroneuria lycorias which is a carnivorous species (Dosdall and Lemkuhl 1979). It could be postulated that if the methoxychlor remained on the particles, nymphs of this species would have no way of taking up the methoxychlor formulation because of their method of feeding. The uptake values recorded in Table 11 seem to a large extent to verify this hypothesis. The reason for the limited methoxychlor uptake by nymphs of Acroneuria lycorias was probably due to desorption of some methoxychlor from the particles to the water where it could then be taken up by the insects and act as a contact poison. Pteronarcys dorsata nymphs are detrivores (Dosdall and Lemkuhl 1979) and the reason for the greater accumulation of the particulate formulation in this species as compared to Acroneuria lycorias may be due to the nymph actually consuming particles of the particulate formulation inadvertently while scraping for food. The delay in response by nymphs of Pteronarcys dorsata when exposed to the particulate formulation

(Fig. 17) verifies this and suggests that the particulate must be ingested in order to initiate an adverse effect on nymphs of this species. An alternative explanation could be that it takes about 10 hrs for enough methoxychlor to desorb from the celite particles to the water and then become available to initiate an adverse response in the stonefly nymphs.

Stonefly nymphs of both species showed a negative reaction almost immediately (within 15 min.) when exposed to the E.C. formulation. The emulsifiable concentrate formulation acted very rapidly in these experiments and was unquestionably detrimental to both species of stonefly nymphs.

D. Chironomid Larval Studies

1) Toxicity of Each Formulation

Larvae (4th instar) of Chironomus tentans when exposed to the emulsifiable concentrate formulation at a level of 0.1 mg/litre showed about 98% total morbidity (Fig. 19) after a period of 96 hrs whereas larvae exposed to the particulate formulation showed about 55% morbidity (Fig. 19). At this exposure level there was 98.3% mortality in larvae exposed to the E.C. formulation and 21.4% mortality in larvae exposed to the particulate formulation after 96 hrs. At an exposure level of 0.3 mg/litre all larvae were moribund in the E.C. exposure after 96 hrs whereas 87% morbidity was observed in the particulate exposure (Fig. 20). At this concentration there was 98.6% mortality in larvae exposed to the emulsifiable concentrate formulation and 35.7% mortality in larvae exposed to the particulate formulation after 96 hrs. Residues in water samples for these experiments are listed in Table 14.

2) Rate of Uptake of Each Formulation Using ^{14}C-Labelled Methoxychlor.

The methoxychlor uptake curves into Chironomus tentans larvae for both formulations of methoxychlor when applied at an initial concentration of 0.1 mg/litre are shown in Fig. 21. Values of 41.2 ppm recorded in one larvae exposed to the particulate formulation 4 hrs after the start of the experiment, and 8.5 ppm recorded in one larvae exposed to the E.C. formulation 96 hrs after initiation of the experiment were not included in the data because they were so different from other values in their respective sample groups.

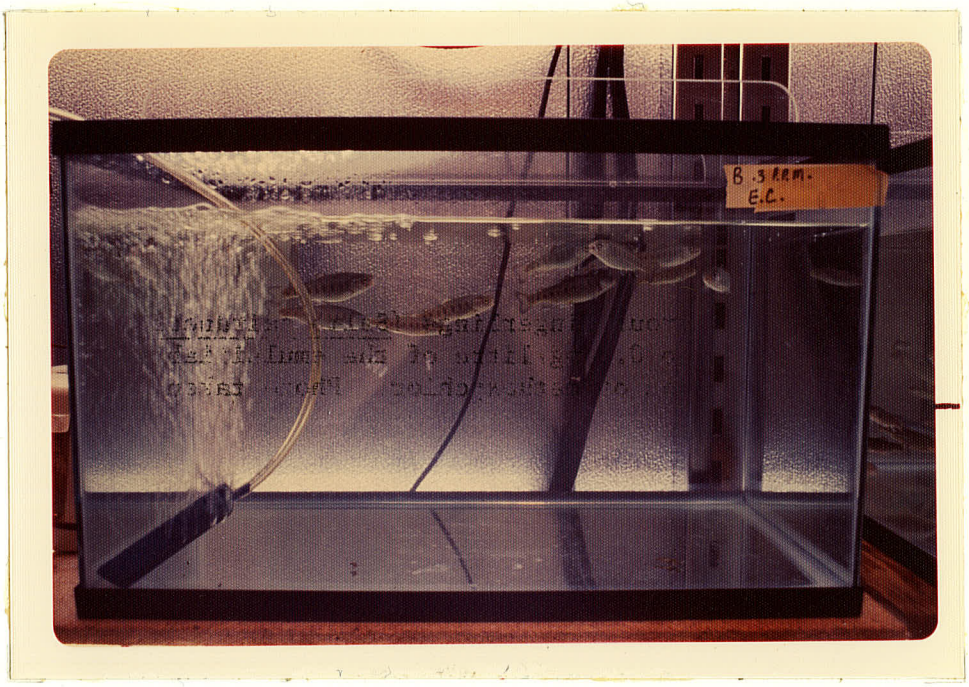
Chironomid larvae exposed to the emulsifiable concentrate formulation accumulated a significantly higher level of methoxychlor than larvae exposed to the particulate formulation throughout the duration of the experiment (96 hrs). The uptake curves for both formulations when the initial exposure level was 0.3 mg/litre are diagrammed in Fig. 22. Uptake values of 178.9 ppm recorded in one larvae after 1 hr of exposure to the E.C. formulation, 126.7 ppm in one larvae 2 hrs after exposure to the E.C., and 0 ppm in three larvae collected 4 hrs, 12 hrs and 24 hrs., after exposure to the E.C. formulation were not included in the data. Also an uptake level of 0 ppm recorded in one larvae exposed to the particulate formulation 96 hrs after exposure was not included. Again at this concentration chironomid larvae exposed to the emulsifiable concentrate formulation accumulated significantly higher levels of methoxychlor than larvae exposed to the particulate formulation at each sample time during the experimental observation period of 96 hrs. Residues in water and substrate for methoxychlor for this experiment are listed in Table 15.

The particulate formulation acted to protect chironomid larvae in these experiments, but not to the same degree as observed in fish or stonefly nymphs. Chironomus tentans is a detritus feeder and would probably consume a wide range of particles including some in the same size range as particles in the particulate formulation of methoxychlor. The reason for the adverse effects on chironomid larvae exposed to the particulate formulation was probably due to the larvae actually ingesting some of the particulate particles while "foraging" for food. The delay in uptake of methoxychlor after exposure to the particulate formulation at 0.1 mg/litre (Fig. 21) and the corresponding delay in response of the larvae after exposure to the particulate formulation (Figs. 19 and 20) supports this idea and suggests that it requires a certain number of hours after exposure for larvae to ingest enough of the particulate larvicide to become moribund. Alternately, the larvae may also be affected by methoxychlor desorbing from celite particles and partitioning into larvae without the ingestion and digestion steps.

The emulsifiable concentrate formulation initiated an almost immediate response in the chironomid larvae at both exposure concentrations examined. The majority of larvae were induced to leave the substrate and begin rapid wiggling movements within one hour after introduction of the chemical. Obviously the E.C. was acting directly on the larvae as a contact poison and showed no protection to larvae of Chironomus tentans.

Figure 10. Rainbow trout fingerlings (Salmo gairdneri Richardson) exposed to 0.3 mg/litre of the emulsifiable concentrate formulation of methoxychlor. Photo taken 3 hrs. after exposure.

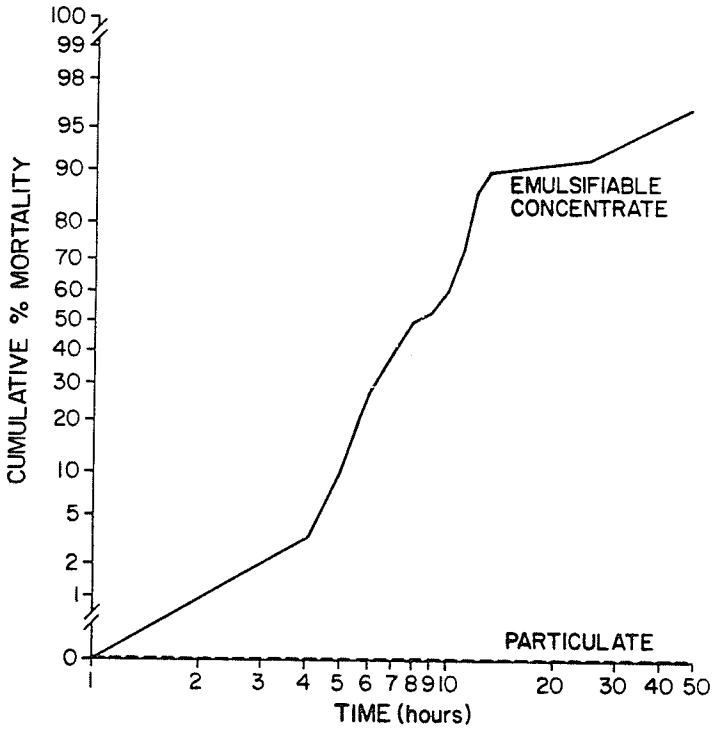
Figure 11. Rainbow trout fingerlings (Salmo gairdneri Richardson) exposed to 0.3 mg/litre of the particulate formulation of methoxychlor. Photo taken 3 hrs. after exposure.


