Fate and Degradation of Permethrin in a Model Aquatic Ecosystem

by

Gary Peter Rawn

A thesis
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ABSTRACT

In 1979 and 1980, outdoor artificial ponds treated with the synthetic pyrethroid permethrin at 0.028 kg/ha (15 ug/L) resulted in 100% mortality of lab-reared fourth instar Aedes aegypti larvae immediately and for 12 hours post-treatment; only 24% mortality was found after 24 h and none was observed at 72 h. Residue analyses were conducted by direct combustion or by TLC-autoradiography, HPLC, GLC, and liquid scintillation counting. Radiotracer data indicated a rapid loss of permethrin from the water which corroborated the bioassay results. Permethrin degradation products in the water were found to be more persistent than permethrin itself. Five degradation products detected in the water at concentrations never exceeding 2.0 ug/L were cis- and trans-cyclopropyl acid, phenoxybenzoic acid, phenoxybenzyl alcohol, and an unknown non-cleaved product of permethrin. Permethrin was readily sorbed by duckweed, fathead minnows, and hydrosoil. In 1979 and 1980 the maximum permethrin concentrations reached in the duckweed were 30 and 55 ug/g, respectively, which decreased to less than 0.1 ug/g by 29 days post-treatment. A growth inhibition of the duckweed was observed in the ponds treated with the permethrin. Permethrin concentrations in the fish reached 1.1 mg/kg but were undetectable by four weeks post-treatment. In 1979, permethrin residues in the hydrosoil reached a maximum of 40 ug/kg and in 1980, 120 ug/kg. Permethrin was persistent in the hydrosoil with 0.25 ug/kg detected at 420 days (1979) and 4.9 ug/kg at

323 days (1980). Cis-permethrin was more persistent in the hydrosoil than the trans-permethrin. The results indicated that permethrin bioactivity against Aedes aegypti larvae was short-lived at an application rate of 15 ug/L as a result of the rapid degradation of permethrin in the water and the sorption of permethrin by the hydrosoil, vegetation, and fish.

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Chapter I

INTRODUCTION

Since the 19th century pyrethrum has been extracted from the flower heads of Chrysanthemum cinerariaefolium to control insect pests. The flower extract was widely used as an insecticide due to its characteristic low mammalian toxicity, low persistence, fast knock-down, and effective repellency. The main disadvantage of pyrethrum was that it was very easily photodegraded.

It was not until 1947 that the chemical structure of the pyrethrins was determined. By 1973, synthetic pyrethroids had been developed which had the attributes of pyrethrins but were also photostable. The pyrethroids are of very low toxicity to mammals, birds, and plants, but are of high toxicity to insects and fish. All pyrethroids are lipophilic, and in this respect, resemble the chlorinated hydrocarbons; however, pyrethroids are readily metabolized by mammals and birds. Several of the recently developed synthetic pyrethroids, permethrin, cypermethrin, fenvalerate, and decamethrin provide superior control of insect pests compared to the older organophosphorus, carbamate, and chlorinated hydrocarbon insecticides.

Wide use of permethrin (NRDC 143, FMC 33297, WL 43479, PP 550, Ambush) for insect pest control in Canadian agriculture and forestry is anticipated within the next few years; experimental results to date indicate that permethrin effectively controls many agricultural and forestry pests and is effective as a mosquito larvicide.

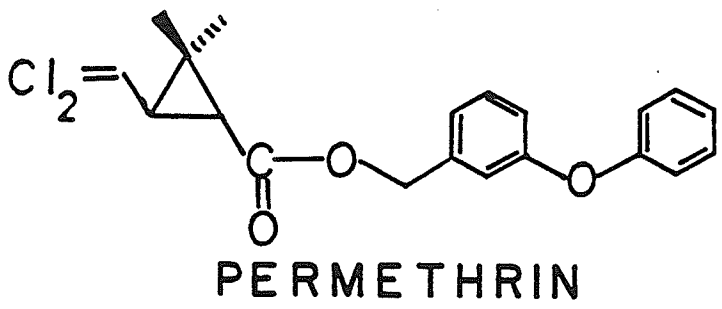
Research was undertaken to appraise the bioavailability and residue levels of permethrin in treated aquatic systems. Outdoor artificial ponds were treated with ^{14}C-permethrin followed by bioavailability tests with Aedes aegypti larvae and residue analysis of permethrin and degradation products in water, hydrosoil, duckweed, and fathead minnows by gas-liquid chromatography, thin-layer chromatography, high-pressure liquid chromatography, autoradiography, and liquid scintillation counting.

The objectives of this thesis are to determine the bioavailability of permethrin in a pond system, the degree of loss of permethrin isomers by photochemical means, and the fate of residues of permethrin and major metabolites in water, hydrosoil, duckweed, and fathead minnows.

Chapter II

LITERATURE REVIEW

Permethrin, (3-phenoxyphenyl) methyl 3-(2,2-dichloroethenyl)-2, 2-dimethylcyclopropane-1-carboxylate, is a synthetic pyrethroid that was discovered at Rothamstead Experimental Station, England. Permethrin has the empirical formula $C_{21}H_{20}O_3Cl_2$ (molecular weight 391.28) and the following structural formula:



PERMETHRIN

Permethrin is a viscous liquid with a boiling point of 210-220°C/0.05mm and a vapour pressure of 3.4×10^{-7} mm Hg. Its water solubility is 0.2 mg/L.

This review surveys the literature on pyrethroids with emphasis on permethrin as a mosquito larvicide and its effect on the aquatic environment. Since there are no published reports on permethrin degradation in an aquatic system, the degradation mechanisms for permethrin in various biological and environmental conditions are presented to indicate the type of degradation pattern that may occur in ponds. Methods for permethrin analysis are also reviewed. Topics of special interest to

the thesis such as pesticide volatilization models, model ecosystems, use of Glenlea ponds, and modelling of pesticide distribution in ponds are also discussed.

2.1 TOXICITY

Pyrethroids, a new generation of insecticides, have the advantage of being very toxic to insects and relatively less toxic to mammals than previous classes of insecticides as indicated in Table 1 (Elliot 1977).

TABLE 1

Toxicity of Classes of Insecticides to Insects and Mammals (Elliott 1977)

Class	Rats	Insects	Ratio
carbamates	45 mg/kg	2.8 mg/kg	16
organophosphates	67 "	2.8 "	33
organochlorine	230 "	2.6 "	91
pyrethroids	2000 "	0.45 "	4500

The variation in toxicity between insects and mammals may be due to the pyrethroids' ability to penetrate rapidly to, and interact with, the site of action in insects, whereas, in mammals, they are eliminated intact or metabolically modified before reaching the site of action (Elliot et al. 1978).

A comparison of rat oral LD_{50}s is given in Table 2 for some pyrethroids (Elliott et al. 1978) and other common insecticides (Matsumura

1975). The values indicate that the pyrethroids are moderately to slightly toxic to mammals. Of the pyrethroids, permethrin is one of the least toxic to mammals.

TABLE 2

Mammalian toxicity of some pyrethroid insecticides (Elliott et al. 1978, Matsumura 1975)

Insecticide	Rat oral LD_{50} (mg/kg)
Permethrin	2000
Cypermethrin	500
Decamethrin	70-140
Fenvalerate	450
DDT	250
Methoxychlor	6000
Parathion	3.6-13
Fenitrothion	250-670
Malathion	900-5800
Chlorpyrifos	97-276

Toxicity values for permethrin to a variety of mammals are shown in Table 3 . The values indicate that permethrin is only slightly toxic to animals and non-toxic to birds (Chipman Chemicals 1978).

Table 4 indicates the toxicity of permethrin and some major metabolites to mice (Gaughan et al. 1977). The results indicate that permethrin metabolites are more toxic to mice than permethrin itself.

In a permethrin toxicity study, Ruzo and Casida (1977) concluded that permethrin did not cause eye or skin irritation or skin sensitizing effects and that tests showed no indication of permethrin being mutagenic

TABLE 3

Permethrin toxicity to mammals (Chipman Chemicals, 1978)

Mammal	Oral LD_{50} (mg/kg)
Guinea pig	> 4000
Rabbit	> 4000
Mallard duck	> 23000
Pheasant	> 23000
Japanese quail	> 13500

TABLE 4

Toxicity of permethrin and metabolites to mice (Gaughan et al., 1977)

Metabolite	48 h Mouse ip LD_{50} (mg/kg)
[1RS,t]permethrin	> 1000
[1RS,c]permethrin	925
PBalc	575
PBacid	350
[1R,t]Cl_2CA	210
[1R,c]Cl_2CA	370

or teratogenic. The low permethrin toxicity was further indicated by the determination, after six months of feeding, of the rat diet no-effect level to be 1500 mg/kg permethrin.

As a result of the pyrethroids' high insect toxicity, low mammalian toxicity, and increased photostability, the pyrethroids have a very high potential for eventual registration and use in biting fly control and crop and forest protection.

Permethrin has greater insecticidal activity than equal amounts of organophosphates or carbamates against members of the insect orders Lep-

idoptera, Coleoptera, Diptera, Heteroptera, and Homoptera (Ruscoe 1977). For lepidopterous insects, the effectiveness ratio of permethrin to organophosphates and carbamates is approximately ten to one.

As a result of the initial reports outlining the high toxicity of pyrethroids to insects, they have been tested against a variety of insects. Mulla et al. (1975) applied permethrin to experimental ponds and found that application rates of 0.028 kg/ha produced complete control of larvae and pupae of Culex species, while Aedes species were controlled at 0.011 kg/ha. Permethrin toxicity against Culiseta and Culex larvae in ponds was further shown by Mulla and Darwazeh (1976). Seven days after treatment of ponds with permethrin at 0.028 kg/ha, larval control of 98 and 100 %, respectively, was recorded. The effective permethrin dosage against stagnant water mosquitoes was estimated to be ca. 0.028 kg/ha. Population monitoring of non-target insects such as mayfly naiads (Baetidae) and dragonfly naiads (Libellulidae) indicated drastic population decreases after treatment at the larvicidal rates. However, usually within two or three weeks, the non-target population recovered.

Table 5 indicates the LC_{50}s for permethrin and decamethrin against mosquito larvae (Mulla et al. 1978). The results indicate that the larvae were more susceptible to decamethrin than permethrin and that decamethrin was about 500 times as effective as methyl parathion on a weight for weight basis.

Field tests indicated that permethrin controlled mosquito larvae and pupae for one week at 0.028 kg/ha, while decamethrin applied at 0.001

TABLE 5

Toxicity of permethrin and decamethrin to mosquito larvae (Mulla et al., 1978)

Isecticide	LC_{50} (ug/L)	
	Culex quinquefasciatus (lab)	Aedes nigromaculis (field)
Permethrin	1.40	---
Decamethrin	0.02	0.40
Methyl parathion	----	210.00

kg/ha was still 80% effective at two weeks post-treatment. However, both compounds adversely affected the mayfly naiad population which required two to three weeks to recover (Mulla et al. 1978 and 1980).

Rettich (1980) studied the efficacy and effect on non-target organisms of permethrin and decamethrin in aquatic systems. Permethrin applied to mosquito breeding sites at 0.1 mg/L had no affect on Rhynchelmis species, Turbellaria, Hirudinea, Mollusca, and Ostracoda. However, at levels of 0.002 mg/L and 0.005 mg/L the Daphnis species, Gerris lacustris, Tricoptera larvae, Dytiscidae, and Dixidae larvae were severely affected, as were Chironomidae larvae at 0.01 mg/L. At the rate of 0.02 mg/L, decamethrin had no effect on Turbellaria, Rhynchelmis species, and Mollusca. The more sensitive non-target organisms severely affected were Gerris species at 0.0002 mg/L, Daphnia species and Trichoptera larvae at 0.0005 mg/L, and Dytiscidae and Helodidae larvae at 0.01 mg/L decamethrin.

A further advantage of the pyrethroids is their toxicity to mosquito pupae as well as larvae. Mulla et al. (1978) stated that pyrethroids were the first compounds they had evaluated with such high biological activity against both larvae and pupae. Table 6 indicates the susceptibility of both life stages to pyrethroids.

TABLE 6

Susceptibility of larvae and pupae to pyrethroids

	LC_{50} (ug/L)			
	larvae		pupae	
	Deca-methrin	Per-methrin	Deca-methrin	Per-methrin
Culex tarsalis*	0.02	2.00	0.30	6.00
Culiseta incidens*	0.30	3.00	0.07	0.70
Aedes sticticus**	0.02	2.7	0.05	1.0
Aedes vexans**	0.09	2.1	0.1	0.8

* Mulla et al. (1978).
** Rettich (1979).

Rettich (1979) studied, under laboratory conditions, the effect of pool bottom substrate on the residual larvicidal action of decamethrin, permethrin, and bioresmethrin. The substrates tested were water plants, decaying leaves, and mud. The results (Table 7) indicate that the substrate does affect the efficacy levels and that decaying leaves were most efficient at reducing the length of mosquito control.

