EFFECTS OF THE INSECTICIDE PERMETHRIN ON THE AQUATIC LIFE STAGES OF THE BURROWING MAYFLY HEXAGENIA RIGIDA (EPHEMEROPTERA:EPHEMERIDAE)

A THESIS

SUBMITTED TO THE FACULTY

OF

GRADUATE STUDIES

THE UNIVERSITY OF MANITOBA

BY

MARGARET KATHERINE FRIESEN

IN PARTIAL FULFILLMENT OF THE

REQUIREMENTS FOR THE DEGREE

OF

MASTER OF SCIENCE

DEPARTMENT OF ENTOMOLOGY

SEPT 1981

EFFECTS OF THE INSECTICIDE PERMETHRIN ON THE AQUATIC LIFE STAGES OF THE BURROWING MAYFLY HEXAGENIA RIGIDA (EPHEMEROPTERA: EPHEMERIDAE)

BY

MARGARET KATHERINE FRIESEN

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ABSTRACT

In a laboratory study, eggs and nymphs of the burrowing mayfly Hexagenia rigida were exposed to permethrin in water and/or sediment at levels corresponding to field application rates at and below 730.8ug/m^2. In the first set of experiments, nymphs were transferred to containers to which permethrin had already been added. In nymphs exposed for 0.25 days to permethrin in water, mortality ranged from 20.9±7.22% at 15.3ug/m^2 (=0.15ug/L) to 100.0±0.00% at 730.8ug/m^2 (=7.63ug/L). Mortality of nymphs exposed to sediment treated with permethrin depended on concentration of permethrin, length of exposure and length of time since treatment of sediment. Exposure of nymphs to sediment treated at the rate of 15.3ug/m^2 resulted in 20.0±0.00% mortality when nymphs were exposed for 7 days to freshly contaminated sediment, but no significant ($P<0.05$) mortality when they were exposed for 1 day to 1 day "old" sediment i.e. sediment contaminated 1 day previously, or when nymphs were exposed continuously to 8 day old sediment. Exposure of nymphs to sediment treated at the rate of 730.8ug/m^2 resulted in 73.7±24.1% mortality when they were exposed for 1 day to 1 day old sediment, 93.3±11.54% when they were exposed to 1 day old sediment for 7 days, and 100.0±0.00% when they were exposed continuously to 8 day old sediment. Treatments of 55.6ug/m^2 and 197.3ug/m^2 resulted in mortalities intermediate between those observed for 15.3ug/m^2 and 730.8ug/m^2 in all but one case; no significant ($P<0.05$) mortality occurred at 55.6ug/m^2 after continuous exposure to 8

day old sediment. Mortality checks up to 1 week and possibly 4 weeks from the start of the experiment were necessary in some cases to determine final nymphal mortality regardless of exposure regime.

In the second set of experiments, referred to as the simulated application, permethrin was applied at the rate of 55.6 and 730.8ug/m^2 directly to the containers in which nymphs were being cultured. Results similar to those observed in the contaminated water and contaminated sediment experiments were obtained. Nymphs in containers contaminated at the rate of 55.6ug/m^2 showed 27.8±16.94% mortality after a 1 day exposure and 74.2±10.93% mortality after continuous exposure to freshly contaminated sediment. Nymphs exposed to sediment treated at a rate of 730.8ug/m^2 showed 88.0±16.18% mortality after a 1 day exposure, and 100.0±0.00% mortality when nymphs were exposed continuously to freshly contaminated conditions. Any deaths attributable to permethrin toxicity had occurred within 1 week after treatment in this part of the experiment. The beakers which had been treated at 55.6 ug/m^2 with nymphs removed after 1 day, and all beakers which had been treated at 730.8ug/m^2, were reinoculated 8 days post-application and nymphs were left indefinitely. Final mortality (after 10 weeks) at the 55.6ug/m^2 application rate was 45.0±4.05%, and at 730.8ug/m^2 it was 100.0±0.00%. The concentration of permethrin in sediment at day 7 in beakers that had been treated at 730.8ug/m^2 was estimated to be in the range of 7.6ug/kg to 69.0ug/kg dry weight of sediment.

Eggs were exposed for 3 day periods at four different stages of development to solutions of permethrin at estimated concentrations ranging from 0.14 to 27.64ug/L. At 2.02ug/L, 89.5±4.17% of eggs exposed

in the earliest developmental stage hatched whereas no hatch occurred in any of the other three stages. No effect on hatch in any stage tested was noted at 0.14ug/L. Effects on success of hatch of early stage eggs after exposure periods of 3 days and less were studied. No significant difference ($P<0.05$) was found in success of hatch of eggs exposed for 3h and 3 day periods.

From these findings it appears that nymphs and eggs of H. rigida are killed at levels which may occur in water bodies as a result of aerial application of this pesticide. Nymphs and eggs may be affected while permethrin is in the water column, and at least the nymphal stage may be affected once permethrin has become associated with sediment. This latter effect would be exerted for days and possibly weeks after the initial application.

To Dr. H.E. Welch, (Department of Zoology, University of Manitoba) for enticing me to study the fascinating world of biology.

ACKNOWLEDGEMENTS

I wish to acknowledge, with thanks and appreciation, the Freshwater Institute, Department of Fisheries and Oceans, for financial assistance and for permission to use data obtained while being an employee of the Department.

I also wish to thank:

Dr. T. D. Galloway, my thesis supervisor, and Mr. J.F. Flannagan, for guidance and advice during the course of the study, and for critically reviewing the thesis manuscript;

Dr. R.A. Brust and Dr. D.C.G. Muir, supervisory committee members, for critically reviewing the thesis;

Dr. D.C.G. Muir and Mr. J.L. Solomon for guidance and assistance with the chemical aspects of the study;

Mrs. B.G.E. de March for advice on statistical aspects of the study, as well as for reviewing the manuscript;

Mr. D.G. Cobb, Mr. K. Laing and Ms. L. Onischuck for able technical assistance;

Mr. K.E. Marshall for maintaining a rich and readily accessible array of scientific literature in the Freshwater Institute library;

Ms. L. McIver for patiently instructing me on the use of the word processor;

Ms. D. Kufflick for drafting figures for the thesis;

Ms. D. Holland for typing the tables;

The staff members at the Freshwater Institute who willingly provided advice and assistance in innumerable ways;

The students and faculty of the Department of Entomology for helping me keep this work in perspective;

Mr. J.F. Flannagan, Dr. S.G. Lawrence and Dr. D.F. Malley for encouraging me to undertake a M.Sc. program;

Ms. S.L. Leonhard, colleague and friend, for encouragement, constructive criticsm and interest throughout the course of the study;

My parents, Katharina and George Friesen, and sister Helene Merrell for moral support.

TABLE OF CONTENTS

Page

Page

LIST OF FIGURES

Figure | Page

LIST OF TABLES

Table | Page

LIST OF APPENDICES

INTRODUCTION

Permethrin (3- phenoxybenzyl (+)- cis, trans- 2, 2- dimethyl- 3- (dichlorovinyl) cyclopropane carboxylate), a synthetic pyrethrin, has been proposed as an agent for the control of a range of agricultural, horticultural and forest pest insects. It is being considered for large scale spray programs for spruce budworm control in Canada. Aerial spraying of an insecticide can lead to its incidental introduction into aquatic ecosystems which may result in adverse effects to parts of the aquatic community (Flannagan 1973; Eidt 1975; Courtemanch and Gibbs 1980). Permethrin is relatively short-lived in the water column (Kingsbury and Kreutzweiser 1979, 1980; Rawn et al. 1979,1980) and adsorbs readily onto particulate matter (Rawn et al. 1979,1980). The toxic action to aquatic arthropods, once this insecticide has become associated with sediment, has not been studied. Since nymphs of the burrowing mayfly, Hexagenia spp., inhabit and ingest sediment (Zimmerman 1977), they are good candidates for studies investigating possible toxic action of a compound once it has become associated with sediment.

The present study was an investigation on the effects of contaminated water and/or contaminated sediment on nymphs of Hexagenia rigida (McDunnough), including post-treatment mortality. Effects of contaminated water on success of hatch of H. rigida eggs were also studied.

CHAPTER I

REVIEW OF PERTINENT LITERATURE

A. PERMETHRIN

The synthetic pyrethrins, commonly termed pyrethroids, are patterned after the insecticidally active constituents in pyrethrum extract. Pyrethrum, which is obtained for commercial purposes from the flower of Chrysanthemum cinerariaefolium (Treviranus) Buccone, has been used as an insecticide in Europe for more than a century, and was in use much earlier than this in Persia (Iran). Pyrethrum was used as a crude powder until an extraction method using kerosene was developed in 1919. This lead to the development of liquid sprays which were fast acting and highly toxic to insects (McLaughlin 1973). In 1935 the usefulness of pyrethrins as contact insecticides against pests of stored products was demonstrated (Elliott et al. 1978). Expansion of use of the natural product is limited largely because of its instability in light and air and its high cost of production. Hence, intensive research into the development of synthetic pyrethrins was begun to develop compounds which would be more stable and more economical to produce (McLaughlin 1973). The first pyrethroids, allethrin and cyclethrin, were produced commercially around 1950 (O'Brian 1967), and were succeeded by numerous other pyrethroids. Some of these products were superior to the natural product e.g. they were more toxic to insects, but all were insufficiently stable in light and air to be used on a widescale basis.

The first photostable pyrethroids, including permethrin, were introduced in 1973 (Elliott et al. 1973). The properties of these chemicals are considered to be sufficiently different by some workers (Elliott et al. 1978) to be regarded as a new class of insecticide, and in some cases these compounds may eventually replace established insecticides. Although more expensive to produce, pyrethroids are effective at doses considerably lower than the "traditional" insecticides. They appear to be readily metabolized by mammals and birds, and breakdown products are thought to be of low toxicity (Elliott et al. 1978).

Natural pyrethrins and pyrethroids are thought to act as neurotoxins and are effective as contact insecticides, and to a lesser degree as stomach poisons (Elliott et al. 1978). Pyrethroids probably act directly on excitable membranes and interfere with ionic conductances (Narahashi 1976). They are well known for their rapid knockdown action, but death may not occur for up to two days after contact with the poison (Briggs et al. 1974). The initial knockdown phase in insects during pyrethroid poisoning results from loss of co-ordination and locomotor activity. Other symptoms generally include hyperexcitation, tremors and convulsions. Advanced poisoning leads to paralysis and death (Wouters and van den Bercken 1978).

Permethrin is patterned after Pyrethrin I, one of six insecticidally active constituents of pyrethrum. Permethrin is an ester of a cyclopropane carboxylic acid and 3-phenoxybenzyl alcohol (Fig. 1). This particular alcohol and the dichlorovinyl side chain at C-3 of the

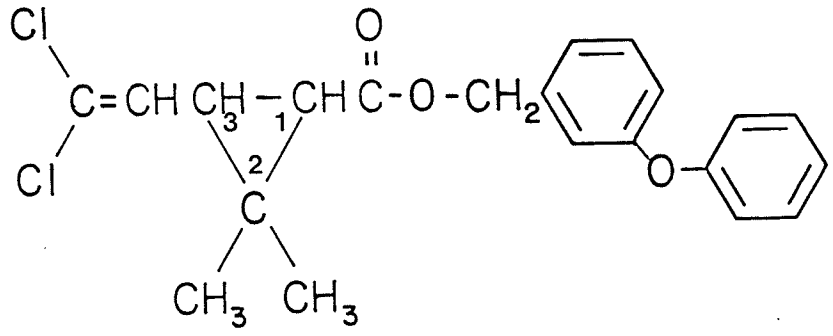

Fig. 1. Structural formula of permethrin (modified from Elliott et al. 1973).

cyclopropane ring result in the photostability of this chemical. The gem-dimethyl group at C-2 of the cyclopropane ring is necessary to obtain the insecticidal activity. Both the cis and trans isomers are insecticidally active (Wouters and van den Bercken 1978). Degradation products are alcohols and acids, and are thought to be inactive as insecticidal agents (Elliott 1976).

Permethrin has been shown to be an effective control agent against the adult, larval and egg stages of a variety of insect species and is thus considered to be a wide spectrum insecticide (Elliott et al. 1978). Registered outdoor uses in Canada include: control of agricultural pests of tobacco, potatoes, cole crops and sweet corn, as well as fruit tree pests of apples, pears, peaches, nectarines and grapes. Suggested trial uses include use against other fruit, vegetable and field crop pests, biting flies, pests of livestock and ornamental, nursery and forest tree pests. The forest tree pests include spruce budworm and gypsy moth species, and the insecticide would be applied through aerial application at the rate of 17.5-35.0 g/ha active ingredient (ai) (Anon. 1981).

Permethrin may enter water bodies as an incidental consequence of aerial spraying. Effects of permethrin in an aquatic system are of concern since some aquatic organisms, including many insects, have been shown to be adversely affected. Studies on the toxic action of permethrin to arthropods once it has become associated with sediment have not been recorded in the published literature.

1. Fate and toxicity of permethrin in water

Permethrin is not detectable in water after a period of hours or days. Two major field trials of aerial applications of permethrin have been conducted in forested areas in Eastern Canada (Kingsbury and Kreutzweiser 1979,1980). In the first trial, conducted in 1978, 17.5g/ha permethrin were applied twice at an interval of 6 days. In the second trial, conducted the following year, 17.5g/ha were applied once. In 1978 the highest level detected in ponds was 2.6ug/L, measured 2h post-treatment and in streams was 0.55ug/L, measured 1/2h post-treatment. Water samples for analyses had been removed 1/2h (in streams only), 2h, 12h and later, and it is possible that the actual peak levels may have been higher. Permethrin levels had dropped to below detectable limits (0.25ug/L) within 48h in the ponds and within 12h in the streams. In the 1979 trial, highest water concentrations in ponds and streams were 147 ug/L and 2.5ug/L, respectively, measured 1h post-application. Levels had dropped to below detectable limits (0.05ug/L) within 48h. Rawn et al. (1979) found that an initial permethrin concentration of 15.5ug/L in outdoor ponds decreased to 1.5ug/L after 18h and to 0.04ug/L after 72h. This disappearance was attributed to breakdown of the parent compound as well as adsorption to sediment (hydrosoil).

Some aquatic arthropods and fish have been shown to be sensitive to low levels of permethrin in water in laboratory and field studies. Certain insects are extremely sensitive to the chemical even after short exposure times. After 1h exposure LC90-95 values were determined to be

approximately 1ug/L for the mayfly Baetis sp. and the caddisfly Brachycentrus sp., and approximately 5ug/L for the black fly Simulium sp. (Muirhead-Thomson 1978). After 15min exposure the LC50 was estimated to be 5-10ug/L for Simulium larvae ; a 15min exposure of the caddisfly Rhyacophila sp. to 10ug/L permethrin resulted in 100% mortality 24h post-treatment (Muirhead-Thomson 1979). Gill (1977) estimated 24h LC50 and 24h LC90 levels for 4th instar Aedes albopictus (Skuse) to be 9.2 and 24ug/L, respectively. Newly hatched and juvenile crayfish were found to have a 96h LC50 of 0.39 and 0.62ug/L, respectively (Jolly et al. 1978). One hundred percent mortality was recorded 24h after the amphipod Gammarus sp. had been exposed to 2ug/L permethrin for 1h (Muirhead-Thomson 1978). Fish are sensitive to permethrin at similar levels: Jolly et al. (1978) estimated the 96h LC50 for channel catfish, bass and mosquitofish to be 1.1, 8.5 and 15.0ug/L, respectively; Kumaraguru and Beamish (1981) reported 96h LC50s for 1g rainbow trout to range from 0.62 to 6.43ug/L, depending on temperature.