B .3 PPM.
E.C.


C .3 PPM.
PARTICULATE

Figure 12. Cumulative percent mortality of rainbow trout fingerlings (Salmo gairdneri Richardson) exposed to methoxychlor at 1 mg/litre presented as either an emulsifiable concentrate (———) or a particulate (-----).

Figure 13. Cumulative percent mortality of rainbow trout fingerlings (Salmo gairdneri Richardson) exposed to methoxychlor at 5 mg/litre presented as either an emulsifiable concentrate (———) or a particulate (-----).


CUMULATIVE % MORTALITY
100
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0
EMULSIFIABLE
CONCENTRATE
PARTICULATE
1
2
3
4
5
6
7
8
9
10
20
30
40
50
TIME (hours)

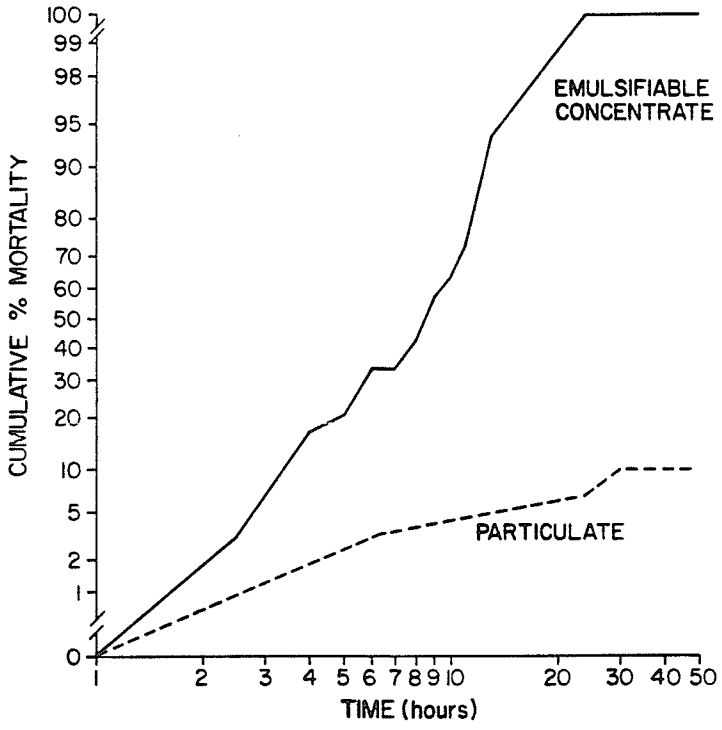
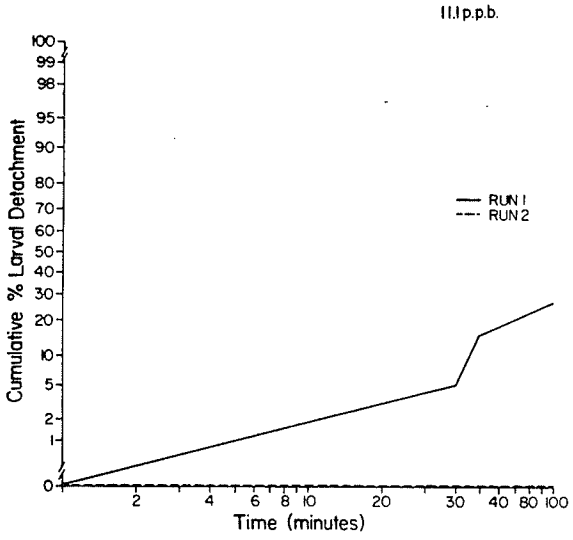
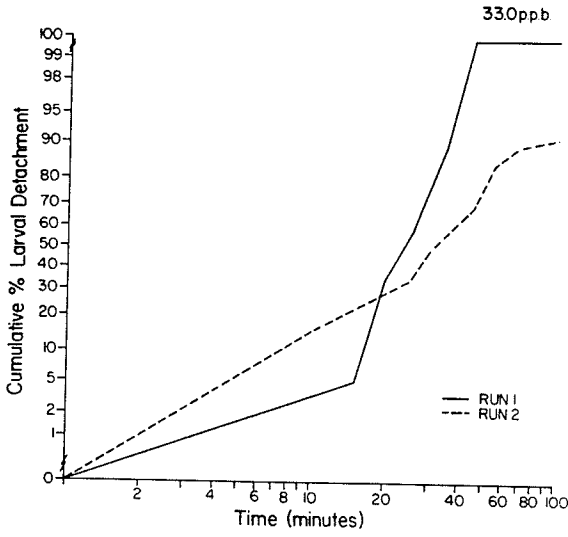

CUMULATIVE % MORTALITY
100
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0
EMULSIFIABLE
CONCENTRATE
PARTICULATE
1
2
3
4
5
6
7
8
9
10
20
30
40
50
TIME (hours)

Figure 14. Cumulative percent detachment of larvae of Simulium decorum Walker exposed to methoxychlor at a) 11.1 ppb, b) 33.0 ppb, c) 100.0 ppb or d) 300.0 ppb presented as an emulsifiable concentrate.

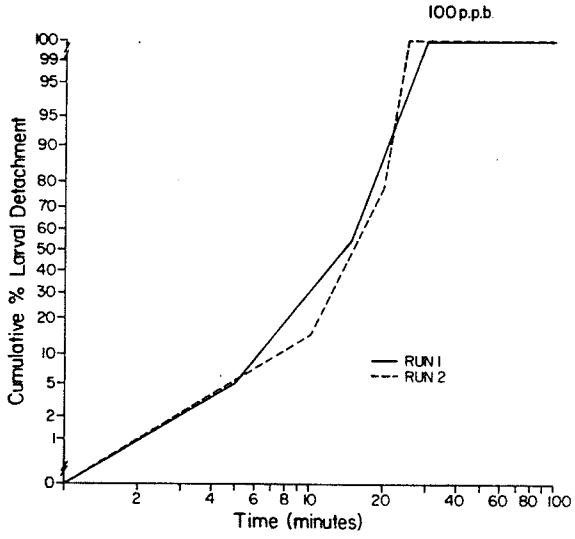
(a)


11.1 p.p.b.
Cumulative % Larval Detachment
100
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0
RUN 1
RUN 2
2
4
6
8
10
20
30
40
80
100
Time (minutes)

(b)


33.0 p.p.b
Cumulative % Larval Detachment
100
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0
RUN 1
RUN 2
2
4
6
8
10
20
40
60
80
100
Time (minutes)

(c)


100 p.p.b.
Cumulative % Larval Detachment
100
99
95
95
90
80
70
60
50
40
30
20
10
5
2
1
0
RUN 1
RUN 2
2
4
6
8
10
20
40
60
80
100
Time (minutes)

(d)

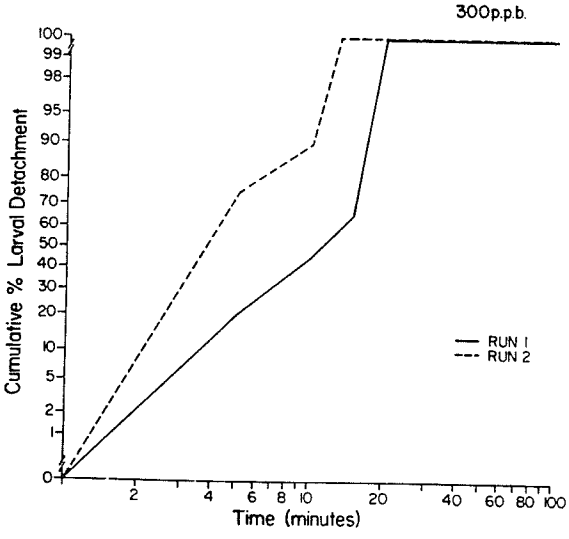

300 p.p.b.
Cumulative % Larval Detachment
100
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0
RUN 1
RUN 2
2
4
6
8
10
20
40
60
80
100
Time (minutes)

Figure 15. Cumulative percent detachment of larvae of Simulium decorum Walker exposed to methoxychlor at a) 11.1 ppb, b) 33.0 ppb, c) 100.0 ppb or d) 300.0 ppb presented as a particulate.