TABLE 7

Effect of substrate on pyrethroid larvicidal effectiveness (Rettich, 979)

Insecticide	Dosage (ug/L)	Effectiveness (days) plants	leaves	mud
Decamethrin	4.0	5-10	3	5-10
	10.0	14-21	4-7	5-10
Permethrin	10.0	1-3	1-2	2
	20.0	1-3	1-2	3-5
	40.0	3-7	1-3	7-10
Bioresmethrin	20.0	1	1	1
	100.0	3-4	1-2	3-5

Permethrin has also been tested in the aquatic environment for pest control of other insects besides mosquitoes. Muirhead-Thomson (1977) compared black fly (Simulium) larvae susceptibility to permethrin and abate (temephos) and concluded that under laboratory conditions, permethrin was about 40 times more toxic than abate. It was noted that permethrin was very fast acting which resulted in large numbers of larval detachments soon after treatment. This early detachment could result in increased efficacy in the field as the result of the detached larvae drifting downstream with the slug of insecticide.

The effect of permethrin on stream non-target organisms was studied by Muirhead-Thomson (1978). The LC_{90-95} 24 h, after a one hour exposure to permethrin was 0.005 mg/L for Simulium while for the non-target organisms Baetis, Gammarus, and Brachycentrus it was 0.001 mg/L and 0.1 for Hydropsyche. These results indicated that the level required for

Simulium control by permethrin could result in the reduction of some of the stream invertebrate population. A further study indicated that after a one hour exposure to 12.5, 25, and 50 ug/L permethrin, rainbow trout appeared to recover completely, while for most of the macroinvertebrates tested, a one hour exposure to 5-10 ug/L resulted in over 95% mortality. Thus, in a stream environment with a short exposure time to permethrin the decrease in fish food population may be more of a problem to the fish than acute toxicity to the fish.

The toxicity of several pyrethroids (technical and formulated) to rainbow trout was studied by Coats and O'Donnell-Jeffery (1979). The LC_{50} values (Table 8) not only indicate the high toxicity of pyrethroids to fish, but also the fact that the formulated material was more toxic to the fish.

TABLE 8

Pyrethroid toxicity to rainbow trout (Coats and O'Donnell-Jeffery, 1979)

Insecticide	24 h LC_{50} (ug/L)	
	technical	formulated
Permethrin	135	61
Cypermethrin	55	11
Fenvalerate	76	21
Fenpropanate	76.7	8.6

Permethrin toxicity to various fish species is indicated in Table 9 (Chipman Chemicals 1978). The point to note is that the fish LC_{50} values are in the same range as those for mosquito larvae. Therefore, per-

methrin applied for mosquito control may be present in levels high enough to affect any fish present.

TABLE 9

Permethrin toxicity to fish (Chipman Chemicals, 1978)

Fish	96 h LC_{50} (ug/L)	No Effect Level (ug/L)
Rainbow trout	2.5	1.0
Channel catfish	5.4	4.2
Coho salmon	17.	7.5
Brook trout	4.7	0.68
Fathead minnow	2.6	1.5

Mauck et al. (1976) studied the toxicity of pyrethroids to several species of fish. The results indicated that pyrethroids were more toxic to fish in cold water (12°C) than in warm (22°C) water. In addition, pyrethroid toxicity was not influenced by a pH range of 6.5-9.5 or by water hardness.

Even though the pyrethroids are very toxic to fish under laboratory conditions this toxicity has not always appeared in field studies. Cypermethrin applied to a pond at 100 g/ha did not affect the fish, fish fry, or amphibia (Breese 1977). Kingsbury (1976) also noted that during a forest spray operation with a deposit rate on a stream of 13.4 g permethrin/ha, the effects on caged or native fish were minimal. An increase in drifting insects was also detected but the population level of bottom fauna did not change.

2.2 PYRETHROID METABOLISM AND DEGRADATION

The potential for widespread use of pyrethroids has resulted in the need to know and understand pyrethroid metabolism and degradation in biological and environmental samples. Permethrin is one of the most studied pyrethroids. A general degradation pathway for permethrin in a number of biological systems is outlined in Figure 1 (Leahey 1979). Degradation of permethrin usually involves ester cleavage and oxidation with the cleavage occuring more readily with the trans-isomer than the cis-isomer. Oxidative attack on the molecule involves oxidation of the benzyl alcohol group to a carboxylic acid, hydroxylation at the 4'-position of the 3-phenoxybenzyl alcohol and oxidation of the gem-dimethyl group on the cyclopropane ring to an alcohol. Table 10 lists the code names for the various permethrin metabolites and the figure in which the structure of each can be seen.

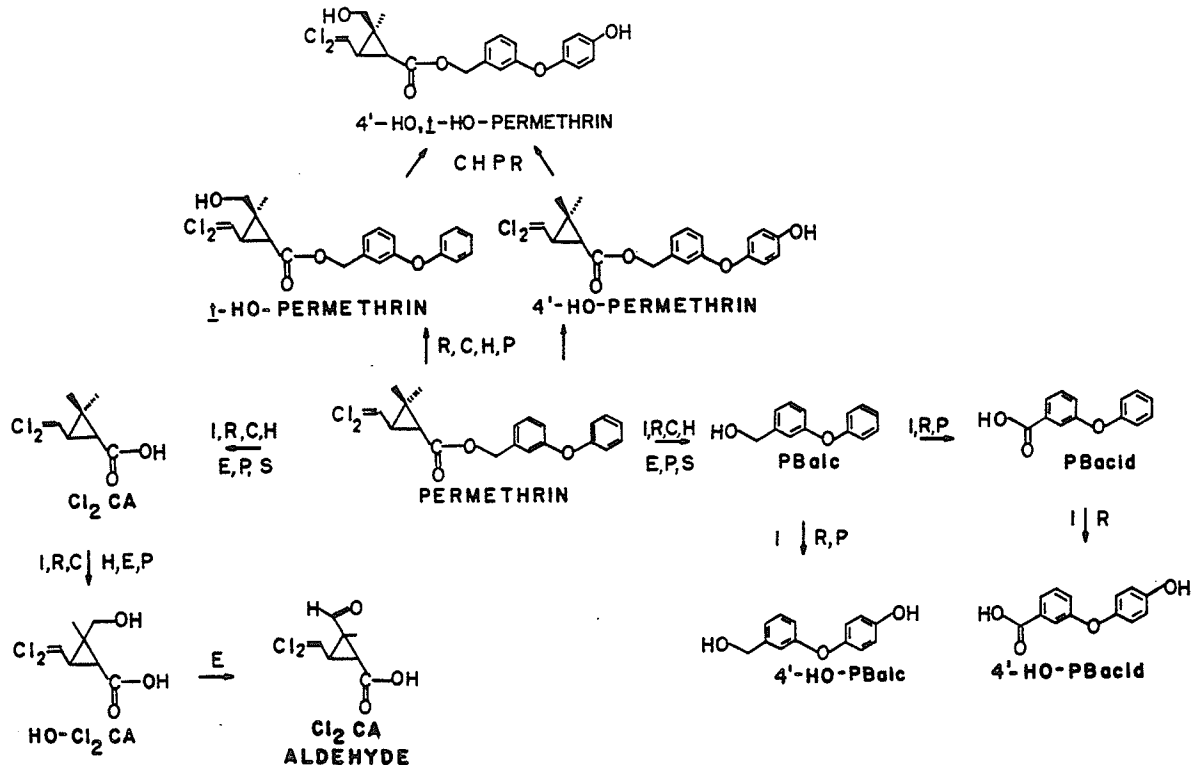


Figure 1: Permethrin metabolism pathways in biological systems (R = rat, C = cow, H = hen, P = plants, I = insects, S = soil, and E = enzymes) (Leahey, 1979)

TABLE 10

Permethrin metabolites

Metabolite	Figure
Permethrin (per)	1
t-HO-per	1
4′-HO-per	1
4′-HO,t-HO-per	1
PBalc	1
4′-HO-PBalc	1
3-HO-Benzyl alcohol	2
PBacid	1
4′-HO-PBacid	1
4′-HO-PBacid-sulfate	4
2′-HO-PBacid-sulfate	4
PBacid-gluc (PBacid-glucuronide conjugate)	4
PBacid-glycine conjugate	4
PBacid-glut (PBacid glutamic acid conjugate)	5
PBacid glutamine conjugate	5
4′-HO-PBacid-gluc (4′-HO-PBacid-glucuronide)	5
Benzoic acid	2
Cl_2CA	1
Cl_2CA-aldehyde	1
HO-Cl_2CA	1
c-HO-Cl_2CA	7
t-HO-Cl_2CA	7
c-HO-Cl_2CA-lactone conjugate	3
Cl_2CA-gluc (Cl_2CA-glucuronide conjugate)	3
Cl_2CA-glycine conjugate	5
Cl_2CA-glutamic acid conjugate	5
Cl_2CA-glutamine conjugate	5

2.2.1 Photolysis

Besides the pathways illustrated in Figure 1, each environment has its own peculiar degradation pathways, and subsequently, its own degradation products. For example, exposure of permethrin to light not only results in ester cleavage of the molecule but also extensive photoisomerization of the cyclopropane ring (Holmstead et al. 1978). In water the isomerization is very rapid and reaches equilibrium in three to four

hours. The main products of permethrin following ester cleavage are the cis- and trans-cyclopropyl acids, phenoxybenzyl alcohol, 3-HO-benzyl alcohol and small amounts of phenoxybenzoic acid and benzoic acid. Photolysis of permethrin in soil resulted mainly in cis- and trans-cyclopropyl acid and phenoxybenzyl alcohol (Figure 2). Unless otherwise indicated, the structures shown refer to both the trans- and cis-isomers.

Decamethrin also undergoes cis- trans photoisomerization but is more sensitive to photolysis than permethrin. Increased photolysis may be due to the presence of the dibromovinyl and cyano substituents in decamethrin. In solution, ester cleavage reactions and loss of bromine are the primary degradation pathways, while in the solid phase, the ester linkage remains intact (Ruzo et al. 1977). Unlike permethrin, the cleavage of decamethrin resulted in free radicals and subsequent photoproducts through loss of carbon dioxide or proton abstraction (Leahey 1979).

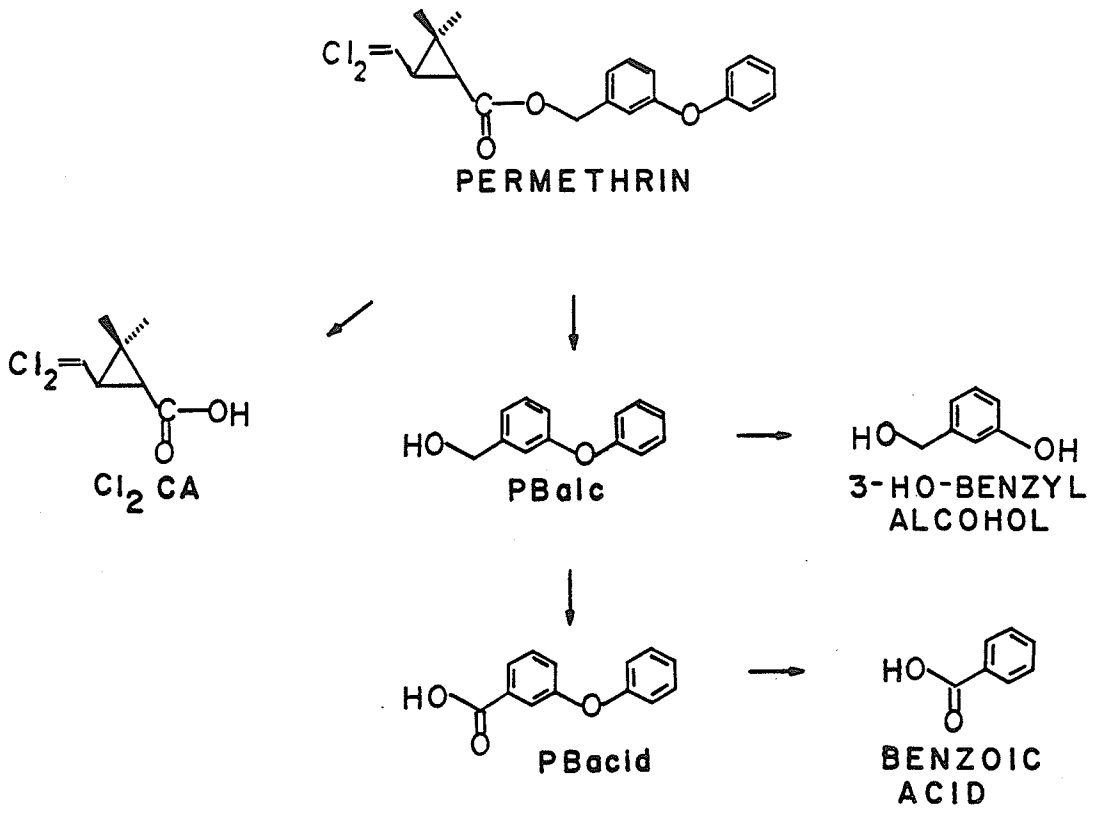


Figure 2: Photolysis of permethrin in water (Holmstead et al., 1978)

2.2.2 Mammals

Permethrin added directly to the rumen of cows at rates equivalent to dietary levels of 33-39 mg/kg showed no adverse affects on the cows and all tissues and organs appeared normal on sacrifice (Gaughan et al. 1978). Residues in the milk were below 100 ppb within 2-4 days post-treatment and consisted mainly of permethrin. By 12-13 days post-treatment, residues in the liver ranged from 72-210 ug/kg (permethrin equivalents) and 35-335 ug/kg (permethrin equivalents) in fat tissue. Within the fat tissue and liver, the levels of cis-permethrin were higher than the levels of the trans-permethrin. This difference was due to the fact that the trans-isomer and its metabolites were eliminated more readily than cis-permethrin and its metabolites. Urinary excretion was a more important elimination route for the metabolites of trans-permethrin than those of cis-permethrin. Metabolism of permethrin, labelled in the carboxyl group of the acid moiety or the methylene position, to carbon dioxide was not detected in the cows. The major metabolic routes were the hydroxylation of the trans-methyl group of permethrin, ester cleavage, cis-methyl group hydroxylation of the acid moieties, and conversion of metabolites to form glucuronide, glutamate, and lactone conjugates. The major permethrin metabolites in cows are illustrated in Figure 3.