In streams studied in the 1978 field trial by Kingsbury and Kreutzweiser (1979), where the highest level measured in water was 0.55ug/L, numbers of drifting organisms increased sharply immediately after the spray and continued for about 15h. Organisms drifting after the first spray consisted of mainly mayfly nymphs (F. Baetidae and F. Heptageniidae), stonefly nymphs and caddisfly larvae. Drift also increased after the second spray but was considerably smaller in magnitude and consisted largely of stonefly nymphs. In a similar spray program in 1979 (not in the same area), mayfly nymphs (F. Baetidae and

F. Heptageniidae) made up the major portion of the postspray drift samples. At a station located 2 km downstream from the treated area , increased drift was made up almost exclusively of early-instar mayfly (F. Baetidae) and stonefly nymphs (Kingsbury and Kreutzweiser 1980).

2. Fate and toxicity of permethrin in soil and sediment

Several studies on fate of permethrin in soils and hydrosoils (sediment) have been conducted. The finding that very little movement of either the cis or trans isomer occurred through soil leached with water, is a good indication that permethrin will remain near the application site (Kaneko et al. 1978; Kaufman et al. 1981), and should not disperse readily in the environment through run-off. Permethrin, in most of the soils tested, has been found to have a half life of less than or about 4 weeks (Kaufman et al. 1977; Kaneko et al. 1978; Williams and Brown 1979). Rawn et al. (1980) found that peak levels of 330ug/kg (dry weight) of methylene-^{14}C-labelled permethrin in hydrosoil 3-4 days post-treatment decreased to half this level within 2 weeks and to less than 70ug/kg in 4 weeks. A similar pattern was noted for cyclopropyl-labelled permethrin except that the peak was at 160ug/L. The lower values for the cyclopropyl labelled permethrin were thought to occur because the more polar acid metabolites of the cyclopropyl portion of the molecule tend to remain in water because of their polar nature, whereas the less polar alcohol metabolites of the methylene portion tend to associate with the hydrosoil. Belanger and Hamilton (1979) found that permethrin applied at rates of 0.56 and 1.12kg/ha ai persisted in soil for 28 days post-application and then declined slowly for the rest

of the season. Sharom and Solomon (1981a), in a laboratory investigation on the fate of permethrin in a water/sediment system, found that after 3 weeks permethrin had not penetrated further than 20mm into the sediment. Microbial activity has been demonstrated to play a major role in the degradation of permethrin in soils (Kaufman et al. 1977; Williams and Brown 1979).

Little work has been done on toxicity of permethrin when associated with soil or sediment. Permethrin applied at a rate of 10ug/g to a sandy loam had no significant effects on non-symbiotic nitrogen fixers, or bacterial or fungal populations examined 6 days post-treatment (Tu 1978). Tu (1980) found that permethrin applied at the above rate showed an inhibitory effect on bacterial and fungal counts in soil after a 1 week exposure but not after a 2 week exposure. From experiments on use of permethrin for the control of soil-inhabiting insects, Harris and Turnbull (1978) found toxicity and spectrum of permethrin activity somewhat inferior to the insecticides chlorpyrifos and carbofuran, but still sufficiently effective to be included in further investigations. Soil type may affect toxicity of permethrin; permethrin (FMC 33297) was about eight times more toxic to crickets in mineral soil than in organic soil (Harris and Kinoshita 1977).

Adverse effects were noted on some benthic insects in the field trial studied by Kingsbury and Kreutzweiser (1979). It should be noted that accurate estimation of numbers of benthic organisms in the ponds is questionable since a Surber sampler, normally used to sample benthic organisms in running water (Merritt and Cummins 1978), was used as the

sampling device in these studies. From the data it appears that Trichoptera larvae may have been totally eliminated since none were found in any of the three treatment ponds after the second spray. Numbers of chironomid larvae declined substanstially in two of the ponds when compared to post-spray levels in the control pond. The highest post-treatment density in the treated ponds was 75 animals/0.093m^2, whereas 500 animals/0.093m^2 was the highest in the control pond. Ephemeroptera were not recorded in any treatment ponds pre- or post-treatment. The diet of one fish species studied shifted from a variety of aquatic insects to primarily midge larvae for at least a 45 day period after the spray. No fish mortality was detected in the ponds. In the streams, bottom fauna numbers decreased after both sprays, the organisms affected most being those which were collected in large quantities in the drift samples. The treated areas were not recolonized in proportions similar to the control station by the affected types of animals for at least 120 days at two stations. Downstream areas were also affected with a reduction in bottom fauna at a station located 2 km from the treatment area. A reduction in bottom fauna at a downstream station was also observed in the field trial conducted the following year (Kingsbury and Kreutzweiser 1980).

B. HEXAGENIA RIGIDA

The genus Hexagenia Walsh is probably restricted to the western hemisphere and has been recorded from Rio Negro, Argentina north to Great Slave Lake, N.W.T., Canada (Edmunds et al. 1976). H. rigida was

chosen for the present study because it is available locally from the Red River in large numbers. This species occurs in eastern and central North America and according to Spieth (1941) is largely confined to the upper part of the Mississippi drainage and the St. Lawrence drainage. It is also abundant in parts of the Churchill-Nelson river system (Flannagan 1979). This species is similar to the four other Hexagenia species recognized by Edmunds et al. (1976), and fills a similar ecological role as food for fish and birds while in the terrestrial phase, and for fish in the aquatic stage. This genus is an important food source for economically important fish such as whitefish and walleye (Neave 1932; Dobie 1966). The distribution of H. rigida overlaps with that of H. limbata and the two species are not distinguishable in the early nymphal stages or between female adults.

The life cycle of Hexagenia species is typical of the order Ephemeroptera and consists of two terrestrial stages, the subimago and imago, and two aquatic stages, the egg and nymph. The terrestrial phases last several days, and it is at this time that dispersal, mating and egg laying occur. Parthenogenetic development has been recorded in H. rigida (Friesen and Flannagan 1976). The terrestrial phases are most familiar to the general public because of the remarkable synchronous mass emergences which occur during the summer in some areas. In extreme cases, mass emergences have created hazardous road and boating conditions and decaying mayfly bodies have created undesirable and potential public health problems (Fremling 1964, 1968). A lesser known problem is the occurrence of allergic reactions in some humans to the powdered subimaginal skin (Perlman 1961).

Hexagenia spp. are among the largest mayflies in the order. Imagines have a wing length of 10-25mm. One female adult may produce up to 8,000 eggs (Edmunds et al. 1976), which are ovoid in shape and in H. rigida have approximate dimensions of 0.2 by 0.3mm (Neave 1932). Eggs are laid directly on or into the water and immediately sink to the substrate. Hatching may occur within days or possibly months depending on environmental conditions such as temperature. Diapause is not known to occur in this genus.

The majority of the life cycle is spent in the nymphal stage. Development is direct and the nymph may undergo as many as forty molts (Zimmerman 1977). Nymphs reach a body length of 12-32mm. Time span of this stage varies and is probably largely dependent on temperature. The life cycle of H. limbata is 2 years in the north of L. Winnipeg (Flannagan 1979) and was found to be less than 5 months in a canal channel in Utah, U.S.A. (Edmunds et al. 1976). Nymphs inhabit silt-type sediments. Early instar nymphs probably inhabit interstitial spaces whereas older nymphs construct burrows. Depth to which nymphs burrow into the sediment depends on body size (Hunt 1953) and type of sediment (Walker 1970). In H. limbata small nymphs inhabit the top 10mm of substrate, whereas larger (more than 10mm) nymphs may burrow as far down as 190mm (Hunt 1953). Animals use the burrows for respiratory purposes and create currents through the burrow by undulating their abdomens and abdominal gills. The nymphal stage is the only stage at which feeding can occur since mouthparts are atrophied in the subimaginal and imaginal stages. The main food source is detritus. Food is pulled to the mouth

from the surface of the substrate using forelegs and mouth-parts. Food particles are likely drawn into the burrow with the currents produced by the gills. Food turn-over in H. limbata was estimated to occur approximately five times in a 24h period at 20C (Zimmerman 1977). Nymphs have been recorded in drift (Flannagan et al. 1979), but do not normally leave their burrows for extended periods unless stressed (Edmunds et al. 1976).

Anon. (1975) has recommended that H. limbata be used as a "tentative" bioassay organism. Fremling and Mauck (1980) describe methods for the collection, culture and use of this genus in toxicity testing. They include a description of artificial substrates which can be used in test vessels when sediment is not desired. Numerous bioassays have been conducted using Hexagenia nymphs in the absence of sediment (Carlson 1966; Fremling 1970, 1975; Leonhard et al. 1980). Bioassays with sediment have been conducted to determine whether sediment removed from various parts of a water system was suitable for habitation of several organisms including Hexagenia (Prater and Anderson 1977a,b). Eriksen (1968) investigated effects of various particle types and sizes on oxygen respiration in Hexagenia nymphs. Oseid and Smith (1975) studied the effects of hydrogen sulfide in sediment on nymphs.

Fremling (1970) found Hexagenia spp. to be excellent indicators of water quality and attributed this to the long nymphal stage and inability of nymphs to swim long distances. Information on water quality requirements and pollution tolerance of mayflies, including Hexagenia spp., has been compiled by Hubbard and Peters (1978). Lewis

(1978) has drawn up a provisional classification of Ephemeroptera according to their tolerance to organic wastes.

Eggs of Hexagenia, namely those of H. rigida, have been used in several toxicity tests (Friesen 1979a, Giles et al. 1979) and procedures are outlined by Friesen (1979b).

C. RATIONALE AND OBJECTIVES

Permethrin in the water column has been shown to be toxic to some aquatic organisms. However, it disappears rapidly, partly through breakdown, but also by adsorbing onto sediment. Nymphs of Hexagenia spp. inhabit sediment, as well as ingest large quantities of sediment while feeding. Since animals seldom leave their burrows, their burrowing behavior could serve to protect them from direct contact when permethrin is in the water column for a brief period or reduce contact if creation of currents ceased temporarily. However, they would be exposed to contaminated sediment eventually by direct physical contact and as a food source. The main objective of this study was to determine whether permethrin in water and/or associated with sediment can be toxic to a benthic organism such as Hexagenia. To more fully evaluate effects of permethrin on this animal in the aquatic environment, studies were also conducted on the egg stage. Various stages of embryonic development in insects may be more sensitive to toxicants than others (Smith and Salkeld 1966; Friesen 1979b; Giles et al. 1979), and effects of permethrin on four stages of egg development were examined.

D. BIOLOGICAL INDICATORS AS SUBSTITUTES FOR CHEMICAL ANALYSES

In the present study it was not feasible to have chemical analyses performed for each experiment. Instead an organism which lives in the water column was used to indicate pesticide levels. The mosquito, Aedes aegypti (L.), was chosen as the bioassay organism because this organism is easy to culture in the laboratory, and has been used widely in toxicity studies (Schoof and Jakob 1964; Madder and Lockhart 1980; and others) including studies on permethrin (Rawn et al. 1979, 1980). Fourth instar larvae were chosen since this is the stage most commonly used by other workers.

A traditional bioassay approach was used and a dose-response relationship determined. Analyses of pesticide levels were performed for one of these bioassays and is referred to as the standard mosquito bioassay (SMB). The dose-response curve obtained was then compared to routine mosquito bioassays (RMB) which were conducted in parallel with toxicity tests with nymphs of Hexagenia for which no chemical analyses were performed. The concentration of permethrin i.e. the dose, was estimated from the response of the larvae, in this case percent mortality. Mosquito larvae were also used to indicate levels of permethrin in situations other than in routine bioassays, including toxicity tests with Hexagenia eggs.

CHAPTER II

MATERIALS AND METHODS

A. GENERAL DESIGN

Five experiments with nymphs exposed to water and/or sediment contaminated with permethrin were conducted. Initial nominal concentrations in water ranged from 0.32ug/L to 10ug/L. Considering the cross sectional area of the vessels (0.00783m^2) and the volume of water (750mL) used, these concentrations would be expected to occur as a result of field application rates ranging from 30.7ug/m^2 to 957.9ug/m^2. In the first experiment nymphs were exposed to contaminated water (CW) for 0.25 days (6h) after which time they were transferred to clean culture conditions. This experiment is called CW 0/0.25; the 0 indicates that there was no lapse of time between preparation of the permethrin solutions and transfer of nymphs to these solutions, and the number below the slash indicates length of time in days that the nymphs were exposed. In the next three experiments, nymphs were exposed to contaminated sediment (CS) for 1 day, 7 days or continuously. In the first two of these experiments, sediment had been treated with permethrin 1 day previously, and in the third experiment sediment had been treated 8 days previously. These experiments are called CS 1/1, CS 1/7 and CS 8/->; the top number indicates length of time in days elapsed between treatment of sediment with permethrin and addition of nymphs, and the bottom number indicates the length of time nymphs were exposed (the arrow (->) indicates continuous exposure). There were 3 replicates for each concentration, and the control, with 8 nymphs per replicate.

In the fifth experiment, referred to as the simulated application (SA), three possible situations which might occur following aerial application of permethrin at nominal rates of 95.8ug/m^2 and 957.9ug/m^2 were simulated: 1) application to a site inhabited by nymphs and subsequent movement of nymphs after 1 day to a non-contaminated site, 2) application as in 1) but with nymphs remaining indefinitely in the contaminated site, and 3) movement of nymphs, not previously exposed to permethrin, into a site where permethrin had been applied 8 days previously and where permethrin had either broken down or become associated with the sediment. These respective situations are referred to as SA 0/1, SA 0/->, and SA 8/->. Permethrin used for the SA experiment was spiked with ^{14}C radio-labelled permethrin, and the fate of the radio-active material in water and sediment was monitored. There were 2 or 3 replicates for each concentration, as well as a control, and at least 30 nymphs per replicate.

Routine mosquito bioassays (RMB) were run in parallel with each experiment. Chemical analyses by gas liquid chromatography (GLC) were conducted for one of these bioassays which is referred to as the standard mosquito bioassay (SMB). The dose-response curve of each individual RMB was then compared to the curve for the SMB to verify that concentrations used were uniform from one experiment to the next.

Two experiments on effects of permethrin in water on success of hatch of eggs were conducted. In the first experiment, eggs at four stages of development were exposed to various permethrin levels for a 3 day period. In the second experiment, eggs in the earliest stage of

development were exposed to permethrin for 3h, 9h, and 1 day periods. These experiments were conducted in Petri dishes in about 20mL of water per dish because the 1-L beakers and 750mL water used in the nymph experiments and the RMB were not suitable for the relatively small eggs. Mosquito bioassays at the same nominal concentrations as the egg toxicity tests were also conducted in Petri dishes and mortality used to estimate actual concentrations from the dose-response curve for the SMB.

B. CHEMICAL ANALYSES

Analyses for non-labelled permethrin in water were conducted for the SMB, and analyses for radio-labelled permethrin in water and sediment for the SA experiment. Two methods were used to prepare the permethrin solutions. In the first method, Method A, nominal concentrations were mixed in 1-L volumetric flasks and poured into beakers or Petri dishes. Animals or eggs were then added. In the second method, Method B, a portion of concentrated permethrin solution (spiked with radio-labelled permethrin) was added to water already in beakers. Method B was used for the SA experiment with nymphs, as well as for a mosquito bioassay run in parallel with the SA experiment, referred to as RMB(B). Method A was used for all other experiments and mosquito bioassays.