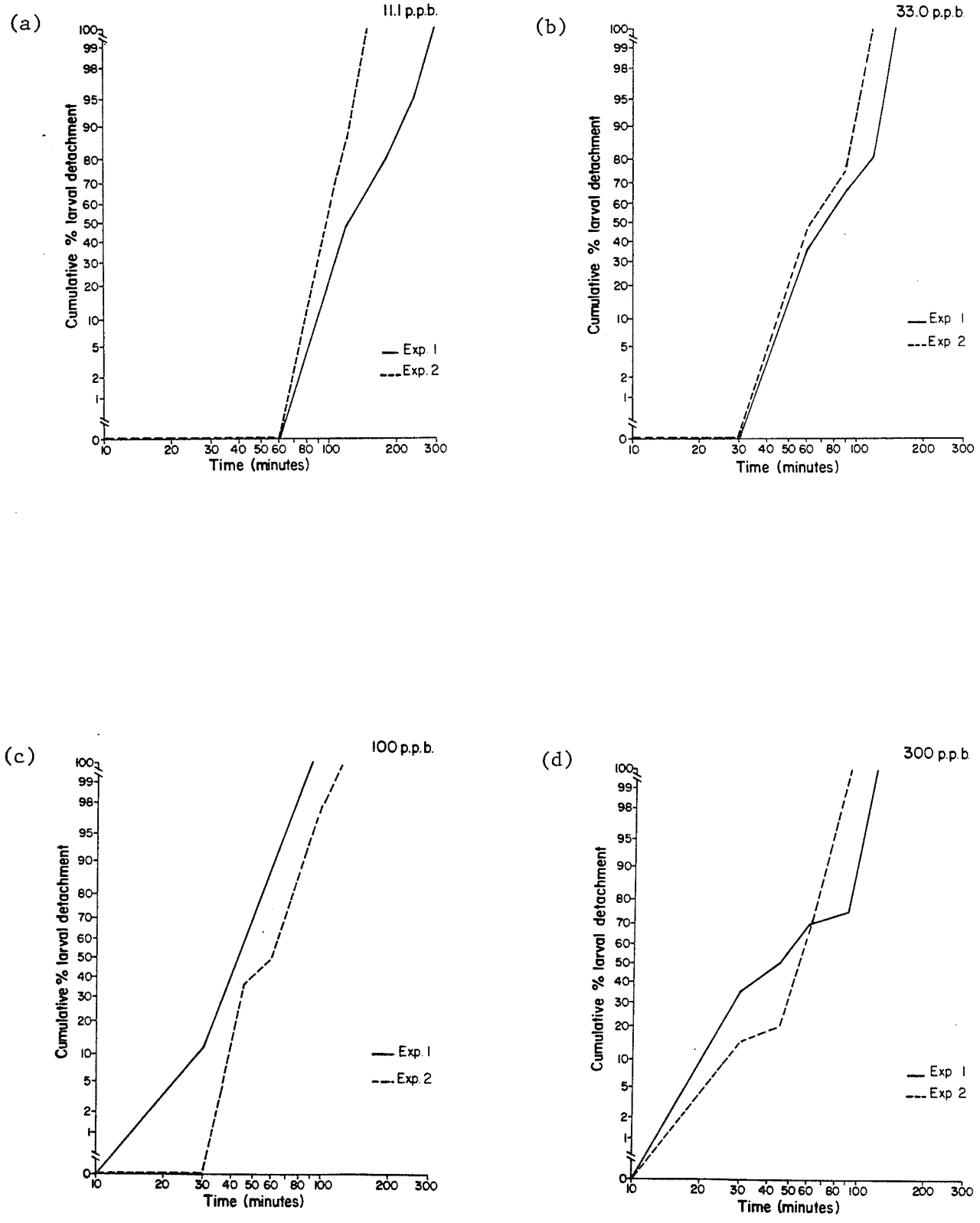

(a)
11.1 p.p.b.
Cumulative % larval detachment
Time (minutes)
Exp. 1
Exp. 2
(b)
33.0 p.p.b.
Cumulative % larval detachment
Time (minutes)
Exp 1
Exp 2
(c)
100 p.p.b.
Cumulative % larval detachment
Time (minutes)
Exp. 1
Exp. 2
(d)
300 p.p.b.
Cumulative % larval detachment
Time (minutes)
Exp 1
Exp 2

Figure 16. Methoxychlor concentrations in single black fly larvae (Simulium decorum Walker) recorded when they detached and drifted after exposure to four concentrations (11.1, 33.0, 100.0, and 300.0 ppb) of the particulate and emulsifiable concentrate formulations of methoxychlor. The regression equation for the particulate formulation is log y = 1.0341+1.1265 (log x) (d.f. = 122). The regression equation for the emulsifiable concentrate formulation is log y = 0.4605+0.3810 (log x) (d.f. = 145).

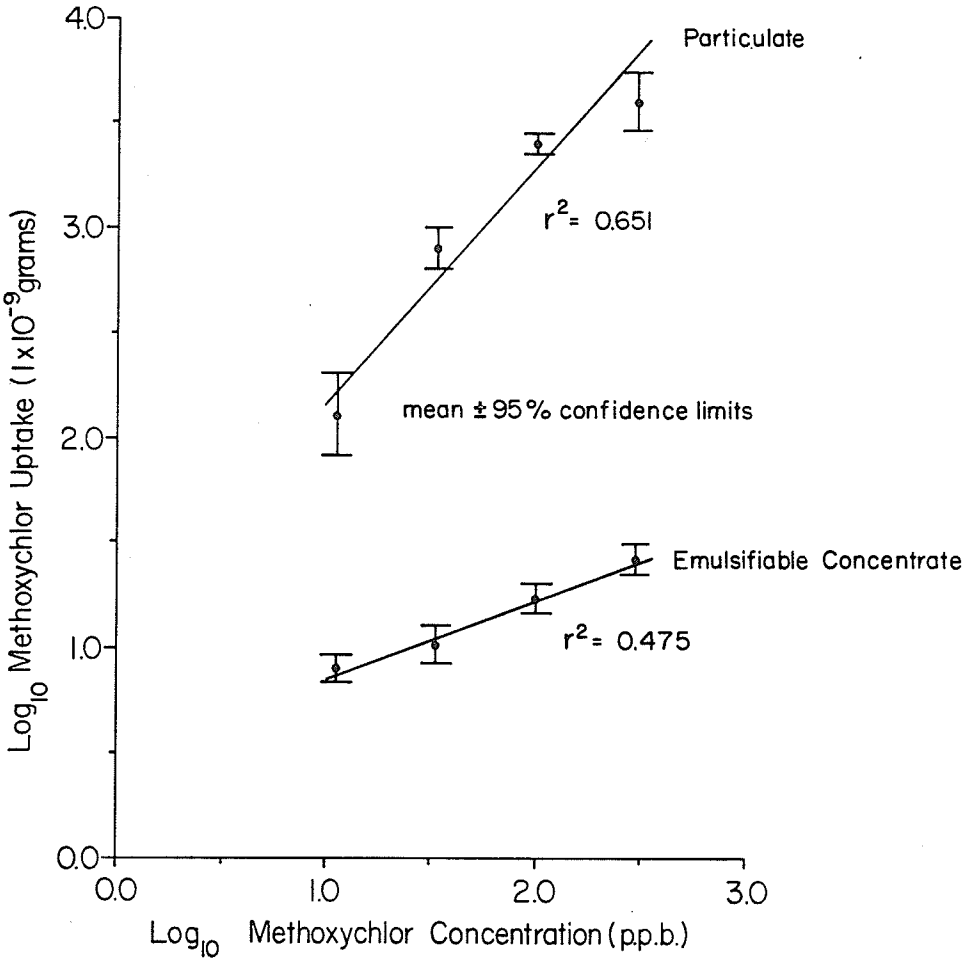

4.0
3.0
2.0
1.0
0.0
0.0
1.0
2.0
3.0
Log10 Methoxychlor Uptake (1x10-9grams)
Log10 Methoxychlor Concentration (p.p.b.)
Particulate
r2= 0.651
mean ± 95% confidence limits
Emulsifiable Concentrate
r2= 0.475

Figure 17. Cumulative percent morbidity of stonefly nymphs (Pteronarcys dorsata Say) at various time periods exposed to methoxychlor at 0.3 mg/litre presented as either an emulsifiable concentrate (———) or a particulate (-----).

Figure 18. Moribund Pteronarcys dorsata Say nymphs after exposure to 0.3 mg/litre of the emulsifiable concentrate formulation of methoxychlor. Photo taken 1 hr. after exposure.