Permethrin, administered orally to male rats at 1.6 to 4.8 mg/kg, was almost completely eliminated from the body within a few days (Gaughan et al. 1977). By 12 days, 97-100% of the radiocarbon was recovered in the excreta, mainly in the urine, and very little as carbon dioxide. As was observed in permethrin metabolism by the cows, cis-permethrin was more persistent than the trans-permethrin. The slower rate of ester cleavage

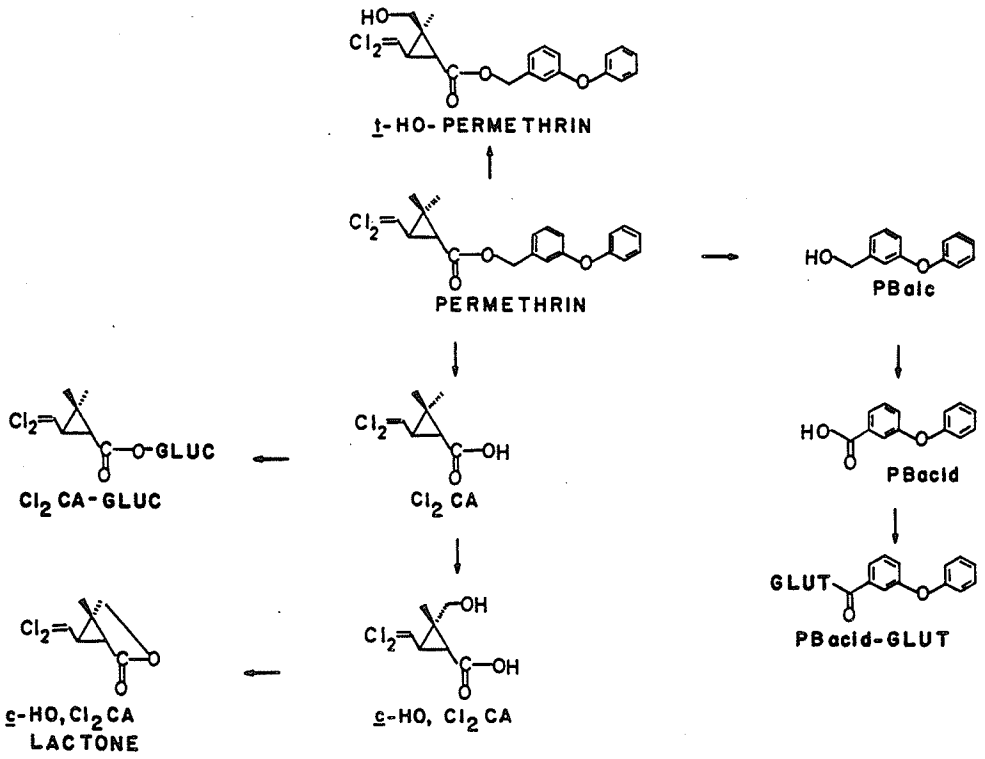


Figure 3: Permethrin metabolism in cows (Gaughan et al., 1978)

of the cis-isomer may explain why 45-54% of the excreted radiocarbon from cis-permethrin appears in the urine whereas 81-90% of the trans-permethrin products are excreted in the urine. The most abundant metabolite detected was 4′-HO-PBacid-sulfate which was not observed in the cow metabolism. The prinicpal sites of metabolic attack on permethrin were found to be ester cleavage, oxidation at the trans- or cis-methyl group at the geminal dimethyl moiety, and oxidation at the 2′ or 4′ position of the phenoxy group. The major permethrin metabolites in rats are shown in Figure 4.

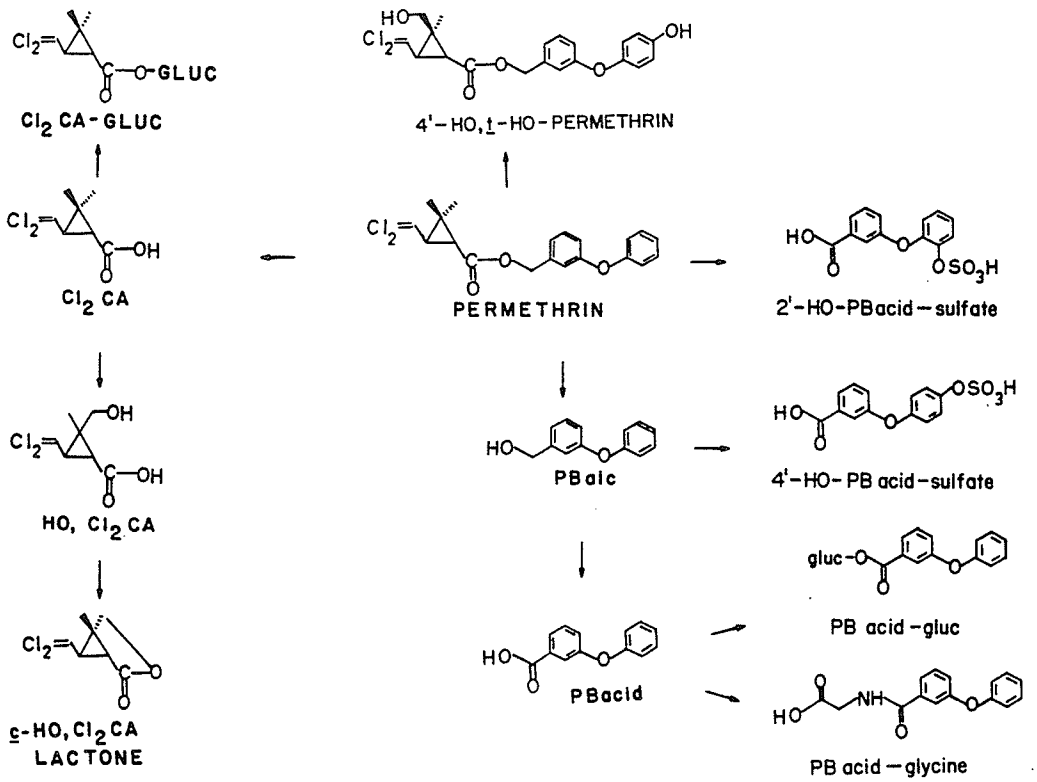


Figure 4: Permethrin metabolism in rats (Gaughan et al., 1977)

2.2.3 Enzyme Metabolism

Microsomal enzyme metabolism of pyrethroids in various animal species is shown in Table 11 (Shono et al. 1979). The results show that trans-permethrin and trans-cypermethrin were more readily hydrolized by esterases than the cis-isomers. The ability of an enzyme system to metabolize a pyrethroid appears to be species dependent. Pyrethroid metabolism by the enzymes occurred by ester cleavage and hydroxylation. The site of hydroxylation (trans- or cis-methyl and the 2', 4', or 6' position of the alcohol moiety) differed with each species.

TABLE 11

Metabolism of Pyrethroids by Esterase and Oxidase Systems (Shono et al., 1979)

		Extent of Metabolism (%)			
		Trans		Cis	
Pyrethroid	Species	Esterase	Oxidase	Esterase	Oxidase
Permethrin	mouse	91.0	83.8	9.1	74.3
	rat	89.3	9.5	6.0	20.7
	housefly	38.0	4.6	9.1	4.2
	looper	37.0	1.5	12.5	11.4
Cypermethrin	mouse	93.2	17.3	41.5	37.6
Decamethrin	mouse			28.3	41.0

2.2.4 Insects

In cockroach adults, house fly adults, and cabbage looper larvae, permethrin metabolism did not lead to the same products (Shono et al. 1978). For example, 6-HO derivatives were detected in house flies only; cis-HO derivatives were found in the cockroaches and house flies but not in the cabbage loopers; and the amino acids used in conjugate formation differed with each species. The major metabolites of permethrin in cockroaches are shown in Figure 5.

Bigley and Plapp (1978) determined that insect metabolism of permethrin involved mainly hydrolysis of the cis-isomer and oxidation of the trans-isomer. The trans-permethrin was found to be more readily metabolized and excreted by the insects than the cis-isomer. The differences in rates of metabolism and excretion may explain why cis-permethrin was 2.5 times as toxic as permethrin (40:60, cis: trans) to the tobacco budworm and the cotton bollworm.

In comparing the metabolism of permethrin and cypermethrin in insects, Holden (1979) found that 80% of the permethrin applied to the cuticle had been absorbed after 24 h compared to only 40% of the cypermethrin. Permethrin metabolism was also faster than the rate for cypermethrin. Eighty percent of the permethrin was metabolized after 17 h while 90-95% of the cypermethrin remained. Permethrin metabolism involved ester cleavage and oxidative degradation of the cis-isomer and hydrolysis of the trans-isomer which was opposite of that reported by Bigley and Plapp (1978). The small amount of cypermethrin metabolism that did occur was the result of ester cleavage and oxidation of both isomers.

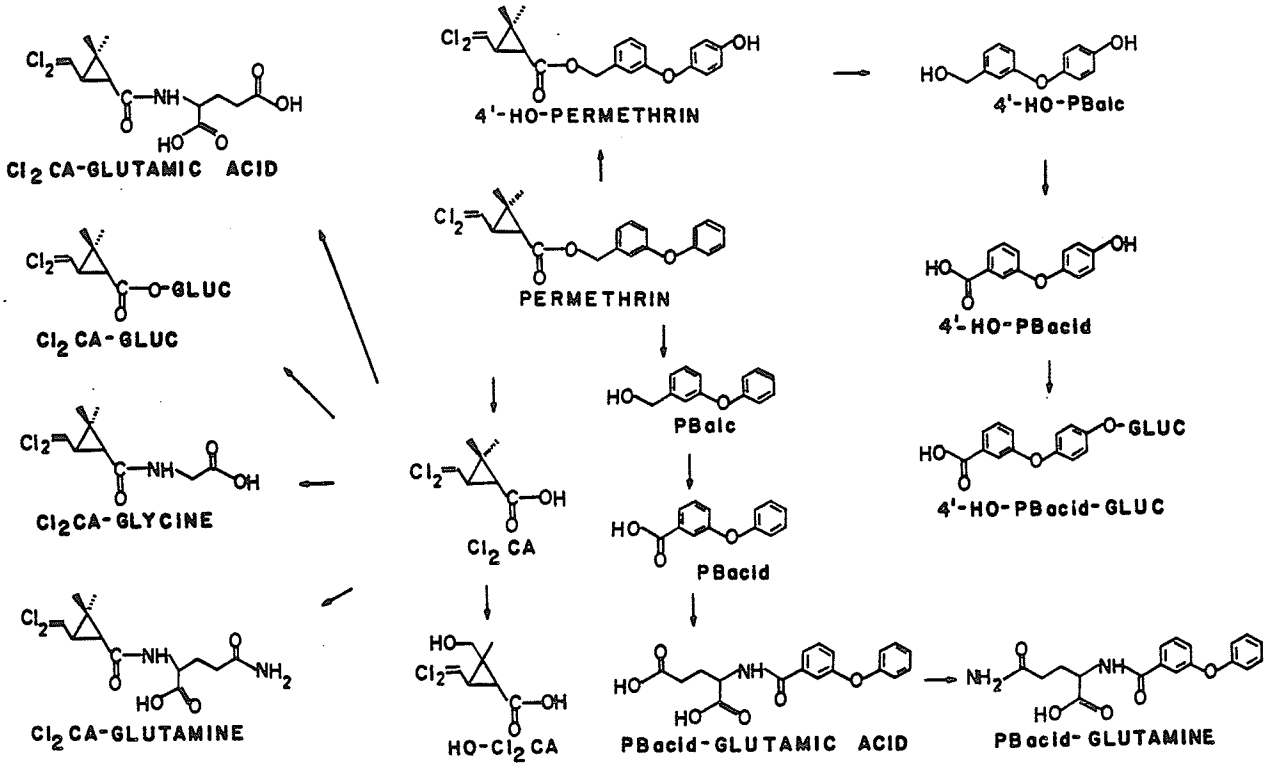


Figure 5: Permethrin metabolism in cockroaches (Shono et al., 1978)

2.2.5 Fish

As occurs in insects, fish liver microsomes also metabolize cis- and trans-permethrin by different pathways (Glickman et al. 1979). Trans-permethrin is metabolized primarily by hydrolysis while cis-permethrin is attacked by oxidation. The oxidation occurs mainly at the 4'-position and, to a lesser extent, at the trans-methyl group. Bile from permethrin exposed rainbow trout contained metabolite conjugates and little or no permethrin. Major permethrin metabolites in rainbow trout are shown in Figure 6.