1. Non-labelled permethrin [1,2]

The permethrin stock liquid was an emulsifiable concentrate (EC) reportedly containing 50% active ingredient (ai). Nominal concentrations of 0.32, 1.0, 3.2, and 10.0ug/L were prepared using Method A as outlined in Fig. 2, and a RMB was established as outlined on page 23. A 50ml sample was removed for analysis from each beaker before mosquitoes were added. After 24h, another set of water samples was removed for analyses. Extra beakers were prepared at nominal concentrations of 1.0ug/L and 10.0ug/L to which no mosquitoes were added, and water samples were removed from these at 6h. Samples were preserved immediately after removal by adding 1mL of hexane and shaking vigorously. Samples were tightly capped and stored at 9C for approximately 2 weeks. At time of analysis samples were allowed to warm to room temperature. Most of the water was withdrawn by suction and samples were centrifuged for several minutes. Five to 10uL were withdrawn from the hexane layer and injected into the GLC. Analyses were carried out with a Tracor 220 GLC equipped with a linearized electron capture detector. A 2m x 4mm glass column packed with 3% OV-17 on Chromosorb W-HP (100-120 mesh) was used. Carrier flow (argon-methane) was 60cc/min. Detector, inlet and column temperatures were 360, 240 and 230C, respectively. A Spectra Physics SP 4100 computing integrator was used to compute analytical results using peak

---

1 Permethrin (Ambush®, JF4674B) was obtained courtesy of Chipman, Inc.
2 The method for analysis of permethrin was developed for this study by Mr. J.L. Solomon, Freshwater Institute.

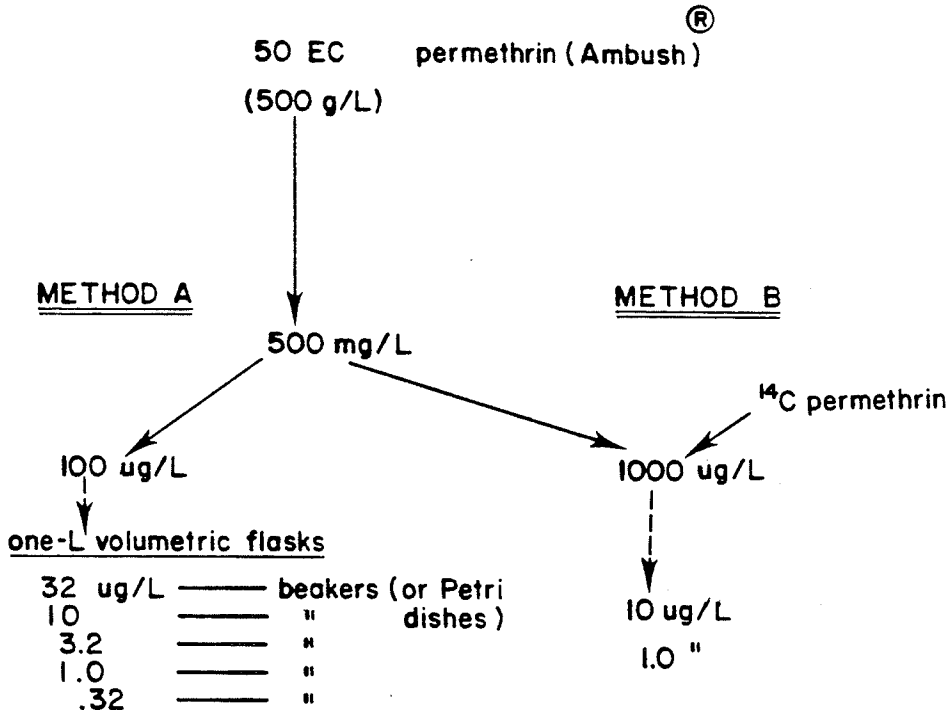

Fig. 2. Dilution procedures used to prepare concentrations of permethrin for the various experiments. In Method A appropriate dilutions were prepared and poured into the beakers or Petri dishes; in Method B concentrated permethrin solution (1,000ug/L), which had been spiked with radio-labelled permethrin, was added to water already in beakers.

areas (attenuation 4, chart speed 0.5cm/min). The retention times of the cis and trans isomers were 14.4 and 15.4min, respectively. Two samples were analyzed for each concentration.

2. Labelled permethrin[3]

One liter of 1,000ug/L ai of the 50% EC was prepared using Method B (Fig. 2). One hundred microliters of radio-labelled permethrin (specific activity 50mCi/mM) dissolved in acetone were added to the stock solution resulting in 6,515.6 DPM /mL. The C^{14} label was located on the cyclopropyl portion of the permethrin molecule. The labelled permethrin consisted of cis and trans isomers, purified separately by thin layer chromatography, and mixed together in a ratio of 40:60, respectively[4]. Nominal concentrations of 1.0 and 10.0ug/L, which correspond to nominal application rates of 95.8 and 957.9ug/m^2, were achieved by adding 0.75 or 7.5mL of the stock solution to the 750mL of water in the beakers. These beakers also contained 50g of standard sediment in which nymphs were being cultured and had been prepared for the SA 0/1 and SA 0/-> experiments as described on page 25. Water and sediment in the SA 0/1 beakers were sampled after 3h, 9h and 1 and 7 days. A water sample was also taken at 0h. Water and sediment in the SA 0/-> beakers were sampled on day 7 only. Only water and sediment

---

3 Labelled permethrin was obtained from Mr. G. Rawn, Dept. Soil Science, U. of Man.

4 Isomers were purified by the Organic Chemistry Lab., Freshwater Institute.

from the higher concentration were analyzed because counts were below detectability at the lower concentration. There were 3 beakers per concentration at the nominal concentration of 10.0ug/L and normally 3 water samples were taken from each beaker at each sampling time. Three milliliters of water were pipetted into scintillation vials and 14.5mL of PCS (Amersham, Oakville, Ont.) added. Samples were shaken until clear and were counted using a Beckman LS-7500 liquid scintillation counter (LSC). Two samples of sediment with individual weights of approximately 0.5g were removed from the top 5-10mm layer of sediment and weighed at each sample time. The 3h and 9h samples were frozen, and thawed and analyzed the next day. The 1 and 7 day samples were analyzed immediately after removal. Samples were oxidized using a Packard model 306 oxidizer for 0.9min with 0.15mL of Combustaid (Packard Instruments). $^{14}CO_2$ from the sample was trapped in 3mL CO_2-M-Met (Amersham) diluted with PCS-xylene (2:1) and analyzed by LSC. Three non-labelled samples were weighed, dried at 110C for 1 day and reweighed to estimate a factor to convert the above samples to dry weight.

C. MOSQUITO BIOASSAYS

1. Culture techniques

Aedes aegypti[5] was cultured following the conditions outlined by Fay (1964). Adults were held at R.H. 60-90% and 28±2C. Mice were used

[5] Eggs from which the Ae. aegypti culture was initiated were obtained from Dr. R. Brust, Dept. Entomology, U. of Man.

as a source of blood for adults. Larvae were reared at 28±1C in water 30-50mm deep, at an approximate density of 1-2 larvae/mL and were fed ground Tetramin®. Third or 4th instar larvae were transferred to bioassay temperatures for acclimation but only 4th instar larvae were used in bioassays.

2. Bioassay techniques

a) Bioassays run with nymph toxicity tests.

(1) Routine 24h bioassay procedure.

The routine mosquito bioassay (RMB) consisted of a 24h exposure of 4th instar Ae. aegypti larvae in 750mL of water in 1-L glass beakers with nominal permethrin concentrations of 0.32, 1.0, 3.2 and 10.0ug/L prepared using Method A. Three replicates per concentration, and a control, with 10 larvae per replicate were used. Water was not aerated and food was not added during the bioassay. No sediment was present in the beakers. After the 24h exposure, larvae were rinsed and transferred to clean water in plastic Petri dishes. A small amount of Tetramin® was added at this time, and was replenished if consumed.

Checks for dead larvae were made 24h after removal of animals from test containers. By this time animals were obviously dead or appeared to have recovered completely. A RMB was conducted in parallel with each of the five experiments conducted for the nymphs.

Mosquito larvae, using the procedure described above, were also exposed to permethrin concentrations in water prepared using Method B. This additional bioassay, referred to as RMB(B), was conducted in parallel with the simulated application (SA) experiment in addition to

the RMB, and the same nominal concentrations as those applied to beakers with nymphs and sediment, namely 1.0 and 10.0ug/L, were used. Regression curves of mortality, using arc sine transformed data, versus log "measured" concentrations were computed for each bioassay and compared to the SMB by covariance analyses. Statisitical analyses are described on page 33. "Measured" concentrations were those determined by GLC for the SMB.

(2) Other bioassays.

Larvae were used throughout the study to assay for permethrin levels in water where sediment was present and to which mayfly nymphs would be added or where they were already present. Larvae were exposed for 24h and bioassay procedures as described in (1) were followed. Unless stated otherwise, 3 replicates per concentration and 10 larvae per replicate were used. Concentrations were estimated from the observed mortality using the dose-response curve of the SMB. These bioassays will be described later.

b) Bioassays run with egg toxicity tests.

Bioassays with mosquito larvae were conducted in Petri dishes so that concentrations in the egg toxicity tests, also conducted in Petri dishes, could be estimated. Nominal permethrin concentrations of 0.32, 1.0, 3.2, 10.0, 32.0 and 100.0ug/L were prepared using Method A. Fifteen to 20mL were added to the respective Petri dishes to a depth of approximately 10mm, and larvae were added with about 1mL of clean water. After 10min approximately 18mL of water were pipetted out and

the dishes were refilled with the appropriate concentrations. There were 2 replicates per concentration, plus 2 replicates for a control and 10 larvae per replicate. A total of four bioassays in Petri dishes were conducted - one for each egg toxicity test. Regression curves were computed as described above, using concentrations determined for the SMB, and compared to the SMB dose-response curve using covariance analyses.

D. MAYFLY TOXICITY TESTS

1. Culture techniques

Laboratory cultures of H. rigida were initiated from either field collected nymphs, or from eggs obtained from imagines collected in the field, and were maintained as outlined by Friesen (1981). Nymphs were collected in fall of 1979 from the Red River just south of the University of Manitoba campus, Winnipeg, Manitoba, and were reared in 20-L aquaria with sediment from the Red River and dechlorinated City of Winnipeg tap water. Chemical characteristics of the water used are listed in Appendix A. Culture temperature was approximately 20C and water was aerated. Light was continuous and at room light intensity. Tetramin® was added as a food source. These nymphs were used the following summer for the contaminated water (CW) and contaminated sediment (CS) experiments. Body lengths at time of toxicity testing were 10-20mm. Sexes were not differentiated. A second group of nymphs was reared from eggs extracted from 10 imagines collected in June, 1980. Eggs were incubated in water at 22±2C under constant light conditions. Newly hatched nymphs were cultured in small containers

initially, and were transferred to 20-L aquaria after several weeks. At the end of August, 70 nymphs were transferred to each of 15 1-L beakers containing 50g of standard sediment and 750mL of water. Standard sediment was prepared by sieving Red River sediment, which had been dried at 65C, through a 1mm mesh screen. Its characteristics are listed in Appendix B. Beakers were aerated and about 10mg of ground Tetramin® were added to each beaker twice per week. These nymphs were used in the simulated application (SA) experiment conducted approximately 4 weeks later. Body lengths at time of toxicity testing were 3-10mm.

Eggs used in toxicity tests were handled as outlined by Friesen et al. (1979) and Friesen (1981). General handling techniques for eggs during tests conducted in this study are described on page 32. Eggs were from the same pool as those from which the nymph culture for the SA experiment was initiated.

2. Toxicity tests with nymphs

a) Contaminated water (CW) experiment.

(1) CW 0/0.25; 0.25 day (6h) exposure to CW (no sediment).

Nymphs were exposed to nominal permethrin concentrations of 0.32, 1.0, 3.2 and 10.0ug/L in 750mL water contained in 1-L glass beakers which had a cross sectional surface area of 0.00783m^2. No sediment was present. After 0.25 days nymphs were rinsed and transferred to clean culture conditions which were similar to those described under Culture Techniques (see above), except that aquaria were 1-L beakers. Checks for mortality were made 1, 4 and 8 weeks from the start of the

experiment. Checks were made by sieving the sediment through 1mm mesh screen and sorting out live nymphs. This experiment was conducted at 22±2C. The exposure, removal and mortality check regime is outlined in Fig. 3. There were 3 beakers per replicate and 8 nymphs per beaker.

The RMB, in this experiment only, was conducted in the same beakers as the nymph toxicity test and exposures were begun at the same time.

b) Contaminated sediment (CS) experiments.

Sediment was contaminated by adding 50g of standard sediment to 750mL of the same nominal permethrin concentrations used in CW 0/0.25 which correspond, respectively, to nominal application rates of 30.7, 95.8, 306.5 and 957.9ug/m^2. The mixture was stirred thoroughly for several seconds with a glass rod and allowed to settle. After various time periods about 600mL of contaminated water were decanted and replaced with clean water. Nymphs were then added. Three experiments were conducted with sediment contaminated this way and the regimes are outlined in Fig. 3. Unless stated otherwise procedures were similar to those of the CW experiment.

Routine mosquito bioassays (RMB) parallelled each experiment. Several mosquito bioassays were also conducted in water with contaminated sediment so that concentrations of permethrin in water could be estimated.

(1) CS 1/1; 1 day exposure to 1 day "old" sediment.