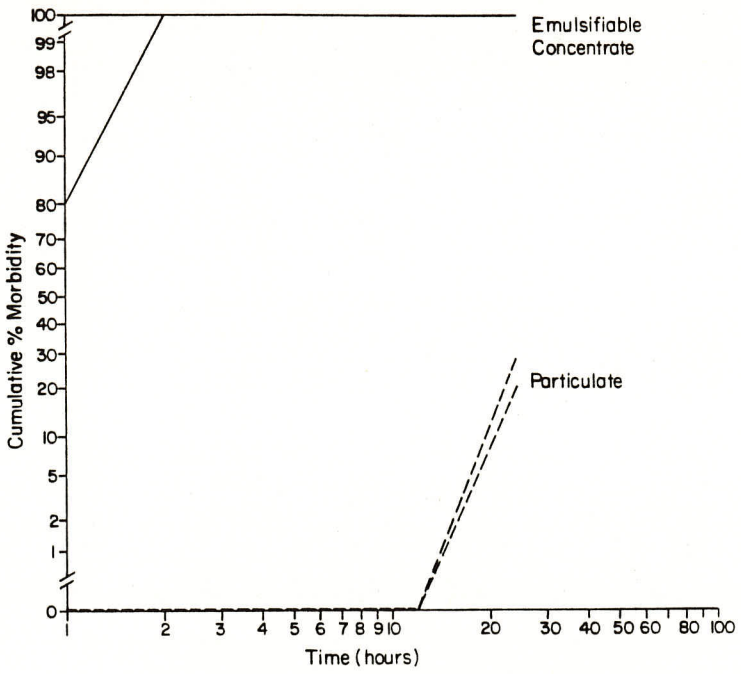
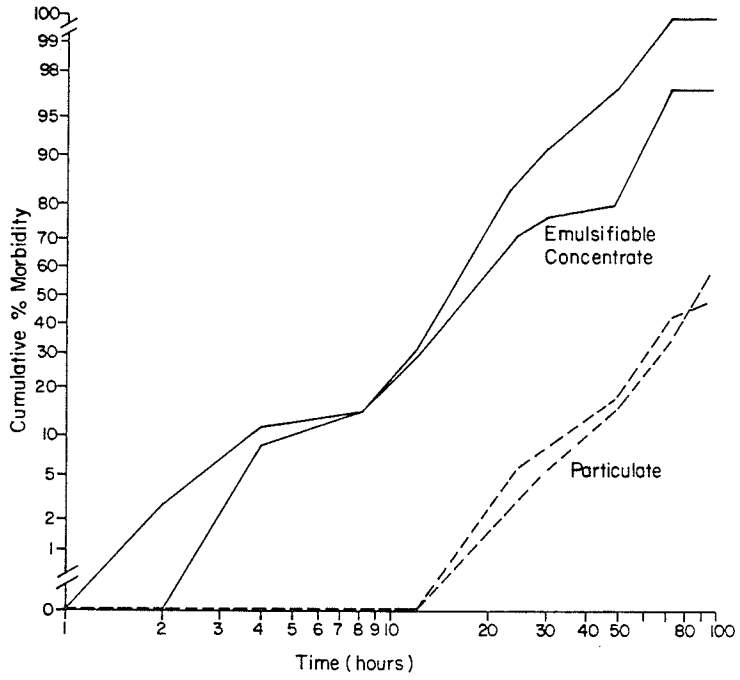

Emulsifiable
Concentrate
Particulate
Cumulative % Morbidity
100
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0
1
2
3
4
5
6
7
8
9
10
20
30
40
50
60
80
100
Time (hours)

Figure 19. Cumulative percent morbidity of 4th instar chironomid larvae (Chironomus tentans Fabricius) at various time periods exposed to methoxychlor at 0.1 mg/litre presented as either an emulsifiable concentrate (———) or a particulate (-----).

Figure 20. Cumulative percent morbidity of 4th instar chironomid larvae (Chironomus tentans Fabricius) at various time periods exposed to methoxychlor at 0.3 mg/litre presented as either an emulsifiable concentrate (———) or a particulate (-----).


Cumulative % Morbidity
100
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0
1
2
3
4
5
6
7
8
9
10
20
30
40
50
80
100
Time (hours)
Emulsifiable
Concentrate
Particulate

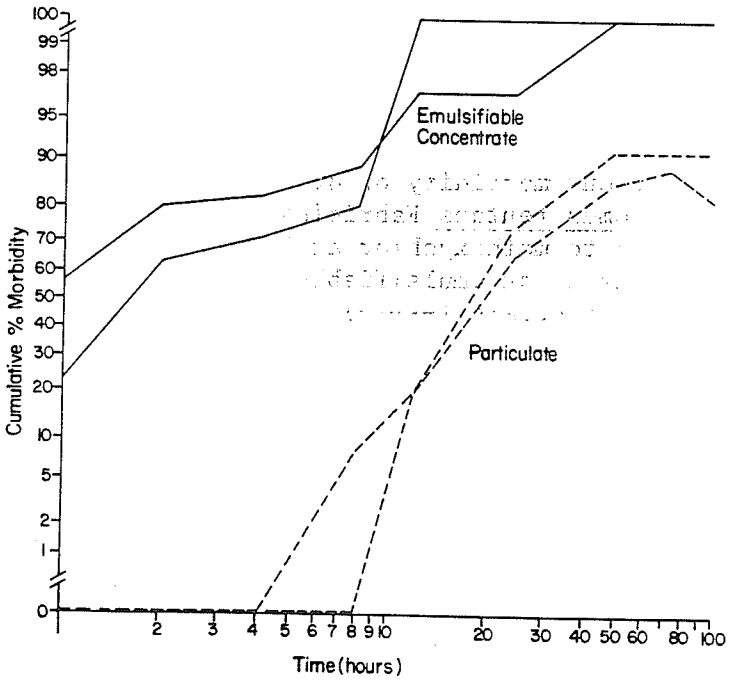
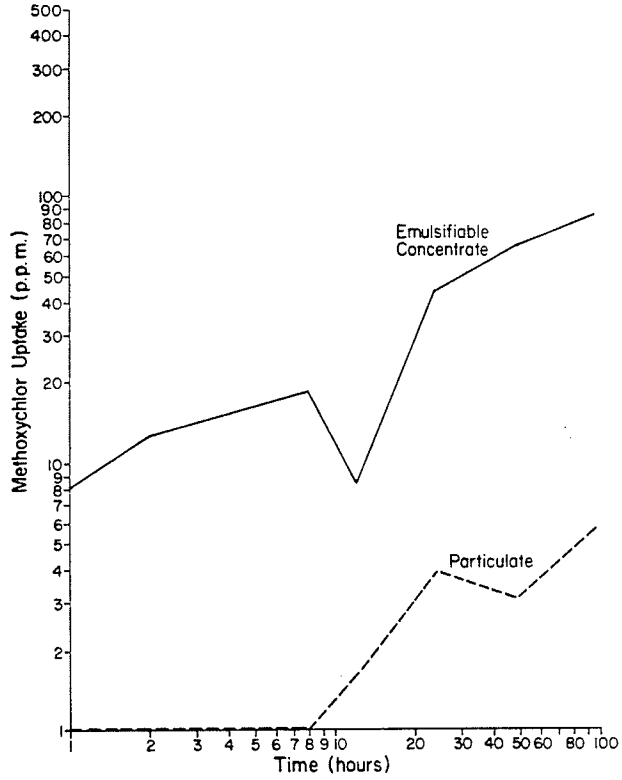

Cumulative % Morbidity
100
99
98
95
90
80
70
60
50
40
30
20
10
5
2
1
0
2
3
4
5
6
7
8
9
10
20
30
40
50
60
80
100
Time(hours)
Emulsifiable
Concentrate
Particulate

Figure 21. Methoxychlor uptake in 4th instar chironomid larvae (Chironomus tentans Fabricius) at various time periods exposed to methoxychlor at 0.1 mg/litre presented as either an emulsifiable concentrate (———) or a particulate (-----).

Figure 22. Methoxychlor uptake in 4th instar chironomid larvae (Chironomus tentans Fabricius) at various time periods exposed to methoxychlor at 0.3 mg/litre presented as either an emulsifiable concentrate (———) or a particulate (-----).