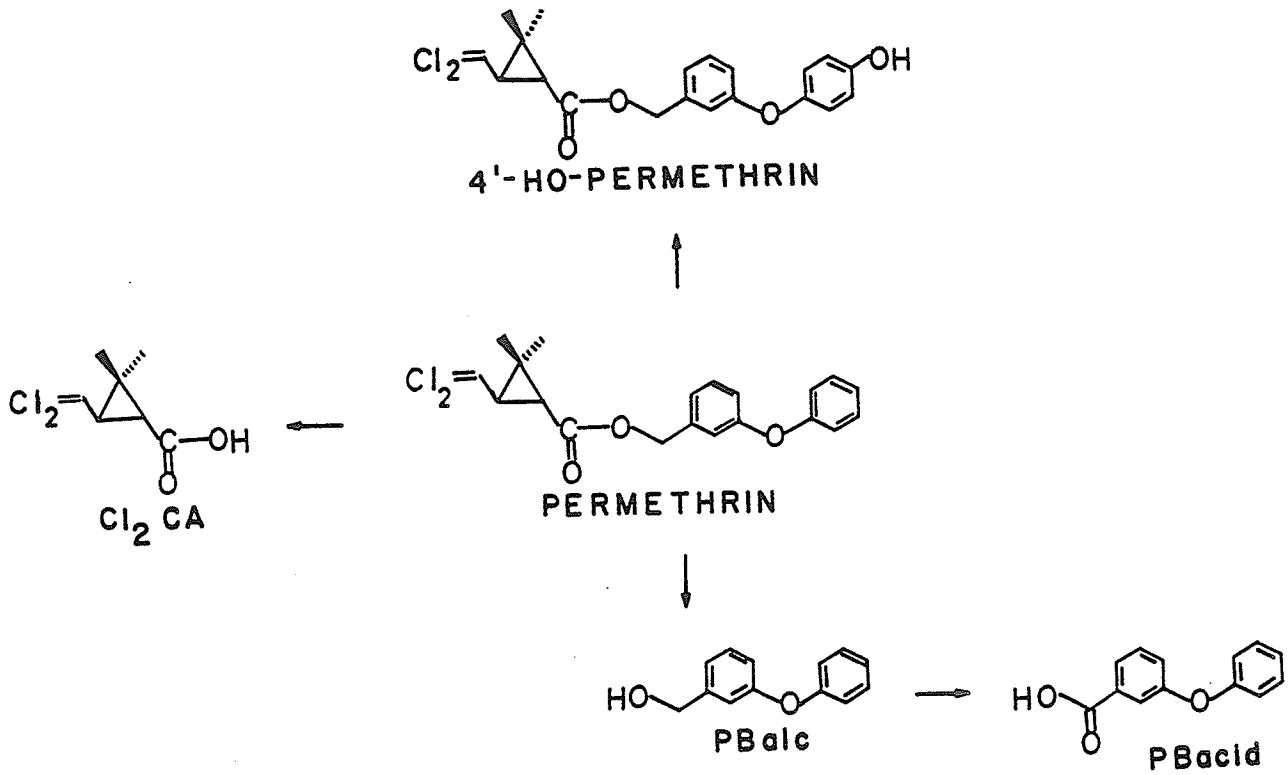


Figure 6: Permethrin metabolism in rainbow trout (Glickman et al., 1979)

2.2.6 Vegetation

Because of the lipophilic nature of pyrethroids, they are readily sorbed by the waxy layers on plant surfaces. Once in the waxy surface, very little xylem or phloem systemic action of permethrin (Ruscoe 1977) or cypermethrin (Breese 1977) occurs.

Permethrin applied to plants undergoes photolysis and metabolism largely by reactions which cleave the ester group (Gaughan and Casida 1978). Isomerization of permethrin at the cyclopropane ring was also noted. As in animals, the trans-permethrin was more readily cleaved than the cis-isomer. After ester cleavage the acid moiety rapidly conjugated either before or after hydroxylation of one methyl group. The resulting alcohol metabolites were also readily conjugated. From this study and that of Wright et al. (1980) who found that the major cypermethrin metabolite in plants was a conjugate of the acid moiety, it appears that the major metabolic route for pyrethroids in plants may be conjugate formation of the metabolic products. Permethrin metabolism in plants is shown in Figure 7.

Very little uptake of permethrin occurred in plants grown in permethrin treated soil (Leahey and Carpenter 1980). However, permethrin metabolites were detected in the plants. Crops which were sown 30 days post-treatment (20 kg/ha) contained total residues of 0.86 ug/g edible tissue in mature plants. Of the residues transferred from the soil to plants, the products derived from the acid moiety (mainly conjugates) were of greater concentration than products from the alcohol moiety.

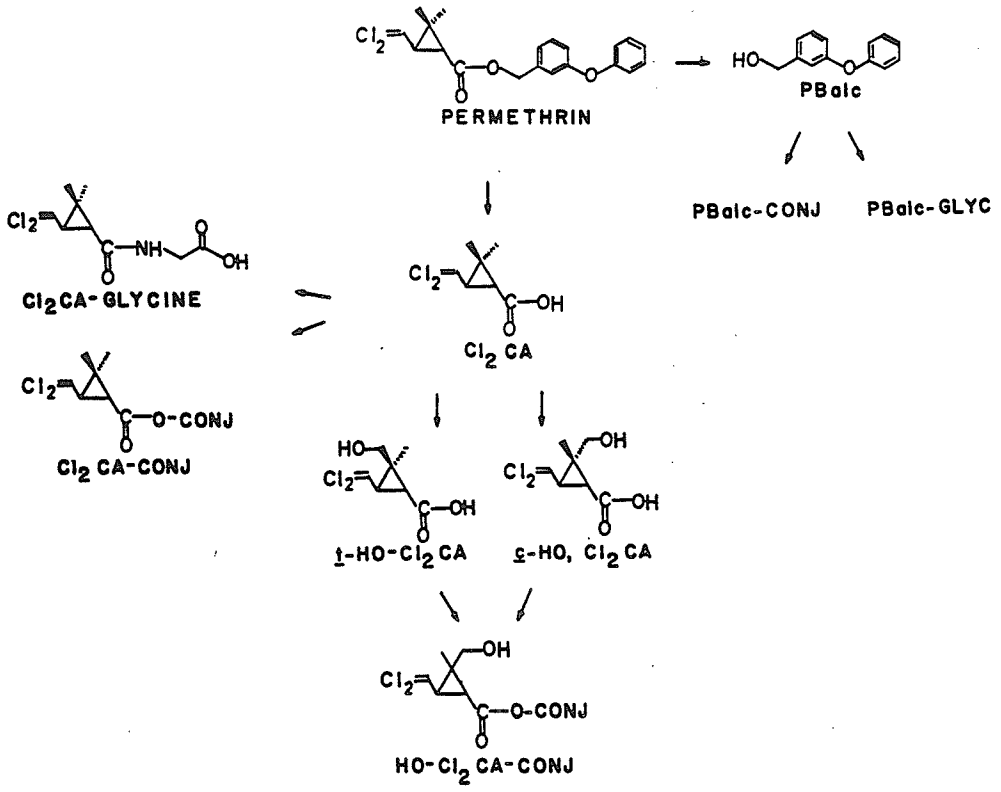


Figure 7: Permethrin metabolism in plants (Gaughan and Casida, 1978)

2.2.7 Soil

Degradation of permethrin in the soil has been found to be rapid with the trans-permethrin degrading more rapidly than the cis-isomer (Kaufman et al 1977). Volatility of permethrin or its degradation products from soil was found to be negligible. The degradation mechanism of permethrin in soil appears to be ester cleavage and hydroxylation with carbon dioxide as an end product. Soil sterilization tests have indicated that soil microbes are involved in permethrin degradation and carbon dioxide formation. Under anaerobic conditions (flooded soil), very little conversion of permethrin to carbon dioxide occurred, but polar metabolites were formed. In both aerobic and anaerobic soil (silty clay loam) the major portion of the ^{14}C residue was associated with the fulvic acid and humin fractions (Table 12).

TABLE 12

Distribution of non-extractable ^{14}C-permethrin in soil organic matter fractions of anaerobically incubated soil (Kaufman et al., 1977)

Label position	% ^{14}C in soil organic fraction		
	fulvic	humic	humin
30 days incubation			
Carbonyl	24.7	4.3	71.0
Methylene	33.4	11.2	55.4
60 days incubation			
Carbonyl	68.5	10.6	20.9
Methylene	55.9	19.9	24.3

Chapman et al. (1981) also noted the effect of soil sterilization upon the degradation rates of several pyrethroids. Table 13 indicates that the pyrethroids are more persistent in sterilized than in natural soil. The cis/ trans ratio for permethrin and cypermethrin was also monitored. The results (Table 14) indicate that the permethrin ratio remained constant while the cypermethrin ratio showed considerable variation.

TABLE 13

Effect of Soil Sterilization on Pyrethroid Degradation (Chapman et al., 1981)

	Percent of Initial Application (1 mg/kg) Remaining 8 wk After Application			
	Mineral		Organic	
Insecticide	Sterilized	Natural	Sterilized	Natural
Permethrin	101	6	100	16
Cypermethrin	93	4	92	16
Fenvalerate	91	12	100	58
Decamethrin	97	52	106	74
DDT	92	89	100	76

Kaneko et al. (1978) found that permethrin applied to soil did not leach and that the soil half-life for permethrin was 6-12 days.

Kaufman et al. (1981) also reported that decamethrin, cypermethrin, and permethrin were immobile in soil. However, the degradation products Cl_2CA, PBalc, and PBacid were all more mobile than any of the parent materials. Of the degradation products, PBalc was the least mobile with 96% of the ^{14}C activity being detected in the upper 13-15 cm for silty

TABLE 14

Cis/trans ratio of permethrin and cypermethrin residues in soil (Chapman et al., 1981)

Soil Type	Time (wk)	cis/trans ratio	
		Permethrin	Cypermethrin
Mineral	0	0.45	0.77
	1	0.63	1.11
	2	0.56	1.43
	4	0.56	0.91
	6	0.53	0.71
	8	0.53	0.71
Organic	0	0.38	0.71
	1	0.59	1.11
	4	0.59	1.43
	8	0.59	1.67
	16	NA	2.00

NA = Not analyzed.

clay and loamy sand soil. PBacid and Cl_2CA were very mobile in leaching studies. For example, in silty clay soil, it was calculated greater than 92% of the Cl_2CA would have reached 18-38 cm with 38.5% of the activity between 25.4 and 27.9 cm. PBacid was slightly less mobile than the Cl_2CA.

Carbon dioxide was also found to be an end product for cypermethrin degradation in soil (Roberts and Standen 1977). After 22 weeks, about 25-40% of the applied cypermethrin was accounted for as carbon dioxide. Under water-logged conditions, the acid moiety products accumulated in the soil as a result of the inhibition of further metabolite degradation to carbon dioxide.

Permethrin degradation in soil is illustrated in Figure 8.