Beakers, prepared as described above, were allowed to stand 1 day after which water was decanted and replaced with clean water. Nymphs were then added to each beaker. Ten mosquito larvae were then added to

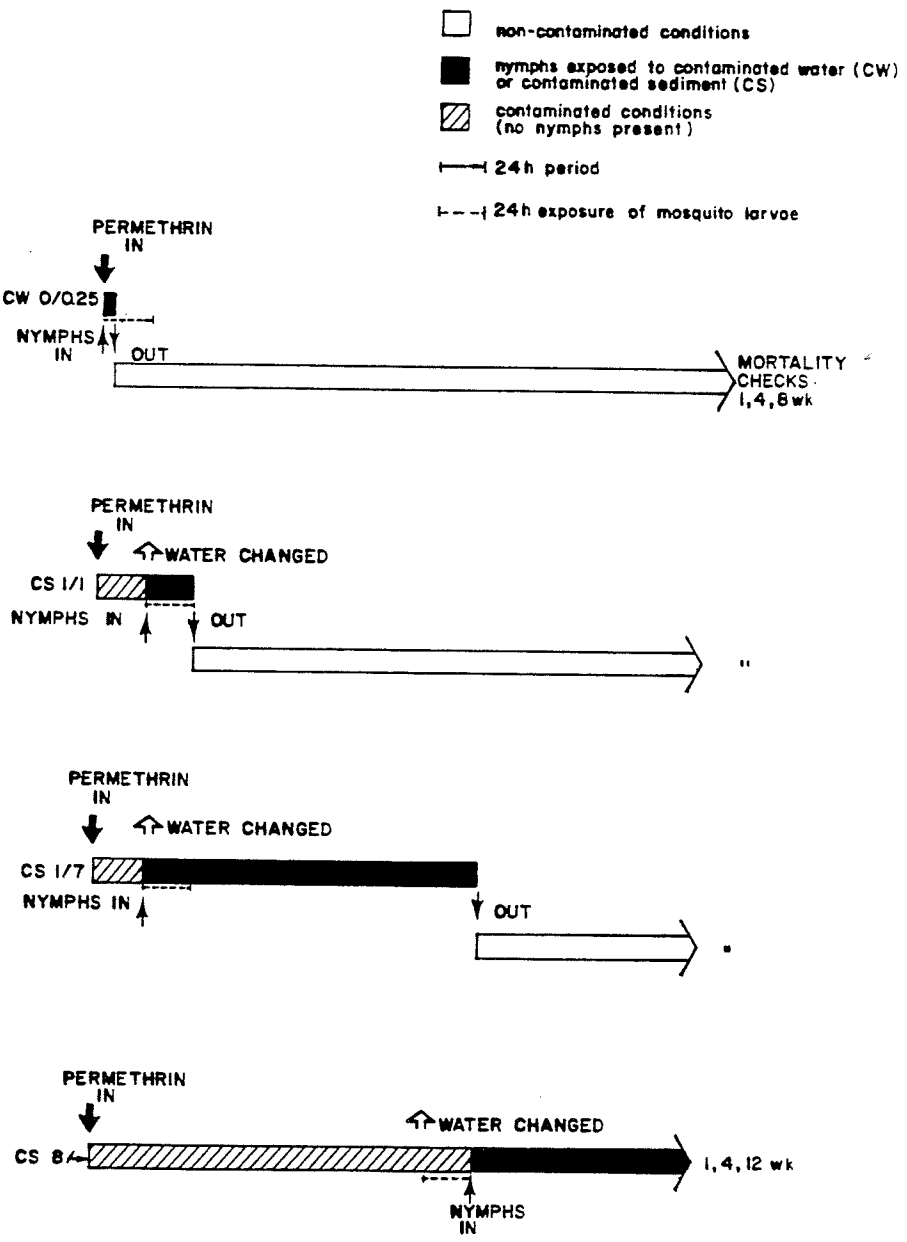

Fig. 3. Exposure, removal and mortality check regime for H. rigida nymphs exposed to contaminated water (CW) or contaminated sediment (CS). CW 0/0.25 indicates exposure of nymphs to CW for 0.25 days, CS 1/1 indicates exposure of nymphs to 1 day old sediment for a 1 day period, CS 1/7 indicates exposure of nymphs to 1 day old CS for 7 days, and CS 8/→ indicates exposure of nymphs to 8 day old CS continuously. Water and sediment were contaminated with permethrin using Method A.

the control beakers and to beakers containing the highest concentration of permethrin. There was no food or aeration provided during the exposure period. After 24h, nymphs and larvae were transferred to appropriate post-treatment conditions. Mortality was assessed as in CW 0/0.25. "Old" refers to time since sediment was treated with permethrin.

(2) CS 1/7; 7 day exposure to 1 day old sediment.

The same procedure as outlined for CW 1/1 was followed except that nymphs were exposed for 7 days instead of 1 day.

(3) CS 8/->; continuous exposure to 8 day old sediment.

Beakers, prepared as above, were allowed to stand for 7 days after which water was decanted and replaced with clean water. Mosquito larvae were then added to all concentrations and exposed for 24h. After removal of the larvae, nymphs were added, and aeration and addition of food begun. Nymphal mortality was assessed at 1, 4 and 12 weeks after nymphs were added.

c) Simulated application (SA) experiment.

One-liter beakers with 750mL of water and 50g standard sediment had been inoculated with 70 nymphs 4 weeks earlier. To achieve initial nominal concentrations in water of 1.0 and 10.0ug/L (corresponding to application rates of 95.8 and 957.9ug/m^2, respectively) beakers were treated with 0.75 amd 7.5mL of 1000ug/L stock solution of permethrin. This stock solution was spiked with radio-labelled permethrin, and water and sediment samples were removed as described on page 21. These experiments were conducted at 25±2C. The exposure, removal and mortality check regimes are outlined in Fig. 4.

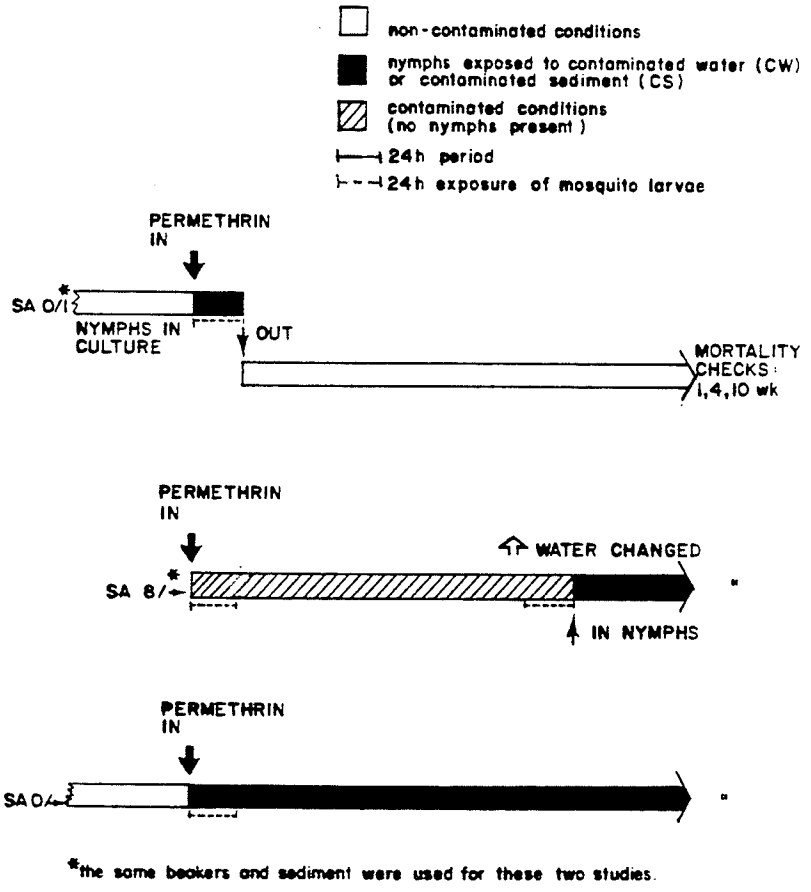

Fig. 4. Exposure, removal and mortality check regimes for H. rigida nymphs exposed in a simulated application (SA) situation. SA 0/1 indicates immediate exposure of nymphs to permethrin in water and sediment for 1 day, SA 0/→ indicates immediate exposure of nymphs to permethrin in water and sediment continuously, SA 8/→ indicates exposure of nymphs to 8 day old contaminated sediment continuously. Permethrin was added using Method B.

(1) SA 0/1; 1 day exposure to freshly contaminated conditions, and SA 8/->(1st); continuous exposure to 8 day old sediment.

Immediately after the addition of permethrin, 10 mosquito larvae were added to all beakers and exposed for 24h. After the mosquitoes were removed the sediment was sieved and nymphs were counted and transferred to clean culture conditions. There were 2 beakers for the control and 3 beakers for each concentration. This part of the experiment is referred to as SA 0/1.

The beakers with contaminated water and sediment were left to stand and 6 days later were decanted and clean water was added. Mosquito larvae were added to the control beakers and to the beakers which had contained the highest concentration of permethrin and were exposed for 24h. After mosquitoes were removed, 30 mayfly nymphs, not previously exposed to permethrin, were added to each beaker. Aeration and addition of food was resumed. This part of the experiment is referred to as SA 8/->(1st). Mortality was assessed at 1, 4 and 10 weeks from the start of the exposure period in both experiments.

(2) SA 0/->; continuous exposure to freshly contaminated conditions, and SA 8/->(2nd); continuous exposure to 8 day old sediment.

Immediately after the addition of permethrin, 10 mosquito larvae were added to all beakers and exposed for 24h. Nymphs were left in the contaminated water and sediment indefinitely, except for periodic checks for mortality. Aeration and addition of food was resumed after the

mosquitoes were removed. There were 2 replicates in the controls and at the nominal concentration of 1.0ug/L, and 3 replicates at the nominal concentration of 10ug/L. This part of the experiment is referred to as SA 0/->. After 7 days sediment in control beakers and beakers at the lower application rate was sieved to assess nymphal mortality.

It was apparent from the lack of burrowing activity that nymphs had not survived at the highest application rate in SA 0/->, and the sediment was not sieved. The water was decanted and replaced with clean water. Ten mosquito larvae were added to each beaker for 24h. After the mosquitoes were removed, 30 nymphs were added to each beaker, and addition of food and aeration was resumed. This part of the experiment is referred to as SA 8/->(2nd) and is not illustrated in Fig. 4. Mortality was assessed at 1, 4 and 10 weeks from the start of the exposure period.

3. Toxicity tests with eggs

a) Exposure of various developmental stages for a 3 day period.

Eggs were stripped from 10 female imagines and pooled. Aliquots of several hundred eggs were incubated in clean water in Petri dishes at 22±2C. Eggs were removed from these dishes at 0, 3, 6 and 9 days, and exposed to permethrin in water for 3 day periods. Nominal concentrations of 0.1, 0.32, 1.0, 3.2, 10.0, 32.0 and 100.0ug/L were used. Eggs were added to approximately 20mL of the appropriate concentration using a pipette or forceps. After all eggs had been added, approximately 18mL of water were withdrawn and fresh permethrin concentrations added. There were 20 eggs per replicate and 2 or 3

replicates per concentration , as well as for the controls, for each 3 day exposure period. Final percent hatch was determined on day 21 when no further hatch was expected to occur (Friesen et al. 1979).

b) Exposure of one egg stage for 3h, 9h or 1 day.

The procedure used here was similar to a) except that eggs were exposed for 3h, 9h or 1 day before eggs were transferred to clean water. Eggs were removed from the pooled stock of eggs at time 0, i.e. eggs were in the earliest developmental stages when they were exposed to permethrin.

E. STATISTICAL ANALYSES

1. Mosquito bioassays

a) Bioassays run with nymph toxicity tests.

Mortality values were transformed using the arc sine transformation (Snedecor and Cochran 1980), and a dose-response relationship was determined for each RMB by computing the regression curves of transformed larval mortality versus log "measured" concentration. Each curve was compared to the SMB curve by covariance analysis (ANCOVA)(Snedecor and Cochran 1980) at a significance level of $P<0.05$. "Measured" concentrations were those determined for the SMB by GLC. Mean mortality and standard deviation of the controls was never more than $3.3\pm0.58\%$, and adjustments for control mortality were not made.

b) Bioassays run with egg toxicity tests.

Regression curves of arc sine transformed larval mortality versus log "measured" concentrations were computed for each of the 4 bioassays conducted in Petri dishes. Slopes and intercepts between curves were compared by ANCOVA as described above. Control mortality in the first bioassay had a mean and standard deviation of 10.0±17.32% and treatment mortality was adjusted using Abbott's formula (Abbott 1925). Values of all bioassays were pooled and the regression line recalculated. This regression curve was then compared to the SMB curve by ANCOVA. Mortality in the controls for the other three bioassays was 10.0% or less in any replicate and adjustments were not made for control mortality.

2. Toxicity tests with nymphs

a) Contaminated water (CW) and contaminated sediment (CS) experiments.

Regression curves of arc sine transformed nymphal mortality versus log of estimated application rate were computed for each check time. Curves between check times within each experiment and curves of CS 1/1 and CS 1/7 at the 8 week check time were compared using ANCOVA at a significance level of $P<0.05$. Differences between exposure types within each application rate were analyzed with a Student t-test at a significance level of $P<0.05$. The application rate was estimated by dividing the total amount of permethrin added per beaker by the cross sectional surface area of the beaker. Treatment mortalities were adjusted for control mortality (Table 1) at each check time using Abbott's formula.

b) Simulated application (SA) experiment.

Mortality, after arc sine transformation of data, between check times was compared using a Student t-test at a significance level of $P<0.05$. Treatment mortalities were adjusted for control mortalities (Table 2) using Abbott's formula.

3. Toxicity tests with eggs

Regression curves for arc sine transformed values of non-hatched eggs versus log estimated concentration in water were computed for each of the four tests and were compared to one another using ANCOVA at a significance level of $P<0.05$. Concentrations had been estimated from the dose-response curve of the SMB based on mortality in mosquito bioassays conducted in Petri dishes. Mean hatch and standard deviations of eggs in the control Petri dishes for the exposure periods ranged from 62.5±10.60% to 85.0±7.07% and adjustments were made in treated eggs using Abbott's formula.

Table 1. Percent mortality (mean ± SD) of H. rigida nymphs in the controls of the contaminated water (CW) and contaminated sediment (CS) experiments at the various check times. There were 3 replicates per experiment and 8 nymphs (initially) in each replicate.

	% Control Mortality			
Check time (weeks)	CW 0/0.25	CS 1/1	CS 1/7	CS 8/->
1	0.0±0.00	0.0±0.00	0.0±0.00	0.0±0.00
4	0.0±0.00	0.0±0.00	4.2±7.22	4.2±7.22
8 or 12	0.0±0.00	20.8±19.09	37.5±0.00	37.5±12.50

Table 2. Percent mortality (mean ± SD) of H. rigida nymphs in the controls of the simulated application (SA) experiment at the various check times. There were 2 replicates per exposure type. Initial number (mean ± SD) of nymphs in each exposure type is given in brackets.

	% Control Mortality		
Check time (weeks)	SA 0/1 (49.0±4.24)	SA 0/> (49.5±0.71)	SA 8/-> (30.0±0.00)
1	0.0±0.00	0.0±0.00	0.0±0.00
4	1.1±1.56	9.1±4.38	1.7±2.33
10	7.1±0.85	17.5±16.23	5.0±7.07

CHAPTER III

RESULTS AND DISCUSSION

A. CHEMICAL ANALYSES

1. Non-labelled permethrin

Initial concentrations for the standard mosquito bioassay (SMB) for the nominal concentrations of 1.0, 3.2 and 10.0ug/L were determined by GLC to be 0.58, 2.06 and 7.63ug/L, respectively. The actual level of the nominal concentration of 0.32ug/L was below detection limits. Levels remained close to initial concentrations for 6h, but had decreased to less than half within 24h in all but one case (Table 3). The initial ratio of cis:trans was approximately 30:70. The cis was more persistent than the trans isomer. The measured concentrations are somewhat lower than the nominal levels possibly due to adsorption of permethrin to glassware during preparation of the solutions (Sharom and Solomon 1981b). The regression line describing the relationship of the initial measured and initial nominal concentrations (means±SD are listed in Table 3) was calculated to be:

$$\log y = -0.2467 + 1.222 \log x$$

where; y = actual concentration (ug/L),

and x = nominal concentration (ug/L). $R^2=0.9979$.

Using this equation the actual concentration for the nominal concentration of 0.32ug/L was estimated to be 0.15ug/L.

Table 3. Nominal and measured concentrations over 24h for non-labelled permethrin analyzed by GLC for the standard mosquito bioassay (SMB). Actual values listed are means (±SD) of two samples. The ratio of the *cis* and *trans* isomers is given in brackets.