Methoxychlor Uptake (p.p.m.)
Time (hours)
Emulsifiable Concentrate
Particulate

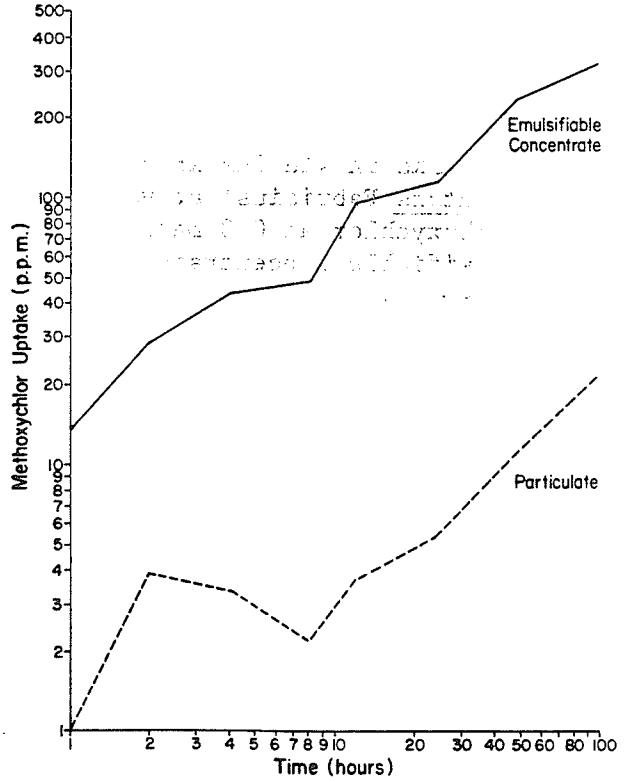

Methoxychlor Uptake (p.p.m.)
Time (hours)
Emulsifiable Concentrate
Particulate

Table 1. Methoxychlor in water (mg/litre) sampled at the start of the exposure to a theoretical concentration of 0.3 mg/litre of particulate and emulsifiable formulations of methoxychlor to rainbow trout (Salmo gairdneri Richardson)

Particulate	Measured Concentration (mg/litre)
Tank 1	0.26
Tank 2	0.28
Emulsifiable	
Tank 1	0.31
Tank 2	0.32

Table 2. Methoxychlor in water (mg/litre) sampled at the start of the exposure to a theoretical concentration of 1.0 mg/litre of particulate and emulsifiable formulations of methoxychlor to rainbow trout (Salmo gairdneri Richardson)

Particulate	Measured Concentration (mg/litre)
Tank 1	0.86
Tank 2	0.96
Emulsifiable	
Tank 1	1.07
Tank 2	0.67

Table 3. Methoxychlor concentrations (mg/litre) in water treated with particulate and emulsifiable formulations at a theoretical concentration of 5 mg/litre to observe toxicity in rainbow trout (Salmo gairdneri Richardson)

Particulate	Start	4 hr.	8 hr.	24 hr.
Tank 1	4.5	5.7	4.8	6.0
Tank 2	5.4	5.5	4.8	4.1
Emulsifiable				
Tank 1	4.3	2.9	2.2	1.6
Tank 2	7.0	2.6	2.2	1.7

Table 4. Methoxychlor concentrations (mg/litre) in water sampled at the start of a one-hour exposure of the particulate and emulsifiable concentrate formulations of methoxychlor at theoretical concentrations of 0.1 and 0.3 mg/litre to rainbow trout (Salmo gairdneri Richardson)

	Methoxychlor residue (mg/litre) After exposure at 0.1 mg/litre	
	Particulate	Emulsifiable
Exp. 1	0.11	0.09
Exp. 2	0.14	0.14
	After exposure at 0.3 mg/litre	
	Particulate	Emulsifiable
Exp. 1	0.23	0.40
Exp. 2	0.21	0.34

Table 5. Methoxychlor residues in whole rainbow trout (Salmo gairdneri Richardson) after one hour exposure to 0.1 and 0.3 mg/litre of particulate and emulsifiable formulations

		Methoxychlor residue (µg/g)	
		After exposure at 0.1 mg/litre	
		Particulate	Emulsifiable
Exp. 1	$\bar{x}$	0.43	2.80
	SD	0.087	0.534
	Range	0.33-0.59	2.01-3.89
	n	10	10
Exp. 2	$\bar{x}$	0.38	2.75
	SD	0.144	0.593
	Range	0.22-0.63	2.18-4.15
	n	10	10
		After exposure at 0.3 mg/litre	
		Particulate	Emulsifiable
Exp. 1	$\bar{x}$	0.71	6.07
	SD	0.176	0.969
	Range	0.45-0.98	4.30-7.68
	n	10	10
Exp. 2	$\bar{x}$	0.42	7.11
	SD	0.173	1.317
	Range	0.31-0.89	5.75-10.5
	n	10	10

Table 6. Corrected % mortality (Abbot's formula) of black fly larvae (Simulium decorum Walker) after 20 hrs. subsequent to exposure to different concentrations and exposure times of the particulate and emulsifiable concentrate formulations of methoxychlor

		0.3 ppm	0.09 ppm	0.03 ppm
120 min.	Particulate	100.0	98.9	100.0
	- - - - - -	- - - -	- - - -	- - - -
	E.C.	100.0	100.0	97.6
30 min.	Particulate	97.4	97.5	80.5
	- - - - - -	- - - -	- - - -	- - - -
	E.C.	94.2	98.8	84.3
7.5 min.	Particulate	87.2	71.5	67.0
	- - - - - -	- - - -	- - - -	- - - -
	E.C.	100.0	84.3	81.8

Table 7. Methoxychlor concentrations (mg/litre) in water sampled at the start of the experiment to expose the particulate and emulsifiable concentrate formulations of methoxychlor at different concentrations and exposure times in order to observe % mortality in black fly larvae (Simulium decorum Walker)

		Theoretical concentration (mg/litre)											
		0.3				0.09				0.03			
		Test 1	2	3	Control	Test 1	2	3	Control	Test 1	2	3	Control
120 min.	Particulate	0.22	0.29	0.32	0.00	0.04	0.07	0.09	0.00	0.02	0.02	0.03	0.00
	E.C.	0.32	0.36	0.33	0.00	0.07	0.07	0.07	0.00	0.02	0.02	0.03	0.00
30 min.	Particulate	0.28	0.32	0.39	0.00	0.05	0.10	0.12	0.00	0.04	0.02	0.03	0.00
	E.C.	0.21	0.23	0.16	0.00	0.08	0.10	0.07	0.00	0.02	0.02	0.02	0.00
7.5 min.	Particulate	0.26	0.35	0.25	0.00	0.07	0.08	0.07	0.00	0.06	0.04	0.05	0.01
	E.C.	0.43	0.45	0.56	0.01	0.06	0.09	0.08	0.00	0.05	0.05	0.04	0.01

Table 8. Analysis of covariance, single classification Bivariate regressions on methoxychlor uptake residues causing single black fly larvae to release and drift when exposed to four concentrations (11.1, 33.0, 100.0, and 300.0 ppb) of the particulate and emulsifiable concentrate formulations of methoxychlor

	d.f.	r^2	b (slope)	a (y-intercept)
Particulate	122	0.6509	1.1265	1.0341
Emulsifiable	145	0.4751	0.3810	0.4605

Test for Equality of Slopes

F = 96.626 for 1,267 d.f. (P>.9995)

Adjusted Group Mean Particulate = 867.810×10^{-9} g at 49.040 ppb

$\log_{10}$ value = 2.9384 at 1.6906

Adjusted Group Mean Emulsifiable = 12.726×10^{-9} g at 49.040 ppb

$\log_{10}$ value = 1.1047 at 1.6906

Test for Equality of Adjusted Group Mean

F = 1658.195 for 1,268 d.f. (P>.9995)

Table 9. Methoxychlor concentrations (mg/litre) in water sampled at the start of the 11.1, 33.0, 100.0, 300.0 ppb exposure of the particulate and emulsifiable concentrate formulations of methoxychlor to black fly larvae (Simulium decorum Walker) to observe uptake residues when the larvae detached

	Exposure Concentration (ppb)			
	11.1	33.0	100.0	300.0
Particulate				
Exp. 1	0.0163	0.0275	0.0965	0.2404
Exp. 2	0.0126	0.0252	0.0930	0.2140
Emulsifiable				
Exp. 1	0.0124	0.0271	0.0861	0.2765
Exp. 2	0.0093	0.0276	0.0830	0.2806

Table 10. Methoxychlor concentrations (mg/litre) in water treated with the particulate and emulsifiable formulations of methoxychlor at a theoretical concentration of 0.3 mg/litre to observe toxicity in the stonefly nymph Pteronarcys dorsata Say

	Start	1 hr	4 hr	24 hr
Particulate				
Trough 1	0.76	0.17	0.08	0.04
Trough 2	0.56	0.18	0.07	0.04
Emulsifiable				
Trough 1	0.26	0.15	0.07	0.01
Trough 2	0.26	0.14	0.06	0.01

Table 11. Methoxychlor residue in Acroneuria lycorias (Newman) (µg/g) after exposure at 0.3 mg/litre for 1 hour