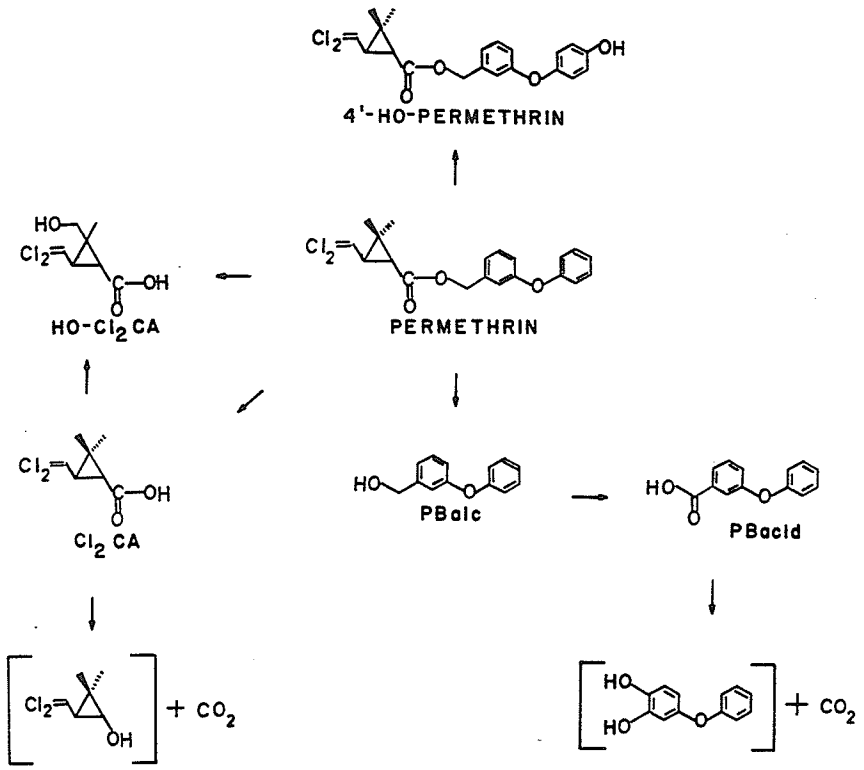


Figure 8: Permethrin degradation in soil (Kaufman et al., 1977 and Kaneko et al., 1978)

Appendix A lists the major and minor products of photolysis and metabolism of permethrin in cows, cockroaches, fish microsomes, plants, and soil.

2.3 PYRETHROID ANALYSIS

2.3.1 Thin-layer Chromatography

One method used to separate and identify the permethrin metabolites is thin-layer chromatography (TLC). Chipman Chemicals Ltd. (1978) reported that three solvent systems were needed to separate the cis- and trans-permethrin and its metabolites. The solvent systems used were hexane-ether (10:1) (HE), cyclohexane (saturated with formic acid)-ether (3:2) (CFE), and chloroform-ethyl acetate-methanol (6:3:1) (CEaM). The HE system was used to separate the permethrin isomers while CFE and CEaM were used to separate metabolites. Other solvent systems have also been reported. For example, photolysis products of permethrin were separated using TLC with the following solvent systems: carbon tetrachloride-benzene (4:1) (CB); benzene (saturated with formic acid)-ether (10:3) (BFE); chloroform (saturated with formic acid)-ether (10:3) (ChFE) (Holmstead et al. 1978).

Gaughan et al. (1978) used two-dimensional development of TLC plates for analysis of cis- and trans-permethrin and metabolites. The solvent systems used were as follows: for ester products, benzene-ethyl acetete (6:1) in the first direction and carbon tetrachloride-ether (3:1) in the second direction; for hydrolysis products and the oxidized derivatives and conjugates, 1-butanol- glacial acetic acid-water (6:1:1) in the first direction followed by two developments with benzene (saturated

with formic acid)-ether (10:3) or benzene (saturated with formic acid)-ether.

2.3.2 Gas-liquid Chromatography

To lower the detection limit of permethrin residues, methodology for cis-, trans-permethrin analysis by gas-liquid chromatography (GLC) was developed. Simonaitis and Cail (1977) used GLC with a packed column of 5% OV-225 and a flame ionization detector to separate and detect the cis- and trans-isomers.

Electron capture detectors (ECD) have also been used in permethrin analysis. Belanger and Hamilton (1979) used a ^{63}Ni-ECD to detect the cis- and trans-isomers separated on a column with a mixture of 1.5% OV-17 plus 1.95% OV-210. A column of 3% OV-17 did not separate the isomers. A method of permethrin analysis developed by ICI (1977) also used a ^{63}Ni-ECD for permethrin detection following separation of the isomers on a 5% OV-210 column. A ^{3}H-ECD was used by Chiba (1978) for permethrin isomer detection following separation on a 5% QF-1 column.

2.3.3 High-pressure Liquid Chromatography

High-pressure liquid chromatography (HPLC) has been another analytical technique used for permethrin analysis. Lam and Grushka (1978) developed HPLC analysis of cis- and trans-permethrin, phenoxybenzyl alcohol, and phenoxybenzoic acid by using a reversed phase column and a solvent system of acetic acid-chloroform-methanol-water (1:5:20:74). The UV detector was set at 254 nm. Cis- and trans-permethrin were also separated on a normal phase column with a mobile phase consisting of

hexane-isopropyl ether (90:10) (Mourot et al. 1979). Detection of the isomers was by UV at 235 nm.

In addition to UV-HPLC, infrared detection can be used in conjuction with HPLC for permethrin analysis (Papadopoulou-Mourkidou et al. 1980). The cis- and trans-permethrin isomers were separated on a Partisil column with a mobile phase of 1% acetonitrile in carbon tetrachloride. At 8.65 microns the minimum detectable level of permethrin for the infrared detector was 1 ug compared to the UV detector (280 nm) limit of 0.1 ug.

2.4 PESTICIDE VOLATILIZATION FROM AQUATIC SURFACES

In studying volatilization from a water surface, certain assumptions must be made. One assumption is that the gas exchange occurs by molecular diffusion through a two-layer gas-liquid interface. The diagram below illustrates Liss and Slater's (1974) model for transport (Figure 9).

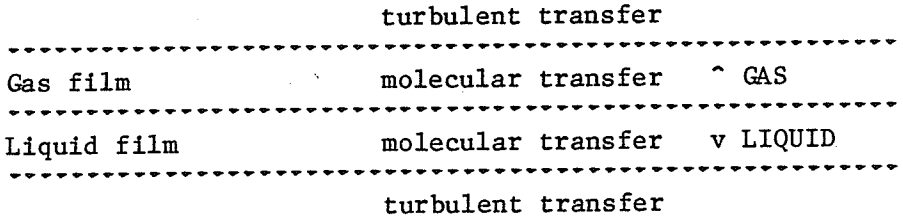


Figure 9: Gas exchange model (Liss and Slater, 1974)

Since the transport through the layer system is by molecular diffusion, Fick's first law is applicable.

$$F = -D \, \partial c/\partial z \qquad (1)$$

where F is flux of gas through layer; D, the coefficient of molecular diffusion of gas in layer material; c, the gas concentration; and z, the

vertical direction in the one-dimensional form. For gas exchange studies (1) is usually written in the form of,

$$F = k \Delta c \qquad (2)$$

Where Δc is the concentration difference across the layer. Assuming that gas transport is a steady state process and that exchanging gas obeys Henry's law (H),

$$F = (c_G - HC_L)/(1/k_G + H/k_L) = (c_G/H - C_L)/(1/k_L + 1/Hk_G) \qquad (3)$$

where k_G and k_L are the exchange constants for the gas and liquid phases, respectively. Equation (3) can then be rewritten as

$$F = K_G(c_G - HC_L) = K_L(c_G/H - C_L) \qquad (4)$$

where

$$1/K_G = 1/k_G + H/k_L \qquad (5)$$

and

$$1/K_L = 1/k_L + 1/Hk_G \qquad (6)$$

Thus the total resistance expressed on either a gas phase $(1/K_G)$ or a liquid phase $(1/K_L)$ basis depends on the exchange constants of the individual phases and the value of the Henry's Law constant for the gas concerned.

The values used by Liss and Slater (1974) for the vapour phase mass transfer rate $(k_{G(water)})$ were 30 m/h and 0.2 m/h for the liquid phase mass transfer rate (k_L). For gases other than water and carbon dioxide (k_L), the following formulae can be used.

$$k_G = (k_{G(water)}) \sqrt{\frac{\text{mol. wt. water}}{\text{mol. wt. compound}}}$$

$$k_L = (k_{L(CO_2)}) \sqrt{\frac{\text{mol. wt. carbon dioxide}}{\text{mol. wt. compound}}}$$

Mackay and Leinonen (1975) combined the two mass transfer coefficient rates to obtain the overall liquid coefficient K_L (m/h).

$$1/K_L = 1/k_L + RT/Hk_G \qquad (7)$$

where T is the absolute temperature (K) and R is the gas constant.

The mass flux, N_i, across the phase boundry can be given in terms of the bulk liquid concentration and the partial pressure in the atmosphere (P_i).

$$N_i = K_L(C_i - P_i/H_i) \text{ mol/m}^2\text{h} \qquad (8)$$

Combining (8) with a mass balance equation leads to

$$dC_i/dt = -K_L(C_i - P_i^*/H_i)/L \qquad (9)$$

The integration of (9) expresses the concentration of the compound as a function of time (10) where C_i is the concentration at time t and C_{i0} the concentration at zero time and depth L.

$$C_i = P_i/H_i + (C_{i0} - P_i/H_i) \exp(-K_L t/L) \qquad (10)$$

Since P_i in the atmosphere is negligible compared to the local level, (10) becomes

$$C_i = C_{i0} \exp(-K_L t/L) \qquad (11)$$

Thus, the total loss from the pond at time t can be calculated as $(C_o - C_i)$ (area) (depth).

The time required for the concentration to drop to half its original value as the result of volatility is

$$t_{1/2} = 0.69\ L/K_L$$

2.5 MODEL ECOSYSTEMS

In order to maintain environmental quality, it is important to know the fate of the many industrial and agriculture chemicals introduced into our ecosystem. Since it is not practical to contaminate large areas of the environment to study the ecotoxicology of each chemical, it is necessary to perform small scale experiments first. These model ecosystems can be a useful screening method for evaluating the environmental toxicology of new chemicals. The design of model ecosystems is greatly varied and can range in size from aquariums to small lakes.

In 1971, Metcalf et al. described a small laboratory model ecosystem in a glass aquarium (25 x 30 x 51 cm) which included a terrestrial-aquatic interface and a seven-element food chain. The model simulated the application of pesticides to crop plants and the eventual contamination of the aquatic environment. Such studies provided information on the environmental degradation pathways of the pesticides and the toxicity of the parent compound and degradation products to typical aquatic organisms. Experimental results indicated that aquarium systems were reproducible and that the data produced was similiar to the results ob-

served in nature. Similiar aquarium model ecosystems have been used to study desorption of herbicides from soil into water (Kearney et al. 1977). Metcalf (1977) reviewed the literature on laboratory model ecosystems.

To duplicate environmental conditions as closely as possible in a model, Hurlbert et al. (1970) used a series of ten outdoor experimental ponds, each ca. 8 x 17 m in size, to study the biological effects and persistence of chlorpyrifos in freshwater ponds. The use of outdoor ponds and the subsequent exposure to sunlight and other weather elements more closely resembles environmental conditions yet provides the necessary samples (water, sediment, vegetation, vertebrates, and invertebrates) to monitor the fate of the compound. Boyle (1980) also used experimental ponds (0.08 ha) to study the effects of aquatic herbicides on pond ecology. Dixon and Brust (1971) used experimental ponds which were lined with polyethylene and covered with sod to simulate an Aedes breeding site for testing larvicides.

It is not always necessary to build experimental ponds. Klaassen and Kadoum (1979) used four farm ponds that naturally occurred in a pasture to study the fate of atrazine and carbofuran in an aquatic system. The advantage of using natural ponds is that the various biological components of the system are of native origin and need not be from laboratory cultures added to the pond.

To study the fate and the factors which control the distribution and persistence of pesticide residues in a lentic system, Hamelink and Waybrant (1976) used a flooded limestone quarry as a large scale model ecosystem. Small scale systems cannot reproduce the temperature cycling or

the sediment-water and air-water interface ratios that occur in large natural lakes.

2.5.1 Glenlea Ponds

In 1975, Rawn et al. (1978) established temporary outdoor pools (one metre square) at the Glenlea Research Station to study the effect of pool bottom substrate on residues and bioactivity of chlorpyrifos. The pools consisted of a wood frame lined with polyethylene and aluminum foil and the bottom of each pool was covered with one of three substrates sod, clay, or sand.

A more complex model ecosystem which included natural fauna and flora was established at Glenlea by Madder and Lockhart (1980). A series of ponds (5 x 3 x 0.5 m) were excavated and lined with polyethylene and 12 cm of sod. A year after construction, by which time the fauna and flora were established, the ponds were used to study the dissipation of insecticides from the pond water and their effects on non-target organisms.

Muir et al. (1980-B) used ponds at Glenlea, which were constructed in a similar manner to those of Madder and Lockhart (1980), to test the persistence of fluridone herbicide in ponds. Samples of water, hydrosoil, duckweed, and fathead minnows were collected for residue analysis. Muir et al. (1980) conducted a similar study in the ponds using phosphate esters.

Malis and Muir (1980) have also used Glenlea ponds to study the fate of fenitrothion in small pond systems. The half-life of fenitrothion in the ponds was found to be 0.8 days which is close to the value of 0.45 days obtained by Roberts et al. (1981) based on a computer model using first-order kinetics.

2.6 SIMULATION MODELLING OF PESTICIDE DISTRIBUTION IN PONDS

Neely and Blau (1977) used a mathematical equation to predict the loss of chlorpyrifos from pond water as well as uptake by fish. Their model was based on a three component system of water, fish, and soil and plants. Based on the rate constants for the above system, an equation was developed to predict the chlorpyrifos concentrations in the water and fish. The predicted results were close to the values observed in an actual pond experiment.