Nominal conc. (ug/L)	Measured conc. (ug/L) means ± SD (*cis*:*trans*)		
	0h	6h	24h
0.32	not detectable	--	--
1.0	0.58±0.07 (34:66)	0.54±0.06 (34:66)	0.26±0.24 (67:33)
3.2	2.06±0.19 (32:68)	no data	0.34±0.13 (70:30)
10.0	7.63±0.18 (33:67)	7.17±0.21 (33:67)	2.1±0.06 (53:47)

2. Labelled permethrin

The fate of ^{14}C in water and sediment as percent of total amount added to the culture container at the nominal permethrin concentration of 10.0ug/L in SA 0/1 is shown in Fig. 5. ^{14}C levels in water decreased steadily over the first 24h. Levels at day 7 were similar to 24h levels. The initial concentration of permethrin in water was assumed to be 7.63ug/L i.e. the same as for the nominal concentration of 10.0ug/L in the SMB. If ^{14}C indicates the intact permethrin molecule then after 24h the mean (±SD) value for the water concentration was 3.5±0.65ug/L and the mean (±SD) value for the sediment concentration for the stratum sampled was 45.7±21.85ug/kg dry weight. At 7 days, the estimated level in water was 3.2±0.24ug/L and in sediment it was 27.5±17.67ug/kg dry weight. The lower levels in sediment on day 7 may be partially due to sieving of sediment to remove nymphs, during which the permethrin would have become distributed throughout the sediment. The estimated level for permethrin in water on day 7 is probably an overestimation because the ^{14}C label was located on the cyclopropyl ring of the molecule which, once breakdown occurs, forms polar metabolites which tend to dissolve in water (Rawn et al. 1979).

^{14}C levels in water and sediment were measured in SA 0/-> beakers on day 7 only; levels in water were estimated to be 4.0±0.64ug/L and in sediment were estimated to be 47.6±15.19ug/kg dry weight. (These beakers are referred to as SA 8/->(2nd) after fresh nymphs were added). The levels in sediment are similar to values found in SA 0/1 on day 1. The variation in permethrin levels in sediment may have been due to

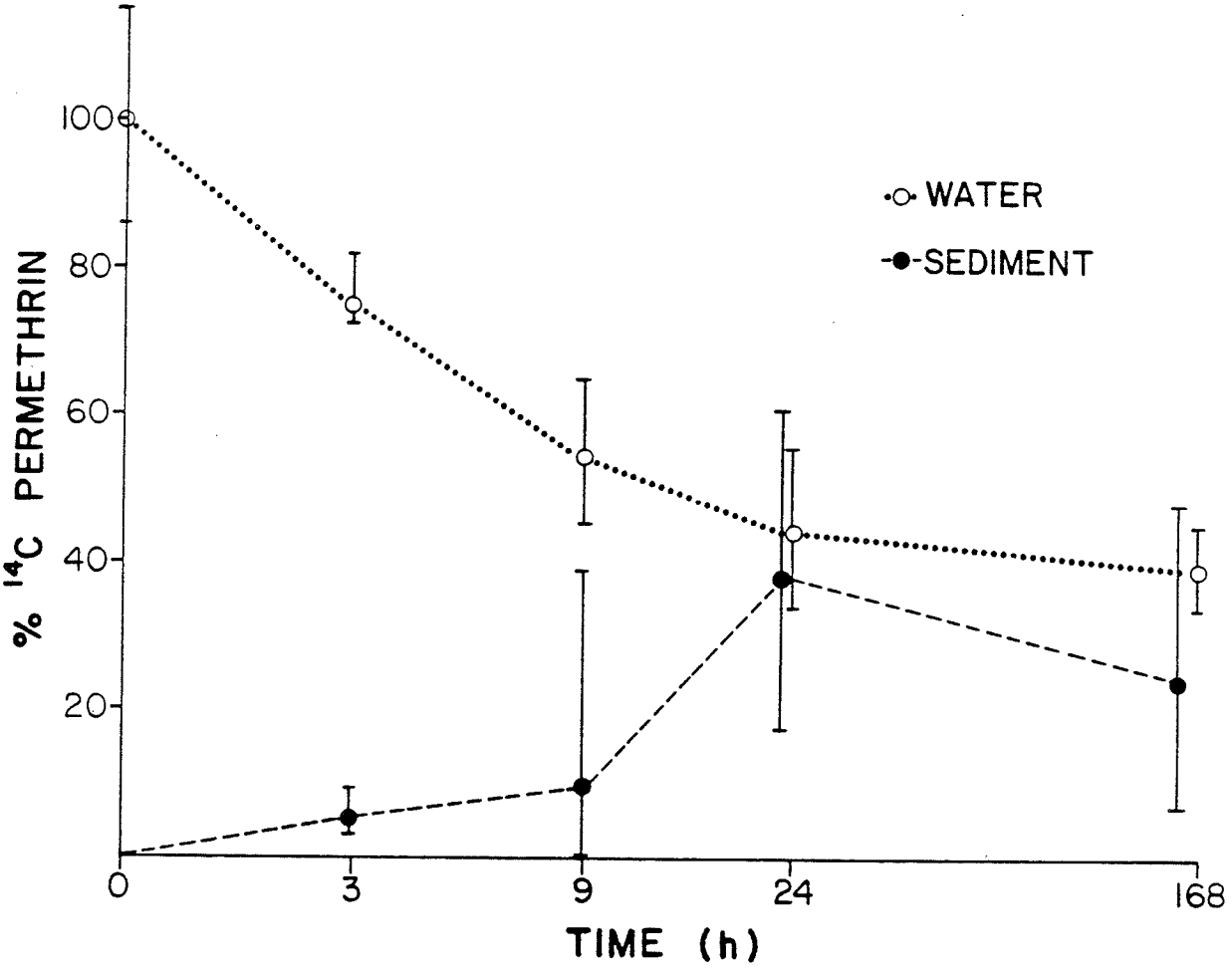

Fig. 5. Disappearance rate in water, and uptake rate in sediment of ^{14}C labelled permethrin over a 168h (7day) period in simulated application beakers (SA 0/1 and (SA 8/➤). Calculations are based on total radioactivity added. Initial estimated concentration in water was 7.63ug/L. Brackets indicate ranges.

variation in combustion efficiencies and/or inadequacies in sampling. If permethrin remained in the top few mm of sediment, then samples which included sediment from a deeper non-contaminated stratum would have lower levels of permethrin. The maximum background ^{14}C detected in sediment in the controls was 2.1% of total ^{14}C added to treated test containers, and adjustments were not made in treatment levels.

B. MOSQUITO BIOASSAYS

1. Bioassays run with nymph toxicity tests

Intercepts and slopes for the routine mosquito bioassays (RMB) were calculated for the equation:

$$y = a + b \log x$$

where; y = arc sine of square root of proportion dead,

a = intercept,

b = slope,

and x = concentration in ug/L.

The actual values and regression curve for the predicted relationship of the dose-response curve for the SMB are shown in Fig. 6. Levels of permethrin were similar for the various concentrations for all experiments since no significant differences ($P<0.05$) were found between the regression curves of the SMB and the other RMBs, nor between the curve for the SMB and RMB(B), the bioassay where concentrations were prepared using Method B (Table 4).

Concentrations of permethrin in water in the CS and SA experiments were periodically estimated by exposing mosquitoes to the contaminated

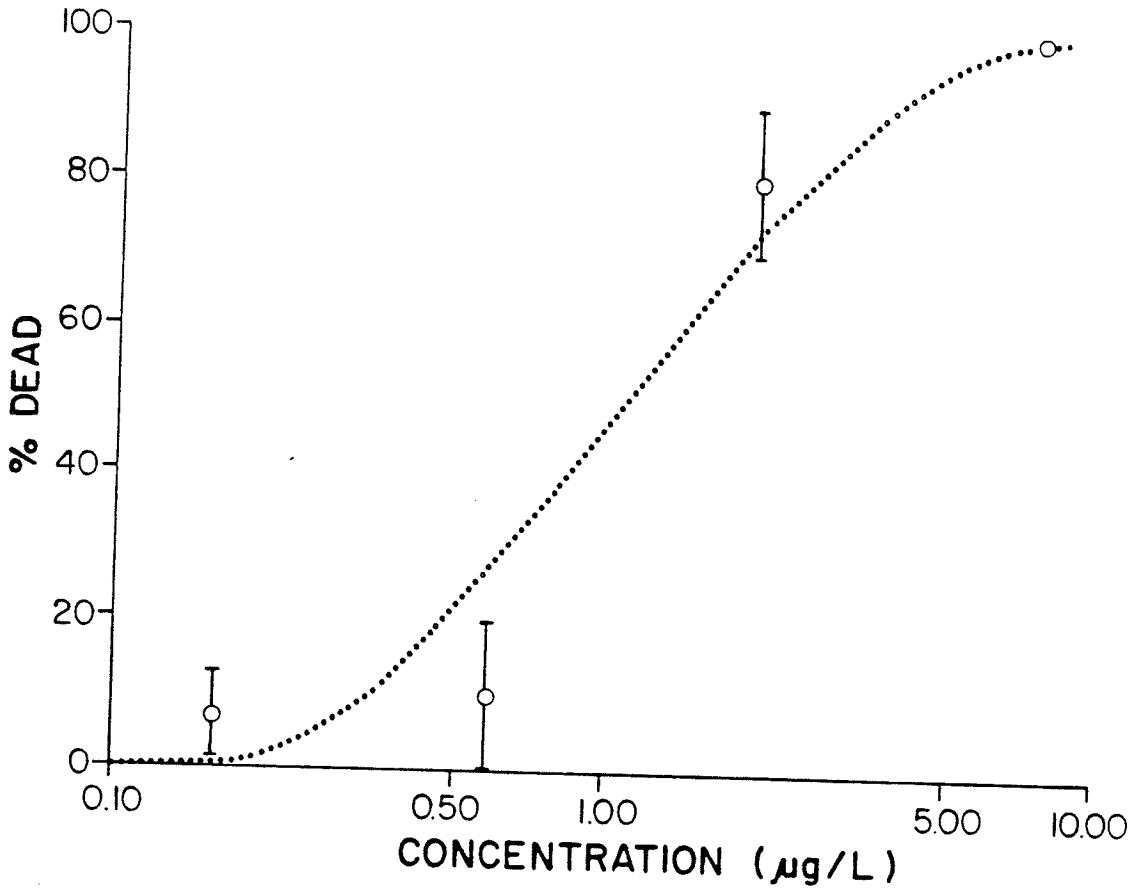

Fig. 6. Predicted dose-response curve for the standard mosquito bioassay (SMB). Symbols indicate means and ranges of actual data points.

Table 4. Intercepts, slopes, degrees of freedom (df) and regression coefficients (R^2) of the dose -response curves for the routine mosquito biossays (RMB) conducted in parallel with the H. rigida nymph toxicity tests, and for the bioassay (RMB(B)) conducted using concentrations prepared using Method B.

Experiment with which RMB was conducted	Intercept[a]	Slope[a]	df	R^2
SA 0/1, SA 0/-> = SMB	43.24	50.47	10	0.87
CW 0/0.25	43.65	44.76	10	0.89
CS 1/1	43.71	44.82	9	0.88
CS 1/7	48.98	41.39	10	0.76
CS 8/->	52.88	46.29	7	0.77
SA 0/1, SA 0/-> = RMB(B)	51.41	43.73	4	0.97

[a] no significant differences ($P < 0.05$) in intercept or slope were found when individual curves were compared to the SMB.

conditions for 24h (see Fig. 3), and then using mosquito mortality to estimate the approximate range of concentrations from the dose-response curve of the SMB. In all cases, control mortality was 3.3±0.58% or less and mortality of the mosquitoes exposed to contaminated conditions was not significantly different ($P<0.05$) from the controls. Levels of permethrin in water were therefore probably below 2.06 or even 0.58ug/L. This represents a reduction of at least 3.7 times of permethrin levels for the 7.63ug/L beakers. By inference, levels in the other beakers had probably been reduced by a similar ratio. In CS 1/7 and CS 8/->, levels of permethrin would be expected to be much lower than this before the introduction of animals, since a longer period of time had elapsed in which breakdown and adsorption could occur. It therefore seems logical to assume that any mortality which would occur in mayfly nymphs occurred as a result of permethrin which had become associated with the sediment.

In mosquitoes added to the SA experiment (see Fig. 4), no mortality occurred in the controls or at the lowest concentration in any exposure type. All larvae died at the highest concentration except for 1 replicate in SA 0/1 where all larvae lived. The permethrin level in this beaker was similar to the other 2 beakers as indicated by LSC. The only noticeable difference was that this beaker had a heavy growth of algae. It is likely that the permethrin adsorbed to or was taken up by these algae and therefore was not available to act on the mosquito larvae as a contact poison. Rawn *et al.* (1980) found that permethrin was readily taken up by duckweed, and peak residue levels in this plant were approximately 100 times higher than in the sediment. Mosquito

larvae may not have been feeding on the algae even before the insecticide was added, or may have stopped feeding after the addition of permethrin. Hence, they would not be poisoned by ingesting contaminated food. Levels of ^{14}C eventually reached the sediment as noted in the sediment analyses, possibly as algae died and settled to the bottom. In any case, the final toxic effect on Hexagenia nymphs was similar in all 3 beakers at this concentration (to be discussed later).

2. Bioassays run with egg toxicity tests

Regression curves for the mosquito bioassays conducted in parallel with the egg toxicity tests were not significantly different ($P<0.05$) in intercept or slope to one another. The regression curve from pooled values of these bioassays was not significantly different ($P<0.05$) from the SMB in slope, but was significantly different ($P<0.05$) in intercept (Table 5). This indicated that actual levels in Petri dishes, for the same nominal concentrations, were lower than in the beakers. These 25mL capacity Petri dishes have a much larger surface area to volume ratio than do the 1-L beakers, and differences in concentrations may be due to proportionally greater adsorption on the dishes. It is also possible that presence of mosquitoes may have led to a faster rate of breakdown in this relatively smaller volume of water than in the 1-L beakers.

To obtain an equation from which actual concentrations in Petri dishes could be estimated, the regression curve of the pooled data for the mosquito bioassays in Petri dishes was recalculated using the intercept of the SMB curve. Actual permethrin levels in Petri dishes were then estimated using this modified equation on the basis of

Table 5. Intercepts, slopes, degrees of freedom (df) and regression coefficients (R^2) of the dose-response curves for the mosquito bioassays conducted in Petri dishes in parallel with the egg toxicity tests.

Egg toxicity test with which bioassay was conducted	Intercept[a]	Slope[a]	df	R^2
1st test	20.34	49.87	10	0.87
2nd "	19.47	46.15	5	0.90
3rd "	7.24	53.93	10	0.79
4th "	9.02	56.05	10	0.89
pooled values	13.51[c]	51.97[b]	41	0.84

[a] no significant differences ($P < 0.05$) in intercept or slope were found between curves.

[b] no significant difference ($P < 0.05$) from the SMB.

[c] a significant difference ($P < 0.05$) from the SMB.

mosquito mortality. Nominal concentrations of 0.32, 1.0, 3.2, 10.0, 32.0 and 100.0ug/L were estimated to be 0.14, 0.25, 0.37, 2.02, 7.77 and 27.64ug/L, respectively.

C. MAYFLY TOXICITY TESTS

1. Toxicity tests with nymphs

In water, permethrin is distributed throughout the water column and an appropriate means of expressing concentration is in ug/L. However, permethrin is not distributed throughout the sediment, but may remain within the top 20mm (Sharom and Solomon 1981a), so that a better means of expressing concentration of permethrin in sediment is the amount applied per surface area, i.e. in ug/m^2. The application rates used in this study, calculated by dividing the total amount of permethrin added to a beaker by the cross sectional area of the beaker ($0.00783m^2$), for the water concentrations of 0.16, 0.58, 2.06 and 7.63ug/L were calculated to be 15.3, 55.6, 197.3 and $730.8ug/m^2$, respectively. Both means of expression are used here.