		Particulate	Emulsifiable
Exp. 1	$\bar{x}$	1.5	11.0
	SD	0.8	2.8
	Range	0.4-2.7	7.5-16.6
	n	7	7
Exp. 2	$\bar{x}$	1.4	14.0
	SD	0.9	3.7
	Range	0.1-2.7	8.4-18.1
	n	8	8
	After exposure at 0.3 mg/litre for 12 hours		
Exp. 1	$\bar{x}$	5.5	32.1
	SD	3.5	9.1
	Range	2.7-12.4	20.2-48.5
	n	7	8
Exp. 2	$\bar{x}$	3.4	22.1
	SD	0.6	5.0
	Range	2.5-3.9	13.9-29.6
	n	8	8

Table 12. Methoxychlor residue in Pteronarcys dorsata Say (µg/g) after exposure at 0.3 mg/litre for 1 hour

		Particulate	Emulsifiable
Exp. 1	$\bar{x}$	3.1	8.0
	SD	1.5	1.3
	Range	1.8-5.6	6.3-10.0
	n	8	8
Exp. 2	$\bar{x}$	5.2	10.8
	SD	3.8	9.7
	Range	2.5-13.6	5.4-34.7
	n	8	8
	After exposure at 0.3 mg/litre for 12 hours		
Exp. 1	$\bar{x}$	7.4	15.3
	SD	3.0	3.2
	Range	4.3-11.8	10.7-19.3
	n	7	8
Exp. 2	$\bar{x}$	6.9	15.6
	SD	3.0	2.8
	Range	3.2-10.9	11.9-19.1
	n	8	8

Table 13 (a). Methoxychlor concentrations (mg/litre) in water treated with particulate and emulsifiable formulations of methoxychlor after exposure at 0.3 mg/litre for 1 hour to observe uptake in Acroneuria lycorias (Newman) and Pteronarcys dorsata Say.

Particulate	Start	1 hr
Exp. 1	0.28	0.20
Exp. 2	0.29	0.21
Emulsifiable		
Exp. 1	0.30	0.22
Exp. 2	0.32	0.23

Table 13 (b). Methoxychlor concentrations (mg/litre) in water treated with particulate and emulsifiable formulations of methoxychlor after exposure at 0.3 mg/litre for 12 hours to observe uptake in Acroneuria lycorias (Newman) and Pteronarcys dorsata Say

Particulate	Start	6 hrs	12 hrs
Exp. 1	0.21	0.05	0.03
Exp. 2	0.21	0.05	0.03
Emulsifiable			
Exp. 1	0.23	0.06	0.03
Exp. 2	0.23	0.06	0.03

Table 14. Methoxychlor concentrations (mg/litre) in water sampled at the start of exposure to 0.1 and 0.3 mg/litre of particulate and emulsifiable concentrate formulations of methoxychlor to Chironomus tentans Fabricius. Fourth instar larvae, toxicity experiment

	Methoxychlor residue (mg/litre)	
	After exposure at 0.1 mg/litre	
	Particulate	Emulsifiable
Exp. 1	0.07	0.13
Exp. 2	0.11	0.12
	After exposure at 0.3 mg/litre	
	Particulate	Emulsifiable
Exp. 1	0.36	0.43
Exp. 2	0.24	0.34

Table 15. Methoxychlor concentrations (mg/litre) in water and (µg/g) in sediment (silica sand) sampled at the start of exposure to 0.1 and 0.3 mg/litre of the particulate and emulsifiable concentrate formulations of methoxychlor to Chironomus tentans Fabricius. Fourth instar larvae, uptake experiment

		Methoxychlor residue (mg/litre) in water and (µg/g) in sediment after exposure at 0.1 mg/litre	
		Particulate	Emulsifiable
Exp. 1	Water	0.05	0.08
	Sediment (1000 mg)	0.05	0.17
Exp. 2	Water	0.03	0.08
	Sediment (1000 mg)	0.10	0.10
		After exposure at 0.3 mg/litre	
		Particulate	Emulsifiable
Exp. 1	Water	0.09	0.24
	Sediment (1000 mg)	0.31	0.55
Exp. 2	Water	0.08	0.26
	Sediment (1000 mg)	0.27	0.22

CONCLUSIONS

As a result of the study described here, the following conclusions are made concerning the use of a particulate formulation of methoxychlor for the control of black fly larvae and its effect on some non-target groups of aquatic organisms.

1. Rainbow trout (Salmo gairdneri) are protected from adverse effects when exposed to the particulate formulation of methoxychlor relative to the emulsifiable concentrate formulation.

2. The particulate formulation decreased methoxychlor uptake in rainbow trout to only 20% or less of the level recorded in fish exposed to the emulsifiable concentrate formulation at exposure concentrations commonly in use for black fly control in large rivers, i.e. 0.1 and 0.3 mg/litre.

3. Black fly larvae (Simulium decorum) were killed by the particulate formulation as effectively as the emulsifiable concentrate at all concentrations and exposure times tested.

4. Black fly larvae exposed to four different concentrations of the particulate formulation accumulated significantly greater levels of methoxychlor before detaching and required much longer periods of time to detach than larvae exposed to similar concentrations of the emulsifiable concentrate formulation. It is concluded that the particulate must be ingested to be effective and once this occurs affected larvae will definitely die. The rapid response observed in larvae exposed to the emulsifiable concentrate suggests that it is taken up through respiratory surfaces and distributed to various organs with no lag time required for digestion, or some component of the formulation

is very irritating to the larvae causing them to detach very rapidly. The behavioural response precedes the lethal one and it may protect larvae from accumulating lethal doses of the emulsifiable concentrate.

5. The particulate formulation protected stone fly nymphs from adverse effects relative to the emulsifiable concentrate formulation. Nymphs of Acroneuria lycorias exposed to the E.C. formulation accumulated at least six times as much methoxychlor as nymphs exposed to the particulate material for the same time periods and concentration. Nymphs of Pteronarcys dorsata after similar time exposures to both formulations of methoxychlor at the same concentration accumulated at least twice as much of the E.C. formulation as the particulate formulation.

6. Larvae of Chironomus tentans were protected from adverse effects by the particulate formulation relative to the emulsifiable concentrate but not to the same degree as observed in fish or stone fly nymphs. At an exposure concentration of 0.1 mg/litre morbidity was reduced by about 40% in larvae exposed to the particulate formulation as compared to larvae exposed to the E.C. formulation for the observation period of 96 hrs. When the exposure concentration was increased to 0.3 mg/litre, larvae exposed to the particulate showed only about a 13% reduction in morbidity compared to larvae exposed to the E.C. formulation after 96 hrs. At this exposure level the particulate formulation proved almost as detrimental to the chironomid larvae as the emulsifiable formulation.

7. Chironomid larvae exposed to the emulsifiable concentrate formulation accumulated significantly higher levels of methoxychlor than

larvae exposed to the particulate formulation at both exposure concentrations (0.1 and 0.3 mg/litre) throughout the duration of the experiment.

8. The overall conclusion that can be made from the data in this study is that this particulate formulation of methoxychlor applied at a rate of 0.1 ppm for 15-30 min. should effectively control black fly larvae and significantly reduce uptake and risk of poisoning by fish, stonefly nymphs and chironomid larvae.

To improve the effectiveness and selectivity of particulate insecticides for the control of black fly larvae, the parameters that will have to be examined are 1) the rate of application, 2) the particle size, and 3) the specific gravity of the formulation.

Particulate larvicides applied in large rivers with a high suspended solids content would be competing with natural particles in the river for ingestion by black fly larvae, therefore the rate of application required for effective control of larvae might be different from the one recommended by this study (0.1 ppm/15-30 min.) or the level used by Helson (1972) and Helson and West (1978) in field trials conducted in shallow, clear-water streams.

The particle size of the formulation would also play a large part in determining the selectivity. By increasing the particle size range, feeding on insecticide particles by detritus feeders such as chironomid larvae, and filter feeders such as philopotamid larvae might be reduced, thus making the particulate formulation more selective to black fly larvae. Chance (1969) suggested that a particulate with a size range of 100-250 μm may be more readily ingested by black fly larvae than by

other stream insects. Perhaps the size range of particles ingested by larvae of the pest species should first be determined, then a particulate formulation could be made according to this information in order to increase the chance of ingestion and hence control of the pest larval species.

The specific gravity of the formulation would also be very important in efficacy as a black fly larvicide. Research is required to determine the distance of effectiveness of a particulate larvicide from the point of application under large river conditions; this would probably vary depending on the flow conditions of the river. A specific gravity that would allow the insecticide to be heavy enough to have a slow rate of sedimentation so it would become an integral part of the running waters of the river where the black fly larvae breed, yet light enough so it would not settle immediately and would exert control for some considerable distance beyond the point of application, would be ideal.

A particulate formulation could probably be made more biodegradable by adsorbing the chemical used on an organic molecule, e.g. (flour, charcoal, starch) as opposed to an inorganic molecule such as the celite particles in the particulate formulation in this study. If a non-persistent chemical such as methoxychlor was used on this organic particle, contamination of the stream or river for any length of time could be avoided.