Roberts et al. (1981) developed a more complex mathematical equation to describe pollutant concentrations in various components (water, sediment, suspended organic matter, and biota) of an aquatic system.

Marshall and Roberts (1977) also developed a simulation model of the distribution of pesticides in ponds based on the dynamics of an insecticide in a well mixed pond system.

The distribution of the insecticide in water and hydrosoil was predicted by the following equations.

$$V_w \, dC_w/dt = k_{hw}V_hC_h - k_{wo}V_wC_w - k_{wh}V_hC_w$$

$$V_h \, dC_h/dt = k_{wh}V_hC_w - k_{hw}C_hV_h - k_{ho}V_hC_h$$

The definitions and solutions to the above equations are shown in Appendix B.

Chapter III

EXPERIMENTAL

3.1 CHEMICALS

Imperial Chemical Industries (ICI, Jealott's Hill, Bracknell, Berkshire, England) provided ^{14}C labelled permethrin as cis-permethrin (^{14}C-methylene, sp. act. 59.7 mCi/mM), trans-permethrin (^{14}C-methylene, sp. act. 59.7 mCi/mM), cis-permethrin (^{14}C-cyclopropane, sp. act. 50 mCi/mM), trans-permethrin (^{14}C-cyclopropane, sp. act. 50 mCi/mM). Prior to use, the ^{14}C labelled permethrin was purified by TLC using solvent system HE described below. Also provided was unlabelled permethrin (94.7% purity), permethrin EC (10% w/v) formulation and analytical standards of trans-and cis-permethrin, 3-(2,2-dichloroethenyl)-2,2-dimethylcyclopropane-1-carboxylic acid, cis isomer (referred to as cis-cyclopropyl acid or c-Cl_2CA) and its trans-isomer (referred to as trans-cyclopropyl acid or t-Cl_2CA), 3 phenoxybenzyl alcohol (PBalc), and 3 phenoxybenzoic acid (PBacid).

All solvents used were of pesticide grade quality (Caledon Ltd., Georgetown, Ontatio).

Methoxy-ethanolamine (CO_2-M-Met) and PCS cocktail were purchased from Amersham Radiochemicals, Oakville, Ontatio.

3.2 APPARATUS

The GLC used for the photolysis study was a Tracor 220 instrument with a linearized ^{63}Ni electron capture detector.

The HPLC used for permethrin analysis in environmental samples was a Waters Associates model 6000 A pump, model 440 UV detector (280 nm), and a μBondapak C_{18} column.

The liquid scintillation counter was a Beckman LS-7500 instrument.

TLC analysis used chromatoplates (20 x 20 cm) precoated with silica gel UV-254 (fluorescent indicator) (0.25 mm) (Brinkmann Instruments, Rexdale, Ontario).

Cellulose extraction thimbles (25 x 80 mm) were purchased from Whatman, W & R Balston Ltd., England.

Stainless steel ball-mill tubes were constructed by J. Solomon, Winnipeg (Solomon and Muir 1981).

3.3 ANALYTICAL METHODS

3.3.1 Gas-liquid Chromatography

Unlabelled trans- and cis-permethrin from the photolysis study were analyzed by GLC utilizing a Tracor 220 with a ^{63}Ni ECD and a glass column (1.2m x 4mm i.d.) with 5% OV-210 on Chromosorb W-HP (100-120 mesh). To improve isomer separation, glass wool was not used at the injector end of the column. GLC operating conditions were as follows: injector, 235°C; detector, 350°C; column, 220°C with a flow rate of 40 mL/min (5% methane in argon); retention time for cis-/ trans-permethrin was 4.0/4.6 min, respectively.

3.3.2 High-pressure liquid chromatography

Labelled trans- and cis-permethrin from the environmental samples were separated by HPLC and analyzed by LSC. The HPLC method was similiar to that reported by Lam and Grushka (1978). The solvent flow rate was 1.5 mL/minute, the solvent consisting of 10 mL formic acid, 50 mL chloroform, 188 mL water, and 752 mL methanol. Elution time was 10.0 minutes and 11.9 minutes for the trans- and cis-isomers, respectively. After sample injection, fractions (0.75 mL) were collected in scintillation vials (total elution volume was 24 mL) for LSC.

3.3.3 Thin-layer Chromatography

Cis- and trans-permethrin and degradation products were separated by TLC (Chipman Chemicals 1978) developed in the following solvent systems: hexane-ether (10:1) (HE); cyclohexane (saturated with formic acid)-ether (3:2) (CFE); chloroform-ethyl acetate-methanol (6:3:1) (CEaM). Radioactive silica gel regions detected by radioautography were scraped from the glass plate and the fraction recovered by extraction of the silica gel with ethyl acetate followed by LSC of the extract. Labelled degradation products were identified by TLC cochromatography with standards in each of the solvent systems.

3.3.4 Oxidation

Total ^{14}C content in field samples was determined by combustion on a Packard 306 oxidizer. Duplicate samples (0.2-0.6 g) were oxidized and $^{14}CO_2$ was trapped in CO_2-M-Met, diluted with PCS-xylene (2:1) followed by LSC.

3.3.5 Calculations of Scintillation Counter Results

Prior to the addition to the ponds, the specific activity of the spiking solution was determined to be 6.3 disintegrations per minute (dpm)/ng for the cyclopropyl labelled permethrin and 5 dpm/ng for the methylene labelled permethrin. The dpm results from the scintillation counter for permethrin residues were then expressed as ng/g by multiplying the dpm by the specific activity and correcting for dilutions. Results for total ^{14}C residues and metabolite residues were expressed as permethrin equivalents. The value obtained is not the concentration of the compound but represents the amount of labelled permethrin from which the product was derived.

3.4 EXTRACTION PROCEDURES

3.4.1 Water

Permethrin and metabolite extraction from the water was initiated in the field immediately after the water sample (1 L) was collected, by the addition of 25 mL of dichloromethane (methylene chloride). In the laboratory the water sample was transferred to a 2 L separatory funnel and the pH adjusted to pH 1 with 6M sulfuric acid. Following extraction with dichloromethane (25 mL x 3) the organic extract was dried (sodium sulfate) and reduced (roto-evaporator) to about 1 mL and stored at 4°C while awaiting analysis.

3.4.2 Hydrosoil

Hydrosoil samples were extracted using a reflux technique (Chipman Chemicals 1978). The hydrosoil sample was weighed into a cellulose extraction thimble and refluxed for 18 h in hexane-acetone (80:20, 150 mL) and a further 18 h in methanol-water (1:1, 150 mL).

After refluxing, the hexane-acetone extract was reduced in volume by roto-evaporation and transferred to a separatory funnel containing 30 mL distilled water which was extracted as in 3.4.1.

Following the reflux extraction, the methanol from the methanol-water extract was removed by roto-evaporation. The remaining water was extracted as outlined in 3.4.1.

3.4.3 Duckweed

The duckweed (Lemna minor) samples were extracted by the ball-mill technique (Grussendorf et al. 1970). Duckweed samples (8-10 g wet weight) were weighed into 30 x 103 mm stainless steel tubes containing two steel balls. After the addition of hexane-acetone (80:20, 25 mL) the tubes were placed on a wrist-action shaker for 30 min and then centrifuged in the tubes for 5 minutes. The organic extract was recovered by decanting into a graduated cylinder (volume recorded) followed by drying (sodium sulfate) and volume reduction (roto-evaporation) before the extract was stored while awaiting analysis.

3.4.4 Fathead minnows

Fathead minnows (Pimephales promelas) were also extracted by the ball-mill technique. One to three minnows were sliced into small pieces with a scapel and placed in a stainless steel tube and extracted in a manner similiar to that described for duckweed (3.4.3).

Prior to analysis, the fish extract required clean-up by the following procedure: the fish extract (hexane-acetone) was taken to almost dryness (nitrogen) and dissolved in 0.5 mL 2% ethyl acetate-hexane and introduced onto a micro column (Pasteur pipette) containing 5 cm 5% deactivated Florisil packed in hexane. The column was eluted with 2% ethyl acetate-hexane; the 0-3 mL fraction was collected for analysis.

3.5 POND CONSTRUCTION

The field testing of permethrin was conducted in artificial temporary ponds (5 x 3 m) constructed in 1975 at the University of Manitoba's Glenlea research station (Madder 1978). Holes were machine excavated and lined with polyethylene (10 mil). On the bottom of the pond a layer of 2-5 cm of sand was spread over the polyethylene. Sod, to a depth of 15 cm, was then placed over the polyethylene and sand. The water level in the ponds was maintained at a depth of 38 cm throughout the experiment by the addition of run-off water pumped from a nearby man-made pond (dugout). Table 15 lists the pond water characteristics at the time of permethrin application.

Natural flora and fauna were allowed to develop in and around the pools and were kept under control by periodic cutting and pond maintenance.

TABLE 15

Pond water characteristics at time of permethrin application

Characteristic	Pond					
	1979			1980		
	2	3	7	2	5	9
Total suspended solids (mg/L)	8	5	2	4	13	11
Chlorophyll-α (ug/L)	54	14	5	7	65	38
Suspended carbon (mg/L)	3.3	1.5	1.1	2.4	3.6	3.8
pH	8.2	8.1	8.1	7.7	7.4	8.9

3.6 PRELIMINARY STUDIES

3.6.1 Dursban Application to Ponds (1978)

In 1978, one year prior to the permethrin application to the Glenlea ponds, separate ponds were treated with Dursban 2.5G (chlorpyrifos), an organophosphorus mosquito larvicide, to establish the bioassay and sampling techniques. Four ponds were treated at the rate of 0.028 kg/ha. Following the application of the Dursban, water samples were collected for bioassay with Aedes aegypti larvae and residue analysis by HPLC.

The chlorpyrifos analysis consisted of solvent extraction with dichloromethane and HPLC analysis with a UV detector operated at 280 nm. Separation was carried out on a µPorasil column using 2.8% acetic acid in hexane at a flow rate of 1.2 mL/minute with a chloropyrifos retention time of 3.68 min.

3.6.2 Permethrin Photolysis in Water

Individual cis- and trans-permethrin isomers in water were exposed to sunlight to determine the effect of photolysis on permethrin. Two sets of nine 1000 mL erlenmeyer flasks (pyrex glass) were filled with 800 mL of pond water. To each flask in one set, 9.11 ug of cis-permethrin (analytical standard) in 1.0 mL acetone was added to give a concentration of 11.4 ug/L. One foil wrapped flask served as a darkened control. To each flask in the other set was added 8.10 ug (10.1 ug/L) of trans-permethrin (analytical standard). Immediately following treatment the flasks were partially submerged in an outdoor artificial pond so that the bottom 5-8 cm of eack flask was immersed in water. At 0, 1, 6, 12, 24, 48, 96, and 144 h (light) and 144 h (foil-wrapped) post-treatment, one flask from each set was removed for permethrin extraction and analysis. Permethrin was extracted from the water as outlined in 3.4.1. The organic extract was analyzed for residues of the permethrin isomers by GLC analysis.

3.7 POND TREATMENT

Prior to the treatment of the ponds, the purified ^{14}C-methylene labelled cis- and trans-permethrin were combined in a 40:60 ratio respectively, as was the ^{14}C- cyclopropyl labelled cis- and trans-permethrin. Therefore, distinct ^{14}C labelling on each side of the permethrin molecule was obtained (Figure 10).

In 1979 and 1980 two ponds were treated with permethrin emulsifiable concentrate (10% w/v) (cis: trans ratio of 40:60) at the rate of 0.028 kg /ha or 15 ug/L (15 ppb). The formulated material was spiked with 69

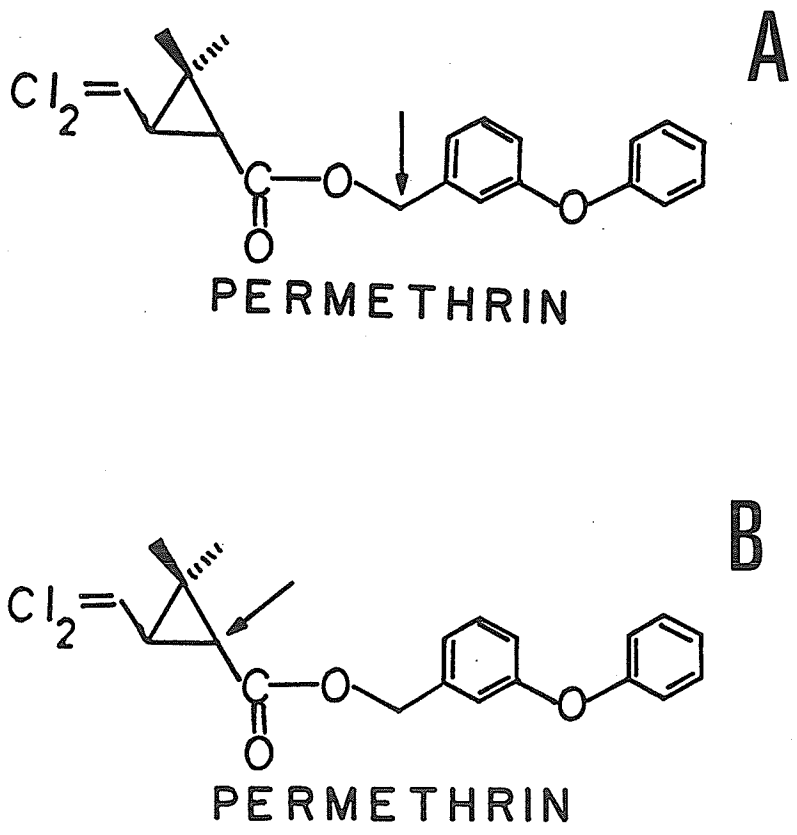


Figure 10: ^{14}C label position in permethrin molecule, methylene position (A) and cyclopropyl position (B)

or 88 uCi of ^{14}C-permethrin labelled in either the methylene or cyclopropyl group, respectively. A third pool served as a non-treated control. Appendix F lists the pond treatments.