In the CW and CS experiments described in this section nymphs were transferred to containers that had already been treated at the above application rates. The final percent dead after the various exposures is given in Fig. 7.

a) Contaminated water (CW) experiment.

In the CW 0/0.25 test where nymphs were exposed to contaminated water for 6h (0/0.25 days), mortality ranged from 20.9±7.22% at $15.3ug/m^2$ (0.16ug/L) to 100.0±0.00% at $730.8ug/m^2$ (7.63ug/L). The 6h

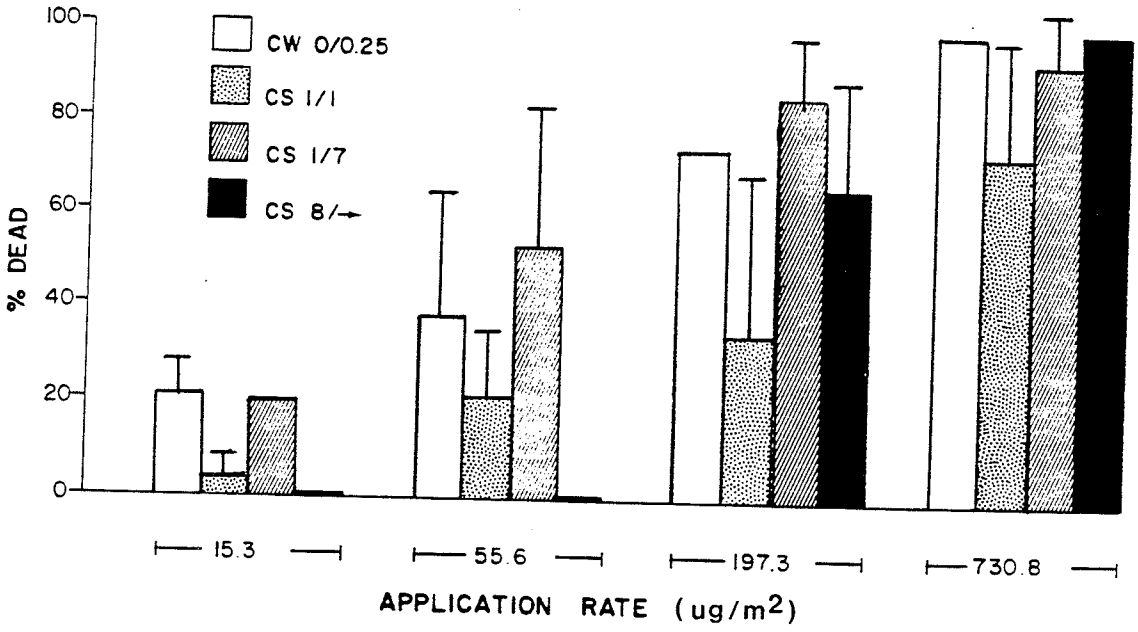

Fig. 7. Final mortality of H. rigida nymphs exposed to contaminated water (CW) or contaminated sediment (CS). Brackets indicate standard deviations.

LC50 lies between 55.6ug/m^2 (0.58ug/L) and 197.3ug/m^2 (2.06ug/L). Toxic action of permethrin in water appears to be similar for H. rigida nymphs and Ae. aegypti larvae in that the LC50's both lie between 0.58 and 2.06ug/L, and all animals died at 7.63ug/L. Mayflies may be somewhat more sensitive since they were exposed for 6h whereas the mosquitoes were exposed for 24h. The concentration of permethrin in water which is toxic to Hexagenia is similar to that found by other workers for some organisms. Muirhead-Thomson (1978) estimated LC90-95 values at and below 5ug/L for Baetis spp. mayflies, a caddisfly species, and Simulium sp. after animals had been exposed to permethrin for 15min to 1h.

Mortality curves for the various check times were compared by ANCOVA among each exposure type (Table 6). In CW 0/0.25 a significant difference ($P<0.05$) was found between the 1 and 4 week, and 1 and 8 week check times. Apparently permethrin can have toxic effects, which do not necessarily appear immediately but may take several weeks to be manifested, even when animals are exposed for a short period of time. To more fully evaluate the potential impact of this insecticide on organisms such as Hexagenia it would be useful to determine the minimum time animals can be exposed to permethrin without being affected adversely.

b) Contaminated sediment (CS) experiments.

As indicated by the low mosquito mortality (page 41), permethrin in water was probably below toxic values for nymphs when nymphs were added in the CS experiment. Therefore, any toxic action can be attributed to permethrin associated with sediment. In the CS 1/1 test, where nymphs

Table 6. Intercepts, slopes, degrees of freedom (df) and regression coefficients (R^2) of the regression curves for H. rigida nymphs exposed in the contaminated water (CW) and contaminated sediment (CS) experiments at the various check times.

$y = \text{arc sine} \sqrt{\text{proportion dead}} = a + b \log \text{estimated application rate } (ug/m^2)$

Toxicity test	Check time (weeks)	Intercept(a)	Slope(b)	df	R^2
CW 0/0.25	1	-41.54^{ab}	33.88	10	0.69
	4	-52.88^{a}	49.19	10	0.95
	8	-23.50^{b}	38.05	10	0.89
CS 1/1	1	-41.54	33.88	10	0.69
	4	-46.30	38.05	10	0.64
	8	-29.58	30.68	10	0.56
CS 1/7	1	-20.82^{a}	25.51	10	0.81
	4	-31.48^{a}	37.07	10	0.83
	8	-11.68^{a}	33.83	10	0.75
CS 8/->	1	-40.43^{a}	31.74^{ab}	10	0.87
	4	-61.88^{a}	50.56^{a}_{b}	10	0.79
	12	-81.08	57.64	10	0.88

Values followed by the same letter (a or b) are significantly different ($P<0.05$) within exposure types.

____ no significant difference ($P < 0.05$) between slopes of CS 1/1 and CS 1/7 at week 8.

— — — significant difference ($P < 0.05$) between intercepts of CS 1/1 and CS 1/7 at week 8.

were exposed for 1 day to sediment contaminated 1 day previously, mortality ranged from 3.5±3.00% at 15.3ug/m^2 to 73.7±24.13% at 730.8ug/m^2. A similar pattern was noted in CS 1/7 where nymphs were exposed for 7 days. When final percent mortality of individual values are compared between each rate of application using a t-test, the only significant difference ($P<0.05$) between these two exposure types is at the 15.3ug/m^2 application rate where 3.5±3.00% died in CS 1/1 and 20.0±0.00% died in CS 1/7. Such being the case, an exposure of 1 day was nearly as toxic as an exposure of 7 days, and/or permethrin levels decreased below toxic levels after 1 day. The latter point is doubtful, at least for the two highest concentrations, since 8 day old sediment is still toxic. When the mortality curves of CS 1/1 and CS 1/7 are compared using ANCOVA, the 7 day exposure resulted in significantly ($P<0.05$) higher mortality than the 1 day exposure (see underscored values in Table 6).

In the CS 8/-> test where nymphs were exposed continuously to sediment contaminated 8 days earlier, no treatment related mortality occurred at 15.3 or 55.6ug/m^2, final treatment mortality was 66.7±32.1% at 193.7ug/m^2 and 100.0±0.00% died at 730.8ug/m^2. At the 193.7 and 730.8ug/m^2 rates, mortality was not significantly different ($P<0.05$) from animals exposed in the CS 1/1 and CS 1/7 tests where animals were exposed for shorter periods, but at possibly higher levels. It appears that a chronic exposure to a lower concentration can be as toxic to some nymphs as a shorter exposure to a higher concentration is to others.

When mortality curves for the various check times were compared no significant differences ($P<0.05$) were detected between check times in CS 1/1, a significant difference ($P<0.05$) was found between all check times of CS 1/7 and a significant difference ($P<0.05$) was found between the 1 and 4 week check times but not between the 4 and 12 week check times for CS 8/->(Table 6). It appears that the level of permethrin in sediment in 8/->, at least for 197.3ug/m^2 application rate, declined below toxic levels 1 to 4 weeks post-treatment and/or some nymphs were tolerant to this level and did not die even after prolonged exposure. The first point is supported by studies on soils where the half-life is often less than 4 weeks (Kaufman et al. 1977; Kaneko et al. 1978; William and Brown 1979). It is not known when levels in beakers treated at 730.8ug/m^2 decreased below toxic levels since all animals had died by week 4. As in the CW 0/0.25 and CS 1/7 tests it was necessary to assess mortality at least several days from the start of the exposure period, and possibly up to 4 weeks to determine toxic effects.

c) Simulated application (SA) experiment.

In this set of tests, permethrin was added to containers in which nymphs were being cultured. In general, results were similar to those obtained in the CW and CS experiments for the same application rates. In the SA 0/1 test where nymphs were removed 1 day after addition of permethrin, final percent mortality was 27.8±16.94% at 55.6ug/m^2 and 88.0±16.18% at 730.8ug/m^2. In the SA 0/-> test, where nymphs were left indefinitely after the addition of permethrin, 74.2±10.93% died at 55.6ug/m^2 and 100.0±0.00% died at 730.8ug/m^2. The percent mortality values at 55.6ug/m^2 are similar to those found in the CS tests where,

unlike the SA tests, nymphs were transferred to the contaminated containers. Exposure to 730.8ug/m^2 in SA 0/1 and 0/-> tests, as in the CW and CS tests, resulted in total or nearly total mortality of nymphs. Animals inhabiting sediment were not protected to any great extent from effects of permethrin. Further study at intermediate application rates are necessary to determine whether the sediment can have a protective value.

In SA 8/->, where nymphs were exposed to sediment contaminated 8 days earlier, the final percent mortality at 55.6ug/m2 was 45.0±4.05%. This is significantly higher than mortality at CS 8/-> where 0.00±0.00% died. It is not known why different mortalities occurred. In the CS experiments, dry sediment was added to water which contained permethrin, whereas in the SA experiment, concentrated permethrin was added to water and sediment that had been wet for weeks. Permethrin may have adsorbed more strongly to the dryer sediment and therefore been less bioactive. Final percent mortality at the application rate of 730.8ug/m2 in SA 8/->(1st) and SA 8/->(2nd), as in CS 8/->, was 100.0±0.00%. The mean estimated concentration (±SD) of permethrin in sediment on day 7 post-application was 27.5±17.67ug/kg in SA 8/->(1st) and 47.6±15.19ug/kg dry weight in SA 8/->(2nd).

No further treatment related mortality occurred after 1 week in animals exposed in SA 0/1 and SA 0/-> (Table 7). In S/A 8/->, at the 55.6ug/m^2 application rate, and in SA 8/->(1st) and SA 8/->(2nd), at the 730ug/m^2 rate, the difference in mortality between week 1 and week 4 was significant ($P<0.05$), but not between week 4 and 10. These findings are similar to those found for the CS experiments.

Table 7. Percent mortality (mean±SD) of nymphs of H. rigida in the simulated application (SA) experiment at the various check times. There were 2 replicates for 0/→ and 3 for the other exposure types. Number (mean±SD) of nymphs per beaker in each exposure type at the start of the experiment is given in brackets. Initial number of nymphs for SA 0/→ was estimated from mean number of nymphs in beakers checked on day 1 for the whole experiment (n=8).

	% Mortality						
	55.6ug/m^2			730.8ug/m^2			
Check times (weeks)	0/1 (43.0±4.36)	0/-> (45.2±4.64)	8/-> (30.0±0.00)	0/1 (42.0±3.61)	0/-> (45.2±4.64)	8/->(1st) (30.0±0.00)	8/->(2nd) (30.0±0.00)
1	29.7±18.73	62.4±6.26	23.3±15.28[a]	85.9±19.99	100.0±0.00	44.4±8.39[a]	42.4±12.62[a]
4	30.4±17.97	68.5±8.90	43.5±7.06[a]	88.7±15.18	---	95.5±1.96[a]	95.5±5.09[a]
10	27.8±16.94	74.2±10.93	45.0±4.05	88.0±16.18	---	100.0±0.00	100.0±0.00

[a] significant difference ($P<0.05$) between check times.

In field trials where permethrin was applied in double applications at 17.5g/ha, the number of aquatic insects (and other invertebrates) in drift increased dramatically immediately post-treatment. Recovery of numbers of organisms, compared to control areas and pre-treatment numbers, did not begin to occur until about 6 weeks later. The highest permethrin level measured in water was 2.6ug/L, and in sediment it was 40ug/kg. Levels of 10ug/kg in sediment were found 28 days after the second application (Kingsbury and Kreutzweiser 1979). These values are in the same range as levels found to be toxic to Hexagenia nymphs in the present study. The lack of recolonization of benthic organisms in the field trial could have been due to permethrin associated with the sediment.

Permethrin is toxic to nymphs of Hexagenia while in the water column, as well as once it has become associated with the sediment. Although permethrin is fairly short-lived in the water, it can affect animals considerably during this time in a sublethal as well as a lethal manner. Symptoms of permethrin poisoning in insects include an initial stage of hyperexcitation, followed by knock-down, and finally by paralysis and death (Wouters and van den Bercken 1978). Knock-down occurs soon after exposure of animals to the insecticide, but death may not occur until much later. Animals which are knocked down do not necessarily die, but in many cases appear to recover completely. Benthic organisms exposed to permethrin may respond initially by coming out of the sediment or detaching themselves from their substrates, at which time they can be caught in the current and drift downstream

resulting in a dramatic increase in drift in lotic systems. Animals which drift can be exposed to the insecticide for longer periods since they may drift with the insecticide. These animals may eventually recover, but meanwhile they are vulnerable to predation or may drift to an unfavorable habitat. Flannagan (1973) referred to animals which were displaced from their niche as being "ecologically dead". If animals do not drift they will then be exposed to contaminated sediment.

It is not known whether nymphs in the present study were killed because of physical contact with the contaminated sediment, because they were ingesting it, or both. Permethrin, as well as being a contact insecticide, is a stomach poison (Elliott et al. 1978), and it would be of interest to determine whether organisms such as Hexagenia can desorb the intact permethrin molecule from sediment particles that pass through their digestive tracts. If exposure is through the food source, then nymphs may be exposed to concentrations of permethrin different from those determined for whole sediment samples, since nymphs seem to selectively gather materials which have a higher organic content than the sediment surrounding their burrows (Zimmerman 1977).

In the SA tests three situations which might occur as a result of field applications were simulated. At the 55.6ug/m^2 application rate, leading to an initial water concentration of about 0.58ug/L and a concentration in sediment, after 7 days, of about 3 to 5ug/kg dry weight, some mortality would be expected to occur in a natural situation if nymphs moved from a treated area to non-contaminated conditions after

a 24h exposure. Mortality would be somewhat higher if nymphs remained in the contaminated area indefinitely. Considerable mortality would also occur, presumably due to permethrin associated with sediment, in nymphs recolonizing the contaminated area within at least 8 days post-application. An application rate of 730.8ug/m^2 would be expected to have severe adverse effects on these organisms and an exposure of 24h or more on nymphs to contaminated conditions would lead to the elimination of a large proportion of the population. The area would not be suitable for recolonization for at least 8 days post-application.