Kurtak (1978) in studies concerned with efficiency of filter feeding of black fly larvae stated that the ideal particle for ingestion by black fly larval species he studied would be about 100-150 μm in diameter

about as dense as water, and rough surfaced. These criteria might be considered in the formulation of a particulate larvicide for the control of black fly larvae.

The emulsifiable concentrate formulation of methoxychlor tested in this study produced an almost immediate detrimental effect on all organisms examined e.g. fish, black fly larvae, chironomid larvae, and stonefly nymphs. It seems likely that either some component of the formulation (perhaps the emulsifier) was very irritable to these organisms, or it was acting as a contact poison either through the cuticle or through respiratory surfaces and distributed to various organs, or both. In western rivers adsorption of emulsifiable methoxychlor to suspended materials probably creates a situation very like the particulate formulation tested here by reducing uptake and risk of poisoning by fish and non-target insects. However Charnetski et al. (1980) observed a rather excessive amount of methoxychlor in the water not associated with particulate material at locations up to 80 km in the 1974 treatment of the Athabasca River with methoxychlor at a rate of 0.3 ppm for 15 min. Fredeen et al. (1975) observed after an injection of methoxychlor at 0.3 ppm for 15 min. into the North Saskatchewan River that the suspended solids contained only about 47 and 40% respectively of the total methoxychlor extracted from water samples taken at a distance of 6.5 km downstream from the injection site. Therefore a significant portion of the formulation remains mixed in the water where it can adversely affect fish and non-target groups of insects by a contact mode of action for many river miles before it all becomes adsorbed to river particles and is rendered less harmful to

these non-target groups. Thus even under optimum conditions when applied to rivers with high suspended solid content it appears that the emulsifiable concentrate formulation of methoxychlor will never be as selective as an artificial particulate formulation for black fly larvae.

Particulate formulations definitely merit further study as a possible alternative to the emulsifiable formulations presently in use to control black fly larvae.

LIST OF REFERENCES

Abdelnur, O.M. 1968. The biology of some black flies (Diptera:Simuliidae) of Alberta. Quaest. Entomol. 4:113-174.

Anderson, J.R. and R.S. Dicke. 1960. Ecology of the immature stages of some Wisconsin black flies (Simuliidae:Diptera). Ann. Entomol. Soc. Amer. 53:386-404.

Betten, C. 1934. The caddis flies or Trichoptera of New York State. N.Y. State Mus. Bull. #292: 203 pp.

Burdick, G.E., H.J. Dean, E.J. Harris, J. Skea, C. Frisa and C. Sweeney. 1968. Methoxychlor as a black fly larvicide, persistence of its residues in fish and its effect on stream arthropods. N.Y. Fish and Game J. 15(2):121-142.

Burdick, G.E., H.J. Dean, J. Skea and C. Frisa. 1974. Effect of black fly larviciding in some Adirondack streams. N.Y. Fish and Game J. 21(1):1-17.

Carlsson, G. 1967. Environmental factors influencing black fly populations. Bull. Wld. Hlth. Org. 37:139-150.

Chance, M.M. 1970. The functional morphology of the mouthparts of black fly larvae (Diptera:Simuliidae). Quaest. Entomol. 6:245-284.

Charnetski, W.A., K.R. Depner and S. Beltaos. 1980. Distribution and persistence of methoxychlor in Athabasca River water. Alberta Envir. - Special Publication (In Press).

Chutter, F.M. 1968. On the ecology of the fauna of stones in the current in a South African river supporting a very large Simulium (Diptera) population. J. Appl. Ecol. 5:531-561.

Craig, D.A. 1974. The labrum and cephalic fans of larval Simuliidae (Diptera:Nematocera). Can. J. Zool. 52:133-159.

Craig, D.A. 1977. Mouthparts and feeding behaviour of Tahitian larval Simuliidae (Diptera:Nematocera). Quaest. Entomol. 13:195-218.

Cummins, K.W. 1973. Trophic relations of aquatic insects. Ann. Rev. Ent. 18:183-206.

Davies, D.M. 1950. A study of black fly populations of a stream in Algonquin Park, Ontario. Trans. R. Can. Inst. 28:121-263.

Davies, D.M. and P.D. Syme. 1958. Three new Ontario black flies of the genus Prosimulium (Diptera:Simuliidae) Part II: Ecological observations and experiments. Can. Ent. 90:744-759.

*Dejoux, C. and J. Troubat. 1974. Action in situ de l'Abate sur la fauna aquatique non-cible, ORSTOM-NOJAMENA No. 1300, 52 pp., 20 VII 74.

Dosdall, L. and D.M. Lemkuhl. 1979. Stoneflies (Plecoptera) of Saskatchewan. Quaest. Entomol. 15:3-116.

Edington, J.M. 1965. The effect of water flow on populations of net-spinning Trichoptera. Mitt. Internat. Verein. Limnol. 13:40-48.

Edmunds, G.F. 1957. The predaceous mayfly nymphs of North America. Proc. Utah Acad. Sci. 34:23-24.

Egglishaw, H.J. 1964. The distributional relationship between the bottom fauna and plant detritus in streams. J. Anim. Ecol. 33: 463-476.

*Elouard, J., F. Lebtahi, C. Leveque, and P. Venard. 1974. Lutte contre Simulium damnosum vecteur de l'onchocercose humaine in Afrique Occidentale. Effets de 2 formulations d'Abate sur l'entomofaune associee aux larves de simulies et sur l'ichtyofaune, ORSTOM-Bouake 341, 12 pp.

*Escaffre, H., J. Brunhes, Y. Sechan, G. Carlsson, A. Stiles, H. Kulzer, O. Pawlick and J. Mertens. 1974. Lutte contre Simulium damnosum vecteur de l'onchocercose humaine en Afrique occidentale. VII Epandages par helicoptere de nouvelles formulations et de nouveaux insecticides dans la region de Korhogo en Cote d'Ivoire ORSTOM-Bouake 27/Onch/Tech. 74, 20 pp. English version: WHO/VBC/76.620.

Flannagan, J.F., B.E. Townsend, B.G.E. DeMarch, M.K. Friesen and S.L. Leonhard. 1979. The effects of an experimental injection of methoxychlor on aquatic invertebrates: Accumulation, standing crop, and drift. Can. Entomol. 111:73-89.

Fredeen, F.J.H. 1960. Bacteria as a source of food for black fly larvae. Nature, Lond. 187:963.

Fredeen, F.J.H. 1962. DDT and Heptachlor as black fly larvicides in clear and turbid water. Can. Entomol. 94:876-880.

Fredeen, F.J.H. 1964. Bacteria as food for black fly larvae (Diptera: Simuliidae) in laboratory cultures and in natural streams. Can. J. Zool. 42:527-548.

Fredeen, F.J.H. 1969. Outbreaks of the black fly Simulium arcticum Malloch in Alberta. Quaest. Entomol. 5:341-372.

Fredeen, F.J.H. 1974. Tests with single injections of methoxychlor black fly (Diptera:Simuliidae) larvicides in large rivers. Can. Ent. 106:285-305.

Fredeen, F.J.H. 1975. Effects of a single injection of methoxychlor black fly larvicide on insect larvae in a 161 km (100 mile) section of the North Saskatchewan River. Can. Ent. 107: 807-817.

Fredeen, F.J.H. 1977. A review of the economic importance of black flies (Simuliidae) in Canada. Quaest. Entomol. 13:219-229.

Fredeen, F.J.H., A.P. Arnason and B. Berck. 1953a. Adsorption of DDT on suspended solids in river water and its role in black fly control. Nature, Lond. 171:700-701.

Fredeen, F.J.H., A.P. Arnason, B. Berck and J.G. Rempel. 1953b. Further experiments with DDT in the control of Simulium arcticum Malloch in the north and south Saskatchewan Rivers. Can. J. Agr. Sci. 33: 379-393.

Fredeen, F.J.H., J. Saha and M. Balba. 1975. Residues of methoxychlor and other chlorinated hydrocarbons in water, sand, and selected fauna following injections of methoxychlor black fly larvicide into the Saskatchewan River, 1972. Pest. Monit. J. 8(4):241-246.

Gardner, D.R. and J.R. Bailey. 1975. Methoxychlor: Its effects on environmental quality. Nat. Res. Council Canada, N.R.C.C. 14102, 164 pp.

Greiner, E.C., G.F. Bennett, E.M. White and R.F. Coombs. 1975. Distribution of the avian hematozoa of North America. Can. Jour. Zool. 53:1762-1787.

Helson, B.V. 1970. Exploratory studies on the possible selective effects of particulate insecticides on stream invertebrate fauna in regard to black fly control. B.Sc. Thesis, Queen's University, Kingston, Ontario.