The mixture of permethrin formulation and ^{14}C-permethrin was applied to each pond from a pipette held just above the water surface. The pipette was moved over the pond to distribute the permethrin as evenly as possible.

In 1979, ponds 3 and 7 were treated with permethrin on August 8, pond 2 was left as a non-treated control. Samples of water, hydrosoil, duckweed, and fathead minnows were collected at frequent intervals for 29 days post-treatment. Separate sampling apparatus was used for each pond to prevent cross-contamination. On June 17, 1980 permethrin was applied to ponds 2 and 9 while 5 was left non-treated. Sampling of the ponds continued for 16 weeks post-treatment. Table 16 outlines the sampling schedule.

TABLE 16

Sampling schedule of ponds in 1979 and 1980

	1979					1980			
Time	water	hydro soil	duck weed	fish*	bio assay	water	hydro soil	duck weed	fish*
-0 h	"	"	"	"	"	"	"	"	"
+0 h	"				"				
2 h						"	"	"	9
4 h	"	"	"						
8 h	"								
12 h	"	"	"		"				
18 h	"								
24 h	"	"	"		"	"	"	"	9
48 h	"	"	"		"	"	"	"	9
72 h	"	"	"		"				9,2
96 h	"	"	"	3,7	"				
7 d	"	"	"		"	"	"	"	9,2
11 d	"	"	"	3,7					
14 d	"	"	"	3,7					
15 d						"	"	"	9,2
21 d	"	"	"	3,7		"	"	"	9,2
28 d						"	"	"	9
29 d	"	"	"	3					2
5 w						"	"	"	9,2
6 w						"	"	"	2
8 w						"	"	"	9
10 w						"	"	"	9
12 w						"	"	"	2
16 w						"	"	"	
37 w		""							
47 w							""		
52 w		""							
60 w		""							

* numbers under fish indicate the pool in which the fish were caught.

"" hydrosoil samples (37,47,52,60 weeks) were taken as core samples from the pond bottom.

3.8 SAMPLING

3.8.1 Water

In 1979, the water was sampled by compositing ten 100 mL random dip surface samples from each pond from which 100 mL was then removed for the bioassay test. To the remaining 900 mL, dichloromethane was immediately added to preserve the sample and commence extraction. The sampling schedule was outlined in Table 16.

In 1980, surface water (as obtained in 1979) and subsurface water were collected. The subsurface sample was obtained by submerging a one litre bottle in the pond. The lid of the bottle had two pieces of glass tubing in it, a short piece to let the water in and a longer piece to allow the air in the bottle to escape. As in 1979, dichloromethane was added to the samples immediately after collection.

3.8.2 Hydrosoil

Four days prior to the permethrin application to the ponds, 455 mL wide-mouth jars were filled with soil and placed on the bottom of each pond. The soil placed in the jars was subsoil previously excavated during the pond construction. The soil had the following characteristics: pH, 7.5; organic matter, 3.4%; component analysis, 3% sand, 29% silt, 68% clay. At each sampling time (Table 16) four jars per pond were removed to provide samples of hydrosoil for residue analysis. From each set of four jars the top 0.5 cm of hydrosoil was collected and combined and stored at -40°C while awaiting extraction and analysis by HPLC.

3.8.3 Duckweed

Duckweed samples were collected with a dip screen from the pond surface at each sampling time (Table 16). The vegetation samples were stored at -40°C while awaiting extraction and analysis by HPLC.

3.8.4 Fathead Minnows

Papers in the literature have reported high toxicity to fathead minnows of permethrin (LC_{50} = 2.6 ug/L, Chipman Chemicals 1978). Since the application rate of permethrin to the ponds was 15 ug/L, a level predicted to be toxic to the fish, fathead minnows were not added to the ponds until three days post-treatment.

In 1980, fathead minnows were unintentionally added to pond 9 prior to the permethrin application. Fish samples were collected at 2, 24, and 48 h post-treatment. After the collection of the 48 h sample, the remaining small number of fish were removed from pond 9. At 72 h post-treatment, as in 1979, both ponds were stocked with fathead minnows.

Sampling of the fish was conducted using a minnow trap from which a maximum of three fish were taken at each sample period (Table 16). The fish were stored at -40°C while awaiting extraction and analysis.

3.9 BIOAVAILABILITY (BIOASSAY)

In 1979 the presence of bioactive compounds in the water of the permethrin treated ponds was determined by bioassays conducted in the laboratory using fourth instar larvae of laboratory reared Aedes aegypti mosquitoes. At each sampling time, a 100 mL aliquot of a one litre water sample was returned to the laboratory and 25 larvae were added to

the sample. The samples were then stored in the dark at room temperature for 24 hours. After the exposure period the percent mortality of the larvae was recorded.

3.10 REARING OF AEDES AEGYPTI

The Aedes aegypti (Diptera, Culicidae) larvae used in the bioavailability tests were laboratory reared from a colony obtained from the Department of Entomology, University of Manitoba. The female adult mosquitoes were blood fed on caged mice placed in a one cubic foot plexiglass cage. Two to three days post-blood-meal, the females would begin to lay eggs which were collected on paper towel water wicks placed inside the plexiglass cages. The eggs were stored on the water wicks until needed. To induce hatching the eggs were transferred from the wick to a small beaker to which two or three drops of a liver powder (ICN Pharmaceuticals Inc., Cleveland) water suspension had been added. At room temperature the eggs usually hatched within one day. After hatching, about 200 larvae were transferred to a large plastic tray to complete their larval development. This cycle produced a large supply of eggs and, after rearing, a daily supply of fourth instar larvae which were used for the bioavailability test.

Chapter IV

RESULTS AND DISCUSSION

4.1 PERMETHRIN PHOTOLYSIS IN WATER

Results of the photodegradation study (Figure 11) indicate that a number of reactions including photolysis, photoisomerization, and unknown degradation mechanism(s) occurred when permethrin was dissolved in water and exposed to sunlight.

The trans-permethrin degraded rapidly during the first 24 h and generated an approximate first order plot over the first six days. Isomerization of the trans-isomer to the cis-isomer occurred rapidly during the first six hours in the illuminated flasks. In the darkened flasks, no isomerization was detected and thus the conclusion can be made that the conversion of the trans- to the cis-isomer was photo-induced. Appendix G shows the UV spectrafor the permethrin isomers. Holmstead et al. (1978) recorded similar results of rapid isomerization and degradation of permethrin in water exposed to artificial light (> 290 nm). The photoisomerization may have an effect on permethrin bioactivity in the water since the cis-isomer has been reported to be more toxic to insects than the trans-isomer (Bigley and Plapp 1978).

The cis-isomer, present as the result of photoisomerization, followed an approximate first order decay plot from day one to day six. However, the trans-permethrin degraded somewhat more rapidly than the cis-isomer. With an initial concentration of 10.1 ug/L trans-permethrin and follow-

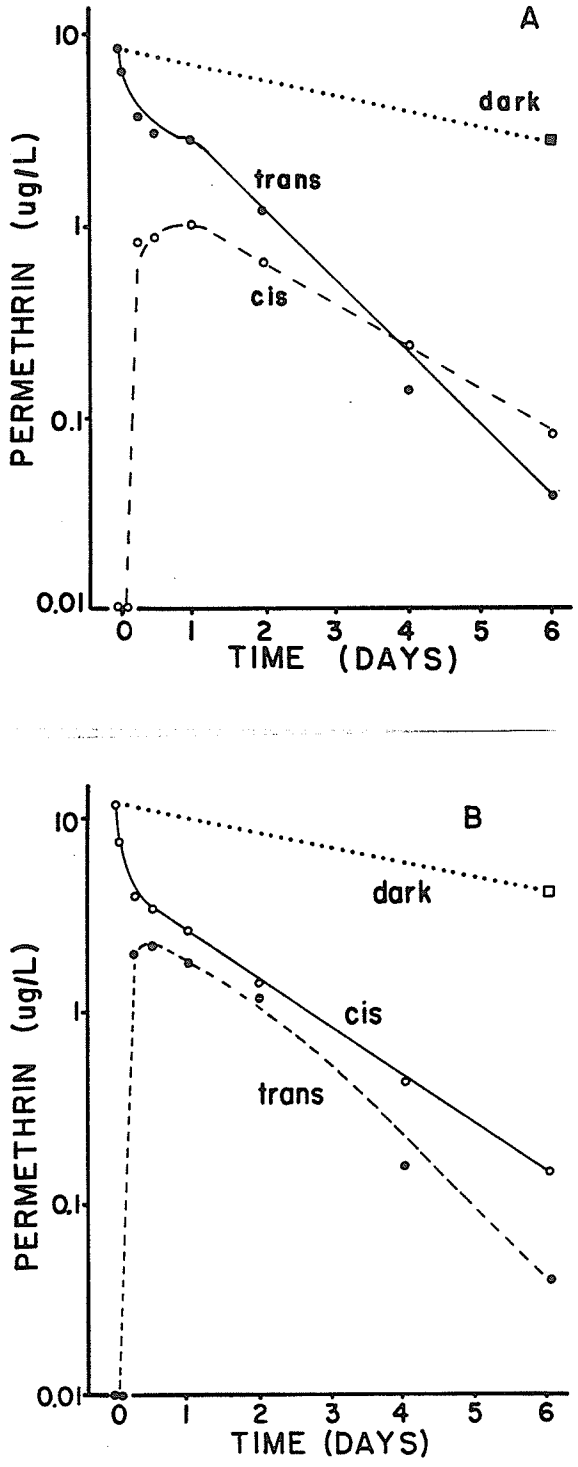


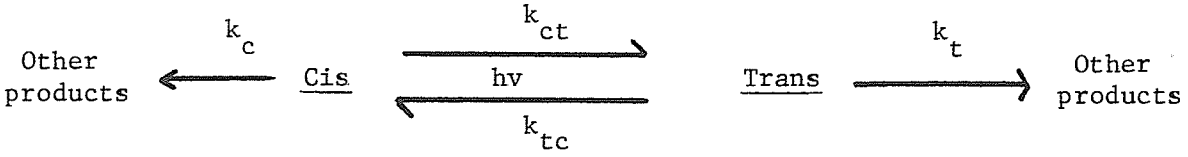
Figure 11: Photolysis of trans (A) and cis (B) permethrin in pond water

ing six days of exposure, 0.04 ug/L trans-isomer and 0.08 ug/L cis-isomer remained in the illuminated flask. After six days, the darkened flask contained 2.7 ug/L of the trans-permethrin, no cis-permethrin was detected. Thus, 73% of the applied trans-permethrin was lost by some mechanism other than photolysis.

Cis-permethrin also degraded rapidly in water in much the same way as the trans-isomer had done (Figure 11-B). Photoisomerization of the cis-isomer to the trans-isomer occurred rapidly during the first six hours in the illuminated flask; no isomerization was detected in the darkened flask. Again, the trans-permethrin degraded more rapidly than the cis-permethrin, and, at the end of six days, 0.15 ug/L cis-isomer and 0.04 ug/L trans-isomer remained from an initial concentration of 11.1 ug/L cis-permethrin. In the darkened flask, only the cis-isomer was detected, where the concentration had dropped from 11.4 to 4.2 ug/L.