In the present study, an application rate of 730.8ug/m^2 led to total or nearly total mortality of nymphs after short and long term exposures. An application rate of 1,750ug/m^2 (=17.5g/ha), the application rate used in the field trials studied by Kingsbury and Kreutzweiser (1979, 1980), would be expected to be even more devastating with longer lasting effects since levels would be maintained for a longer period of time. In evaluating the length of time this insecticide affects benthic organisms such as Hexagenia, the persistence time of the insecticide, particularly in sediment, must be considered, as well as the time it takes for lethality to occur. In the present study, death did not occur for at least 1 or several weeks post-treatment for Hexagenia nymphs, even after a 6h exposure.

Stage of nymphal development may also be an important consideration in the assessment of toxic effects. Early instar mayfly nymphs may be more sensitive than older nymphs since they were the predominant group

of insect (along with early instar stonefly nymphs) collected in drift immediately after aerial spraying in the field trial of Kingsbury and Kreutzweiser (1980). Nymphs used in the present study were longer than 3mm, and the concentrations of permethrin toxic to smaller nymphs may be even lower than those found here.

2. Toxicity tests with eggs

a) Exposure of various developmental stages for a 3 day period.

Depending on the stage of development, eggs were less or more sensitive to permethrin than were the nymphs. Hatch of eggs in the earliest developmental stage (1st stage) was least affected after exposure to permethrin with 89.5±4.17% hatch occurring at 27.64ug/L, whereas no hatch occurred in any of the other stages at 2.02ug/L. The intercept and slope of the regression line of percent hatch versus concentration was significantly different ($P<0.05$) from the other three stages (Table 8). There appears to be a trend of increasing sensitivity to permethrin with increase in embryonic development (Fig. 8). The very last part of development before hatch occurred was not tested and it is possible that this stage may display a different degree of sensitivity than the stages tested.

Varying sensitivity to pyrethroids of eggs in different developmental stages has been found in other insects. Pree and Hagley (1977) found that newly laid eggs of the codling moth were more susceptible to poisoning by permethrin than were eggs prior to hatch. Salkeld and Potter (1953) found that for the pyrethroid, allethrin, eggs of two Lepidoptera spp. in later stages of development were more susceptible than were those in early stages. In studies on effects of saline groundwater on success of hatch of Hexagenia eggs, it was found

Table 8. Intercepts, slopes, degrees of freedom (df) and regression coefficients (R^2) of the regression curves for H. rigida egg toxicity tests where different stages of development were exposed to permethrin for 3 day periods.

$y = \text{arc sine}\sqrt{\text{proportion non-hatch}} = a + b \log \text{concentration (ug/L)}.$

Egg toxicity test	Intercept(a)	Slope(b)	df	R^2
1st - 3 day exposure	28.75[a]	40.32[a]	8	0.89
2nd - 3 day exposure	61.71	95.73[b]	4	0.86
3rd - 3 day exposure	67.81	85.17	4	0.78
4th - 3 day exposure	76.14	64.23[b]	10	0.73

[a]significant difference ($P<0.05$) from slopes and intercepts of other curves.

[b]significant difference ($P<0.05$) between individual values.

Table 9. Intercepts, slopes, degrees of freedom (df) and regression coefficients (R^2) of the regression curves for H. rigida egg toxicity tests where early stage eggs were exposed for 3h, 9h or 1 day. Data from the 3 day exposure from the previous test are also included.

$y = \text{arc sine}\sqrt{\text{proportion non-hatch}} = a + b \log \text{concentration (ug/L)}.$

Egg toxicity test	Intercept(a)	Slope(b)	df	R^2
3h exposure	12.76[a]	52.41	6	0.81
9h exposure	32.97	34.50	10	0.85
1 day exposure	39.61[ab]	38.50	16	0.88
3 day exposure	28.75[b]	40.32	8	0.89

Values followed by the same letter (*a* or *b*) are significantly different ($P<0.05$).

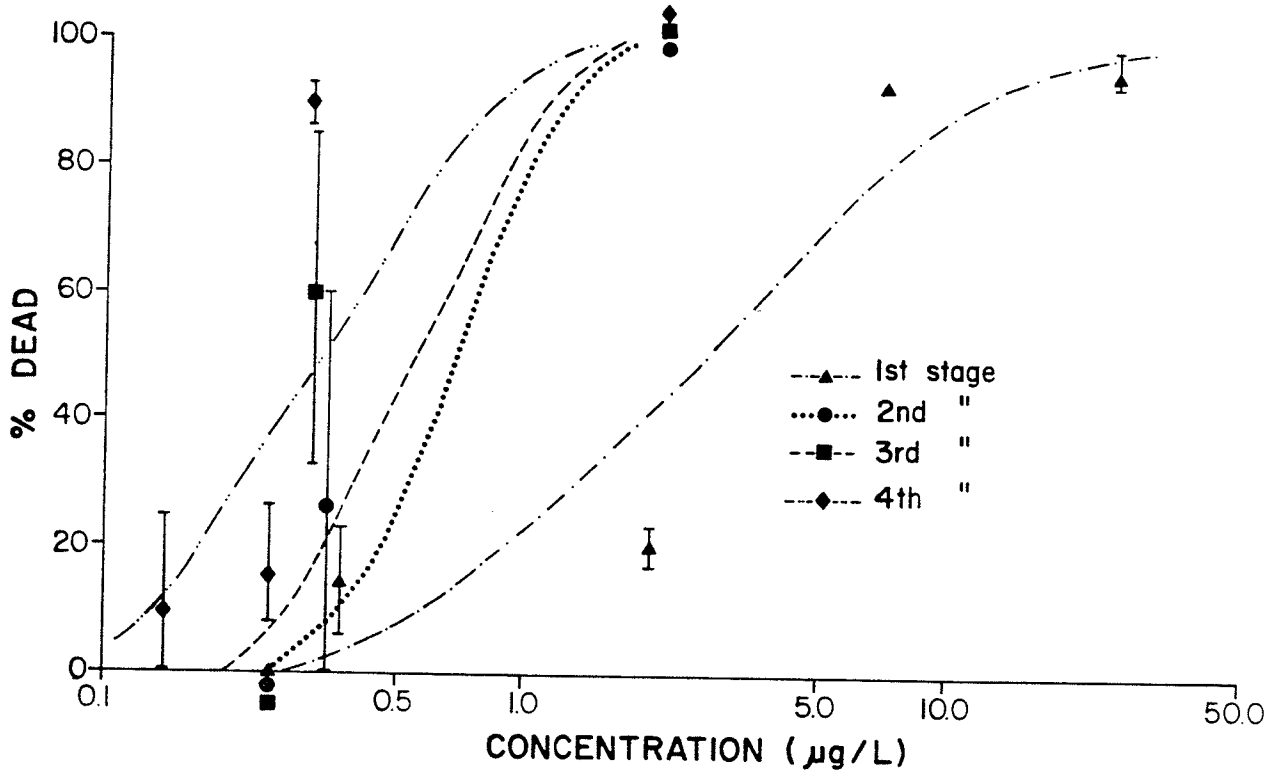

Fig. 8. Predicted regression curves computed from Table 8 for mortality of four different developmental stages of H. rigida eggs after exposure to permethrin in water for 3 day periods. Means and ranges of actual data points are indicated by symbols.

that eggs in later stages of development were not as sensitive as were early stages (Giles et al. 1979). Hence, it appears that time of greatest sensitivity to the action of a toxicant may be dependent on the individual species as well as the toxicant.

b) Exposure of one egg stage for 3h, 9h, 1 or 3 day periods.

Hexagenia eggs were exposed for four-3 day periods so that all but the very last few days of development were exposed to permethrin. Since permethrin may disappear rapidly from the water, exposure periods of shorter times were studied to determine the importance of exposure times. Eggs in the earliest developmental stage were used. Effects on hatch of the various exposure times were all similar to one another (Fig. 9) except that a significant difference ($P<0.05$) in intercept occurred between the 3h and 1 day curves, and the 1 day and 3 day curves (Table 9). The reason for this is not definitely known but the variation noted in the data (Fig. 9), occurs at 2.02 and 7.77ug/L, which is probably the critical range where adverse effects are beginning to occur and small variation in concentration may have a large influence on toxic effects.

As noted for the nymphal stage, a long exposure period does not necessarily result in a large increase in toxicity. In the egg study it appears that much of the damage occurred in a 3h period. However, development in most of the unhatched eggs which had been exposed to permethrin, did not cease till they were in advanced stages of development, probably between day 6 and day 7 from start of incubation. This may indicate that toxic action is delayed and is not manifested until development of the target tissue (i.e. the nervous system) has

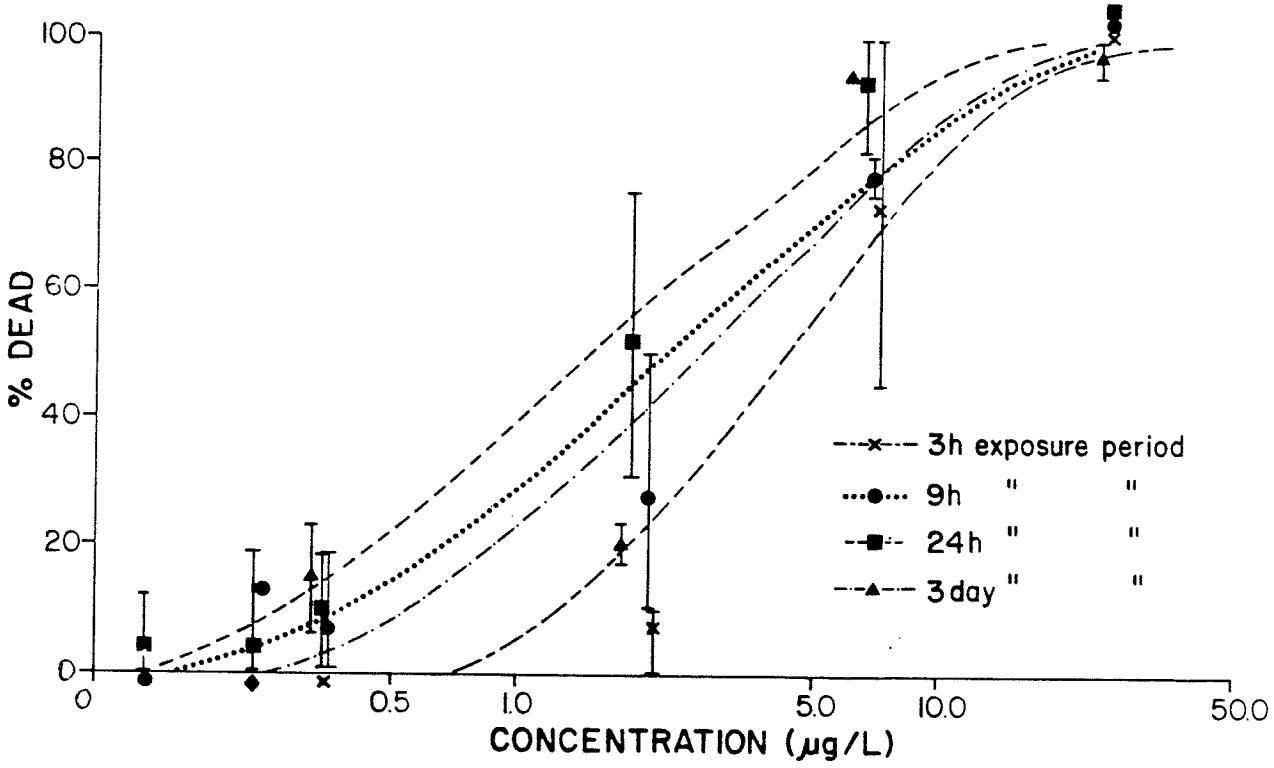

Fig. 9. Predicted regression curves computed from Table 9 of early stage eggs of H. rigida after exposure to permethrin in water for 3 days and less. Means and ranges of actual data points are indicated by symbols.

reached a certain point, or that the insecticide does not come in contact with the target tissue until this time. Susceptibility of the chorion, and other membranes enveloping the embryo, to penetration of insecticides has been found to change with embryonic development in several insect species (Salkeld and Potter 1953). It is possible that the permethrin may have been adsorbed onto or have been retained by the chorion or other structures whose permeability did not allow penetration of the toxicant to the target tissue until a certain developmental stage was reached. This adsorption may occur rapidly so that the total length of exposure to permethrin in water to cause toxic action may be much less than 3h.

Mayflies characteristically emerge in large numbers over a short period of time as an adaptation to increase encounters for mating during their brief terrestrial phase. Eggs are laid immediately after mating. Hence, it is possible that eggs are nearly all at the same developmental stage. This means that they may all be at a vulnerable stage at the time of treatment with permethrin, resulting in the elimination of a substantial portion of the upcoming cohort or generation. If this portion of the population was eliminated it might take at least a year in temperate climates for this organism to become reestablished to pretreatment numbers because of its long life cycle.

CONCLUSIONS

1. Permethrin levels in water at initial concentrations of 0.58ug/L to 7.63ug/L remained constant for at least 6h and declined to less than half initial levels by 24h.
2. Permethrin concentrations in sediment, when applied at a rate of 730.8ug/m^2, and estimated using radio-labelled material, reached a level of 45.7±21.85ug/kg dry weight after 24h. Estimated concentrations after 7 days were in a similar range.
3. The 6h LC50 of permethrin in water to Hexagenia nymphs lies between 0.58 and 2.06ug/L. Nymphs did not survive a 6h exposure to 7.63ug/L.
4. Permethrin associated with sediment can be toxic to Hexagenia nymphs. Exposure of nymphs to permethrin in sediment at an estimated initial concentration of 27.5±17.67ug/kg dry weight resulted in 100.0±0.00% mortality of nymphs. Exposure of nymphs to initial levels at about one tenth this concentration resulted in a final mortality of 45.0±4.05%.
5. Lethal effects to Hexagenia nymphs of water and sediment contaminated with permethrin, even for a period of 6h, were delayed for at least several days and possibly weeks.
6. In a simulated application test, where permethrin was added to nymph cultures, similar results to the above were noted, an indication that sediment would not necessarily "protect" nymphs during and after an application.

7. Eggs, depending on the stage of development, were less or more sensitive to permethrin than the nymphs. There appeared to be a direct relationship between sensitivity to permethrin and advancement of development. Eggs in earliest stage of development had 89.5±4.17% hatch at an estimated concentration of 27.64ug/L, whereas no eggs hatched in the other three stages of development at 2.02ug/L.
8. A short exposure (3h) of eggs to permethrin was as toxic as a long term (3 day) exposure i.e. damage appears to occur over a short period of time, or the egg may retain permethrin which acts past the initial exposure period, so that even though permethrin is very short-lived in the water column, it can have a lethal effect after it is no longer detectable in the water column.
9. Nymphs and eggs of Hexagenia were killed at and below concentrations of permethrin resulting from applications at and below 730.8ug/m^2. This was apparently due to toxic effects of permethrin while in the water column and, at least for the nymphal stage, after it had become associated with sediment.

LIST OF REFERENCES

Abbott, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265-267.

Anon. 1981. Ambush®. Experimental insecticide. Technical Data Sheet. Chipman Inc. 10p.

Anon. 1975. Standard methods for the examination of water and wastewater. 14th Ed. Amer. Public Health Assoc., Wash. D.C.

Belanger, A. and H.A. Hamilton. 1979. Determination of disulfoton and permethrin residues in an organic soil and their translocation into lettuce, onion and carrot. J. Environ. Sci. Health, B14: 213-226.