Helson, B.V. 1972. The selective effects of particulate formulations of insecticides on stream fauna when applied as black fly (Diptera: Simuliidae) larvicides. M.Sc. Thesis, Queen's University, Kingston, Ontario.

Helson, B.V. and A.S. West. 1978. Particulate formulations of abate and methoxychlor as black fly larvicides: Their selective effects on stream fauna. Can. Entomol. 110:591-602.

Hynes, H.B.N. 1970. The Ecology of Running Waters 1st ed. Univ. of Toronto Press. 555 pp.

Jamnback, H. 1976. Simuliidae (black flies) and their control. Bull. Wld. Hlth. Org. 76:653, 64 pp.

Jones, J.R.E. 1949. A further ecological study of calcareous streams in the 'Black Mountain' district of South Wales. J. Anim. Ecol. 18:142-159.

Kershaw, W.E., T.R. Williams, S. Frost and H.B.N. Hynes. 1965. Selective effect of particulate insecticides on Simulium among stream fauna. Nature, Lond. 206:199.

Kershaw, W.E., T.R. Williams, S. Frost, R.E. Matchett, M.L. Mills and R.D. Johnson. 1968. The selective control of Simulium larvae by particulate insecticides and its significance in river management. Trans. R. Soc. Med. and Hyg. 62:35-40.

Kissam, J., R. Noblet and H. Moore. 1973. Simulium: Field evaluation of Abate larvicide for control in an area endemic for Leucocytozoon smithi of turkeys. J. Econ. Ent. 66:426-428.

Kurtak, D.C. 1978. Efficiency of filter feeding of black fly larvae (Diptera:Simuliidae). Can. J. Zool. 56:1608-1623.

Laird, M. and G.F. Bennett. 1970. The subarctic epizootiology of Leucocytozoon simondi. J. of Parasit. 56:198.

*Lauzanne, L. and C. Dejoux. 1973. Etude de terrain de la toxicate sur la faune aquatique non cible de nouveaux insecticides employes en lutte anti-simulies, ORSTOM, Fort-Lamy, 38 pp.

Lloyd, J.T. 1921. The biology of North American caddis fly larvae. Bull. Lloyd Liber. 21:1-24.

Lockhart, W.L. 1980. Methoxychlor Studies with Fish: Athabasca River Exposures and Experimental Exposures. Alberta Env. - Special Publication (In Press).

Lockhart, W.L., D.A. Metner and J. Solomon. 1977. Methoxychlor residue studies in caged and wild fish from the Athabasca River, Alberta, following a single application of black fly larvicide. J. Fish. Res. Board Can. 34:626-632.

Maitland, P.S. and M.M. Penney. 1967. The ecology of the Simuliidae in a Scottish river. J. Anim. Ecol. 36:179-206.

Merna, J.W., M.E. Bender and J.R. Novy. 1972. The effects of methoxychlor on fishes. I. Acute toxicity and breakdown studies. Trans. Am. Fish. Soc., 101:298-301.

Merna, J.W. and P.J. Eisele. 1973. The effects of methoxychlor on aquatic biota. U.S. Environ. Prot. Agency, Ecol. Res. Ser., EPA-R3-73-046. 59 pp.

Millar, J.L. and J.G. Rempel. 1944. Livestock losses in Saskatchewan due to black flies. Can. J. of Comp. Med. and Vet. Science 8:334-337.

Noel-Buxton, M.B. 1956. Field experiments with DDT in association with finely divided inorganic material for the destruction of the immature stages of the Genus Simulium in the Gold Coast. J.W. Afr. Sci. Ass. 2:36-40.

*Pennak, R.W. 1953. Freshwater Invertebrates of the United States. Ronald Press Comp., New York. pp. 501, 616, 650.

Percival, E. and H. Whitehead. 1929. A quantitative study of some types of stream-bed. J. Ecol. 17:282-314.

Peterson, B.V. 1959. Observations on mating, feeding and oviposition of some Utah species of black flies (Diptera:Simuliidae). Can. Ent. 91:147-155.

*Philippon, B., Y. Sechan, F. Riviere, H. Kulzer, O. Pawlich and M.Krupke. 1973. Lutte contre Simulium damnosum vecteur de l'ouchocercose humaine en Afrique Occidentale. VI. Epandages de nouvelles formulations insecticides par helicoptere in region de savane quineenne. English version: WHO/VBC/76.619.

Philipson, G.N. 1953. The larva and pupa of Hydropsyche instabilis Curtis (Trichoptera:Hydropsychidae). Proc. R. Ent. Soc. Lond. A28:17-23.

*Pledger, D.J. 1978. Black flies (Diptera:Simuliidae) of the Swan Hills, Alberta, as possible vectors of Onchocerca cervipedis Wehr and Dikmans, 1935 (Nematoda, Onchocercidae) in Moose (Alces Alces Linnaeus). M.Sc. Thesis, Univ. of Alberta, Edmonton, Alberta. 143 pp.

Rempel, J.G. and A.P. Arnason. 1947. An account of three successive outbreaks of the black fly, Simulium arcticum, a serious livestock pest in Saskatchewan. Sci. Agr. 27:428-445.

Ross, H.H. 1944. The caddis flies, or Trichoptera, of Illinois. Bull. Ill. Nat. Hist. Surv. 23:1-326.

Savage, A. and J.M. Isa. 1945. Case report. An outbreak of Leucocytozoon disease in turkeys. Cornell Veterinarian 35:270-272.

*Schneller, G.H. and G.B.L. Smith. 1949. 1-Trichloro-2, 2-bis (p-methoxyphenyl) ethane (Methoxychlor) Industrial and Engineering Chemistry 41:1027-1029.

Solomon, J. and W.L. Lockhart. 1977. Rapid preparation of micro sample and gas-liquid chromatographic determination of methoxychlor residues in animal tissues and water. J. Assoc. Off. Anal. Chem. 60:690-695.

Solomon, J. and D.C.G. Muir. 1978. A rapid semi-micro method for the determination of chlorinated hydrocarbons in fish tissue and water samples. Proc. Can. Workshop on Pesticide Chem., Vancouver, May 1978.

Swabey, Y.H., C.F. Shenk and G.L. Parker. 1967. Evaluation of two organophosphorus compounds as black fly larvicides. Mosquito News 27:149-155.

Thompson, B.H. 1975. Testing the susceptibility of Simulium damnosum (Diptera:Simuliidae) to insecticides in the laboratory and in the field. Bull. Wld. Hlth. Org. 52:351-357.

Townsend, B.E. (In prep.) Chironomus tentans Fabricius. In S.G. Lawrence (ed.). A manual for the Culture of Selected Freshwater Invertebrates. Jour. of Fish and Aquat. Sciences. Special Publication.

Travis, B.V. and D.P. Wilton. 1965. A progress report on simulated stream tests of black fly larvicides. Mosquito News 25:112-118.

Wallace, R.R. 1971. The effects of several insecticides on black fly larvae (Diptera:Simuliidae) and on other stream-dwelling aquatic invertebrates. M.Sc. Thesis, Queen's University, Kingston, Ontario.

Wallace, R.R. and H.B. Hynes. 1975. Catastrophic drift of stream insects after treatments with methoxychlor (1, 1, 1-trichloro-2, 2-bis (p-methoxyphenyl) ethane). Envir. Pollut. 8:255-268.

Wallace, R.R., H.B. Hynes and W.F. Merritt. 1976. Laboratory and field experiments with methoxychlor as a larvicide for Simuliidae. Environ. Pollut. 10:251-269.

Wallace, R.R., A. West, A. Downes and H.B. Hynes. 1973. The effects of experimental black fly (Diptera:Simuliidae) larviciding with Abate, Dursban, Methoxychlor on stream invertebrates. Can. Ent. 105:817-831.

Williams, T.R., R. Connolly, H.B.N. Hynes and W.E. Kershaw. 1961. Size of particles ingested by Simulium larvae. Nature, Lond. 189:78.

*Wilson, R. and D. Snow. 1972. Non-target effects of Abate, an organophosphorus insecticide. Unpublished report of a field program to evaluate black fly control methods at Baie Verte, Nfld., Manuscript Report No. 72-8, Atlantic Region, Envir. Prot. Serv., St. John's, 177 pp.

Wotton, R.S. 1976. Evidence that black fly larvae can feed on particles of colloidal size. Nature, Lond., 261 (5562):697.

Wotton, R.S. 1977. The size of particles ingested by moorland stream black fly larvae (Simuliidae). Oikos 29:332-335.

Zitko, V. 1974. Uptake of chlorinated paraffins and PCB from suspended solids and food by juvenile Atlantic salmon. Bull. Envir. Contam. Toxicol. 12:406-412.

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*Original article not seen.