Briggs, G.G., M. Elliott, A.W. Farnham and N.F. Janes. 1974. Structural aspects of the knockdown of pyrethroids. Pestic. Sci. 5: 643-649.

Carlson, C.A. 1966. Effects of three organophosphorus insecticides on immature Hexagenia and Hydropysche of the upper Mississippi River. Trans. Am. Fish. Soc. 95: 1-5.

Courtemanch, D.L. and K.E. Gibbs. 1980. Short- and long-term effects of forest spraying of carbaryl (Sevin-4-oil®) on stream invertebrates. Can. Entomol. 112: 271-276.

Dobie, J. 1966. Food and feeding habits of the walleye, Stizostedion v. vitreum, and associated game and forage fishes in Lake Vermilion, Minnesota, with special reference to the tullibee, Coregonus (Leucichthys) artedi. Minn. Fish. Invest., Dep. Conserv., Div. Game and Fish. 4: 39-71.

Edmunds, Jr., G.F., S.L. Jensen and L. Berner. 1976. The mayflies of North and Central America. Univer. Minn. Press, Minneapolis.

Eidt, D.C. 1975. The effect of fenitrothion from large-scale forest spraying on benthos in New Brunswick headwaters streams. Can. Entomol. 107: 743-760.

Elliott, M. 1976. Future use of natural and synthetic pyrethroids. p.163-193. In: R.L. Metcalf and J.J. McKelvey, Jr. (eds.). The future for insecticides. Needs and prospects. Wiley, N.Y.

Elliott, M., A.W. Farnham, N.F. Janes, P.H. Needham, D.A. Pulman, J.H. Stevenson. 1973. A photostable pyrethroid. Nature 246: 169-170.

Elliott, M., N.F. Janes and C. Potter. 1978. The future of pyrethroids in insect control. Annu. Rev. Entomol. 23: 443-469.

Eriksen, C.H. 1968. Ecological significance of respiration and substrate for burrowing Ephemeroptera. Can. J. Zool. 46: 93-103.

Fay, R.W. 1964. The biology and bionomics of Aedes aegypti in the laboratory. Mosq. News 24: 300-308.

Flannagan, J.F. 1973. Field and laboratory studies of the effect of exposure to fenitrothion on freshwater aquatic invertebrates. Manit. Entomol. 7: 15-25.

Flannagan, J.F. 1979. The burrowing mayflies of Lake Winnipeg, Manitoba, Canada. p. 103-113. In: K. Pasternak & P. Sowa (ed.). Proc. 2nd Int. Conf. Ephemeroptera, 1975. Warszawa, Panstwowe Wydawnictwo Naukowe.

Flannagan, J.F., B.E. Townsend, B.G.E. de March, 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.

Fremling, C.R. 1964. Rhythmic Hexagenia mayfly emergences and the environmental factors which influence them. Verh. Internat. Verein. Limnol. 15: 912-916.

Fremling C.R. 1968. Documentation of a mass emergence of Hexagenia mayflies from the upper Mississippi River. Trans. Am. Fish. Soc. 97: 278-280.

Fremling, C.R. 1970. Mayfly distribution as a water quality index. U.S. Environ. Prot. Agency., Water Pollut. Control Res. Ser. 16030 DQH 11/70.

Fremling, C.R. 1975. Acute toxicity of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) to nymphs of mayflies (Hexagenia sp.). U.S. Fish. Wildl. Serv., Invest. Fish Control 58: 1-8.

Fremling, C.R. and W.L. Mauck. 1980. Methods for using nymphs of burrowing mayflies (Ephemeroptera, Hexagenia) as toxicity test organisms. p. 81-97. In: A.L. Buikema, Jr. and J. Cairns, Jr., (eds.). Aquatic Invertebrate Bioassays. Am. Soc. Test. Mater., Spec. Tech. Publ. 715.

Friesen, M.K. 1979a. Toxicity testing using eggs of the burrowing mayfly Hexagenia rigida (Ephemeroptera: Ephemeridae), with methoxychlor as toxicant. p. 266-277. In: P.T.S. Wong, P.V. Hodson, A.J. Nimmi, V. Cairns and U. Borgmann, (eds.). Proc. 5th Annu. Aquat. Toxicity Workshop, Hamilton, Ont. 1978. Fish.Mar. Serv. Tech. Rep. 862.

Friesen, M.K. 1979b. Use of eggs of the burrowing mayfly Hexagenia rigida in toxicity testing. p. 27-36. In: E. Scherer (ed). Toxicity tests for freshwater organisms. Can. Spec. Publ. Fish. Aquat. Sci. 44.

Friesen, M.K. 1981. Hexagenia rigida (McDunnough). p. 127-142. In: S. G. Lawrence (ed.). Manual for the culture of selected freshwater invertebrates. Can. Spec. Publ. Fish. Aquat. Sci. 54.

Friesen, M.K. and J.F. Flannagan. 1976. Parthenogenesis in the burrowing mayfly Hexagenia rigida (Ephemeroptera). Can. Entomol. 108: 1295.

Friesen, M.K., J.F. Flannagan and S.G. Lawrence. 1979. Effects of temperature and cold storage on development time and viability of eggs of the burrowing mayfly Hexagenia rigida (Ephemeroptera: Ephemeridae). Can. Entomol. 111: 665-673.

Giles, M.A., J.F. Klaverkamp and S.G. Lawrence. 1979. The acute toxicity of saline groundwater and of vanadium to fish and aquatic invertebrates. Prep. for the Alberta Oil Sands Environmental Program by Dep. Fisheries and the Environment, Freshwater Institute. AOSERP. Rep. 56: 216p.

Gill, S.S. 1977. Larvicidal activity of synthetic pyrethroids against Aedes albopictus (Skuse). Southeast Asian J. Trop. Med. Public Health 8: 510-514.

Harris, C.R. and G.B. Kinoshita. 1977. Influence of posttreatment temperature on the toxicity of pyrethroid insecticides. J. Econ. Entomol. 70: 215-218.

Harris, C.R. and S.A. Turnbull. 1978. Laboratory studies on the contact toxicity and activity in soil of four pyrethroid insecticides. Can. Entomol. 110: 285-288.

Hubbard, M.D. and W.L. Peters. 1978. Environmental requirements and pollution tolerance of Ephemeroptera. U.S. Environ. Prot. Agency., Environ. Monitoring Ser. Publ. EPA-600/4-78-061.

Hunt, B.P. 1953. The life history and economic importance of a burrowing mayfly, Hexagenia limbata, in southern Michigan lakes. Mich. Conserv. Dep., Bull. Inst. Fish. Res. 4.

Jolly, Jr., A.L., J.W. Avault, Jr., K.L. Koonce and J.B. Graves. 1978. Acute toxicity of permethrin to several aquatic animals. Trans. Am. Fish. Soc. 107: 825-827.

Kaneko, H., H. Ohkawa and J. Miyamoto. 1978. Degradation and movement of permethrin isomers in soil. J. Pestic. Sci. 3: 43-51.

Kaufman, D.D., S.C. Haynes, E.G. Jordan and A.J. Kayser. 1977. Permethrin degradation in soil and microbial cultures. p. 147-161. In: M. Elliott (ed.). Synthetic pyrethroids. Am. Chem. Soc.Symp. Ser. 42.

Kaufman, D.D., B.A. Russell, C.S. Helling and A.J. Kayser. 1981. Movement of cypermethrin, decamethrin, permethrin, and their degradation products in soil. J. Agric. Food Chem. 29: 239-245.

Kingsbury, P.D. and D.P. Kreutzweiser. 1979. Impact of double applications of permethrin on forest streams and ponds. For. Pest Manage. Inst., Sault Ste. Marie, Ontario. Inf. Rep. FPM-X-27.

Kingsbury, P.D. and D.P. Kreutzweiser. 1980. Environmental impact assessment of a semi-operational permethrin application. For. Pest Manage. Inst., Sault Ste. Marie, Ontario. Inf. Rep. FPM -X-30.

Kumaraguru, A.K. and F.W. Beamish. 1981. Lethal toxicity of permethrin (NRDC-143) to rainbow trout, Salmo gairdneri, in relation to body weight and water temperature. Water Res. 15: 503-505.

Leonhard, S.L., S.G. Lawrence, M.K. Friesen and J.F. Flannagan. 1980. Evaluation of the acute toxicity of the heavy metal cadmium to nymphs of the burrowing mayfly, Hexagenia rigida. p. 457-465. In: J. F. Flannagan and K.E. Marshall (eds.). Advances in Ephemeroptera Biology. Plenum Press, N.Y.

Lewis, P.A. 1978. Mayfly communities as indicators of water quality. Presented at 26th Annu. Meet., North Amer. Benthological Soc. Winnipeg, Manit.

Madder, D.J. and W.L. Lockhart. 1980. Studies on the dissipation of diflubenzuron and methoprene from shallow prairie pools. Can. Entomol. 112: 173-177.

McLaughlin, G.A. 1973. History of pyrethrum. p. 3-15. In: J.E. Casida (ed.). Pyrethrum. The natural insecticide. Academic Press, N.Y.

Merritt, R.W. and K.W. Cummins. 1978. An introduction to the aquatic insects of North America. Kendall/Hunt Publ. Co., Iowa.

Muirhead-Thomson, R.C. 1978. Lethal and behavioural impact of permethrin (NRDC 143) on selected stream macroinvertebrates. Mosq. News 38: 185-190.

Muirhead-Thomson, R.C. 1979. Experimental studies on macroinvertebrate predator-prey impact of pesticides. The reactions of Rhyacophila and Hydropysche (Trichoptera) larvae to Simulium larvicides. Can. J. Zool. 57: 2264-2270.

Narahashi, T. 1976. Nerve membrane as a target of pyrethroids. Pestic. Sci. 7: 267-272.

Neave, F. 1932. A study of the mayflies (Hexagenia) of Lake Winnipeg. Contrib. Can. Biol. Fish., N.S. 7: 177-201.

O'Brian, R.D. 1967. Insecticides, action and metabolism. Academic Press, N.Y.

Oseid, D.M. and L.L. Smith, Jr. 1975. Long-term effects of hydrogen sulfide on Hexagenia limbata (Ephemeroptera). Environ. Entomol. 4: 15-18.

Perlman, F. 1961. Insect allergens: Their interrelationship and differences. J. Allergy 32: 93-101.

Prater, B.L. and M.A. Anderson. 1977a. A 96-hour sediment bioassay of Duluth and Superior harbour basins (Minnesota) using Hexagenia limbata, Asellus communis, Daphnia magna, and Pimephales promelas as test organisms. Bull. Environ. Contam. Toxicol. 18: 159-169.

Prater, B.L. and M.A. Anderson. 1977b. A 96-hour bioassay of Otter Creek, Ohio. J. Water Pollut. Control Fed. 49: 2099-2106.

Pree, D.J. and E.A.C. Hagley. 1977. Toxicity of some insecticides to eggs of the oriental fruit moth and codling moth. Proc. Entomol. Soc. Ont. 108: 69-74.

Rawn, G.P., G.R.B. Webster and D.C.G. Muir. 1979. Bioactivity and degradation of permethrin in artificial pools. p. 140-149. In: J.F. Klaverkamp, S.L. Leonhard and K.E. Marshall (eds.). Proc. 6th Annu. Aquat. Toxicity Workshop, Winnipeg, Manit. 1979. Can. Tech. Rep. Fish. Aquat. Sci. 975.

Rawn, G.P., G.R.B. Webster, and D.C.G. Muir. 1980. Analysis and fate of (^{14}C) permethrin in an aquatic ecosystem. p.25-33. In: A.J. Cessna (ed.). Proc. 15th Annu. Pestic. Residue Analysts (Western Canada) Workshop, Regina, Sask. 1980. Can. Plains Res. Center, Univ. Regina, Sask.

Salkeld, E.H. and C. Potter. 1953. The effect of age and stage of development of insect eggs on their resistence to insecticides. Bull. Entomol. Res. 44: 527-580.

Schoof, H.F. and W.L. Jakob. 1964. Insecticides for use against Aedes aegypti. Mosq. News 24: 309-311.

Sharom, M.S. and K.R. Solomon. 1981a. Adsorption, desorption, distribution and degradation of permethrin in sediment-water system. p. 346-349. In: N. Bermingham, C. Blaise, P. Couture, B. Hummel, G. Joubert and M. Speyer (eds.). Proc. 7th Annu. Aquat. Toxicity Workshop. Montreal, Que. 1980. Can. Tech. Rep. Fish. Aquat. Sci. 990.

Sharom, M.S. and K.R. Solomon. 1981b. Adsorption and desorption of permethrin and other pesticides on glass and plastic materials used in bioassay procedures. Can. J. Fish. Aquat. Sci. 38: 199-204.

Smith, E.H. and E.H. Salkeld. 1966. The use and action of ovicides. Annu. Rev. Entomol. 11: 331-368.

Snedecor, G.W. and W.G. Cochran. 1980. Statistical Methods. 7th Ed. Iowa State Univ. Press, Iowa.

Spieth, H.T. 1941. Taxonomic studies on the Ephemeroptera. II. The genus Hexagenia. Am. midl. Nat. 26: 233-280.

Tu, C.M. 1978. Effect of pesticides on acetylene reduction and microorganims in a sandy loam. Soil Biol. Biochem. 10: 451-456.

Tu, C.M. 1980. Analogues on microbial populations, nitrification and respiration activites in soil. Bull. Environ. Contam. Toxicol. 24: 13-19.

Walker, Jr., W.H. 1970. A study of the ecology and distribution of burrowing mayfly nymphs (Ephemeroptera: Hexagenia munda Eaton) in Stone Mountain Lake, Georgia. M.Sc. Thesis, Emory Univ. Georgia. 105 p.

Williams, I.H. and M.J. Brown. 1979. Persistence of permethrin and WL 43775 in soil. J. Agric. Food Chem. 27: 130-132.

Wouters, W. and J. van den Bercken. 1978. Action of pyrethroids. Gen. Pharmacol. 9: 387-398.

Zimmerman, M.C. 1977. Aspects of the life history, feeding behavior, and nutritional dynamics of the burrowing mayfly, Hexagenia limbata in an Ohio pond. Ph.D. Thesis, Miami Univ. Ohio. 173 p.

Appendix A. Chemical characteristics of declorinated City of Winnipeg tap water [1] (16 May, 1980).

pH	8.0	Fe	0.03 mg/L
conductivity	160uS/cm	Mn	<0.01 mg/L
TSS	1 mg/L	Cl	4.2 mg/L
Susp C	440 ug/L	SO_4	5.6 mg/L
Susp N	28 ug/L	NH_4-N	30.0 ug/L
Na	2.1 mg/L	NO_2-N	<1 ug/L
K	1.3 mg/L	NO_3-N	35 ug/L
Ca	21.0 mg/L	TDN	420 ug/L
Mg	5.7 mg/L	TDP	12 ug/L

1. Analyses conducted by the Analytical Unit, Freshwater Institute.

Appendix B. Characteristics of standard sediment.[2]

Textual designation	silty clay-clay
sand	15%
silt	32%
clay	53%
organic matter	2%
pH	7.2
salinity	0.9 mS/cm
nitrate-nitrogen	0.6 ppm
avail. phosphorus	27 ppm
avail. potassium	390 ppm
sulphate-sulphur	20+ ppm

2. Analyses conducted by the Manitoba Provincial Testing Laboratory.