In a number of biological systems (insects, Bigley and Plapp 1978; plants, Gaughan and Casida 1978) the trans-permethrin was more labile than the cis-isomer as occurred in the photolysis study. In the photolysis experiment, the half-life (Table 17) for the trans-isomer was 19.6 $\pm$ 2.3 h compared to 27.1 $\pm$ 4.4 h for the cis-isomer, which agrees with the results reported for biological systems. To determine if the degradation rates of the permethrin isomers (both original and photo-produced) were similiar or not, the regression lines from Figure 11 were subjected to an analysis of covariance (Table 18). The reaction path-

ways are illustrated below.



k_{ct} = photoisomerization of cis- to trans-permethrin
k_{tc} = photoisomerization of trans- to cis-permethrin
k_t = trans-isomer degradation by photochemical and other processes
k_c = cis-isomer degradation by photochemical and other processes

The analysis shows that there is a significant difference (0.005%) between the degradation rates of the two isomers. Therefore, it appears that the trans-permethrin is more readily attacked by a variety of mechanisms than the cis-permethrin. The results for the other isomer combinations indicate that a number of factors are involved in the degradation of the original and photo-produced isomers. Besides the differences in the rate of isomer degradation, the rates are affected by the fact that the photo-produced isomers are being produced by isomerization as well as being degraded at the same time. Thus, the degradation rate for the photo-produced isomers is a function of isomer production and degradation.

The loss of 63-73% of the permethrin in the darkened flasks indicated that photolysis was probably not the most important loss mechanism. Mechanisms such as hydrolysis, microbial degradation, or algal degradation may be occurring in the water.

TABLE 17

Photolysis half-lives of permethrin isomers in water

Compound	Half-life (h)
cis-permethrin	27.1 ± 4.4
trans-permethrin from cis	22.9 ± 5.6
trans-permethrin	19.6 ± 2.3
cis-permethrin from trans	38.8 ± 4.4

Confidence intervals are set at the 0.05 level.

TABLE 18

Analysis of covariance of the rate of permethrin degradation by photolysis

Comparison	F (slopes)	DF	Significance (%)
$(-k_c-k_{ct})$ / $(-k_t-k_{tc})$	16.64	1,10	0.005
$(-k_c-k_{ct})$ / $(k_{tc}-k_c)$	9.22	1,9	0.05
$(-k_t-k_{ct})$ / $(k_{ct}-k_t)$	3.22	1,9	NS
$(-k_c-k_{ct})$ / $(k_{ct}-k_t)$	2.73	1,9	NS
$(-k_t-k_{tc})$ / $(k_{tc}-k_c)$	45.23	1,9	0.005

negative k's refer to losses, positive to gains.

4.2 PERMETHRIN BIOAVAILABILITY IN WATER

Figure 12 shows the average mortality of the A. aegypti larvae in the bioassay tests on the water from the two ponds treated with permethrin EC (0.028 kg/ha). The average concentration of permethrin in the water of the two ponds, derived from TLC separation, autoradiography, and LSC is also shown. The bioassay results indicated 100% mortality of mosqui-

to larvae immediately and for 12 h post-treatment. However, the bioavailable toxic residue decreased very quickly yielding 4% mortality at 48 h and 0% at 72 h. The initial concentration of 15.5 ug/L decreased very quickly to 1.5 ug/L by 18 h and 0.04 ug/L at 72 h post-treatment.

A one to two day period of larval control was similiar to that reported by Rettich (1980) but shorter than the results of Mulla et al. (1978,1980). The shorter period of larval control in the Glenlea ponds may be due to a difference in susceptibility of the larvae tested or differences in pond components such as soil and vegetation and their ability to sorb the permethrin. However, the application rate of 0.028 kg permethrin/ha was effective as a larvicide against Aedes larvae.

Results from the preliminary study with Dursban 2.5G provided a comparison of bioavailability and rate of loss from the water between an organophosphate and a pyrethroid insecticide. The Dursban results (Table 19) indicated that Dursban maintained 96-100% larval control for 24 h post-treatment, 49% at 96 h and 0% by 240 h post-treatment. Permethrin larval control had reached 0% by 72 h. The half-life for Dursban in the water was 75.9 $\pm$ 0.3 h compared to 8.5 $\pm$ 2.3 h for permethrin. Residue values of chlorpyrifos in the water ranged from 4.1 ug/L at two hours and 0.5 ug/L at 240 h post-treatment. Thus, at similar rates of application, the granular Dursban formulation resulted in a longer period of larval control with less active ingredient biologically available in the water than resulted from the permethrin application. However, whereas permethrin was not detectable in the water beyond 72 h post-treatment, the chlorpyrifos was detected at 0.5 ug/L at 240 h post-treatment.

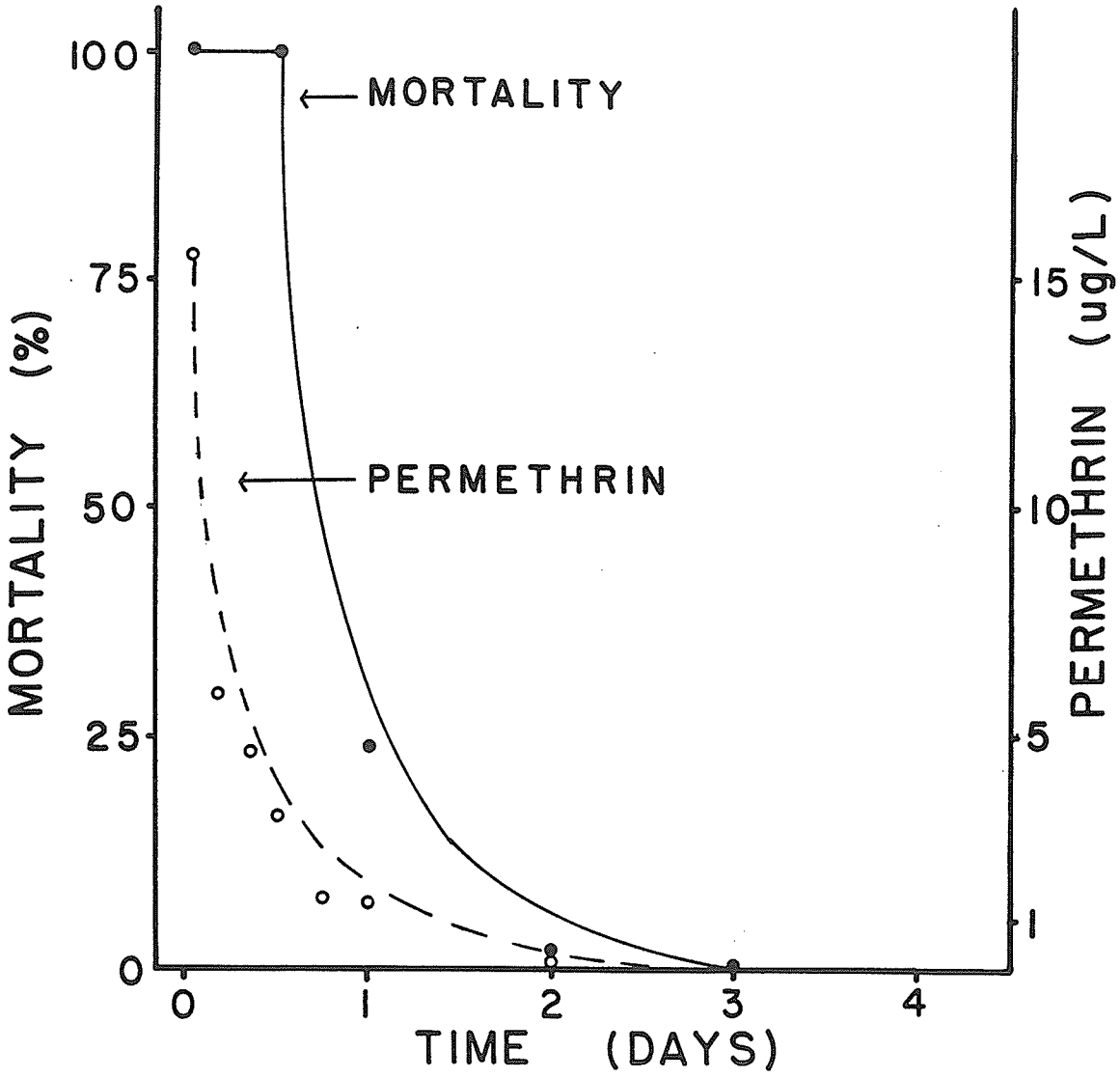


Figure 12: Bioavailability of permethrin in pond water, 1979

TABLE 19

Bioavailability of Dursban (chlorpyrifos) in Glenlea ponds

Time (h)	Mortality (%)	Chlorpyrifos (ug/L)
2	100	4.1
4	100	4.0
8	96	4.0
12	99	3.8
24	98	3.1
48	50	2.3
96	49	1.4
168	5	0.8
240	0	0.5

Data are based on four replicate ponds.

4.3 PERMETHRIN RESIDUES IN POND WATER

Figures 13 and 14 illustrate the average permethrin concentration in the two ponds treated in 1979 and 1980, respectively. The results were derived from TLC separation, autoradiography, and LSC quantitation of the pond water extract. The 1979 data described water samples collected at the pond surface only while the 1980 data included permethrin concentrations in both surface and subsurface samples. Also indicated in the graphs are the calculated values predicted by the simulation model reported by Marshall and Roberts (1977). The model is based on first order kinetics and is derived from the following equation. Calculations and terms are shown in Appendix B.

$$V_w \, dC_w/dt = k_{hw}V_hC_h - k_{wo}V_wC_w - k_{wh}V_hC_w$$

In 1979 the initial concentration of permethrin in the surface water, 15.5 ug/L, decreased very quickly to 1.5 ug/L by 18 h and 0.04 ug/L at 72 h post-treatment. The calculated values of the model indicated

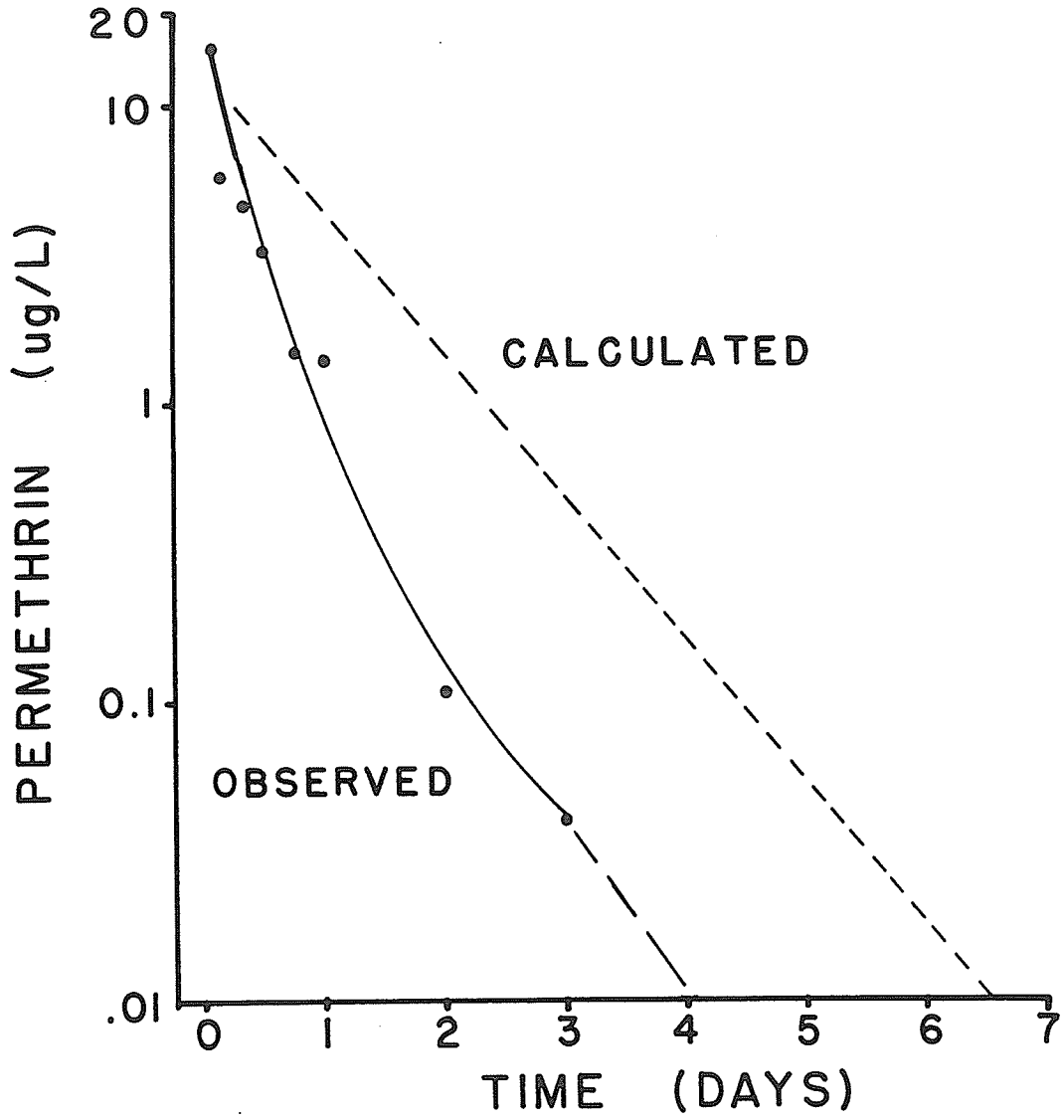


Figure 13: Permethrin concentration in surface pond water, 1979

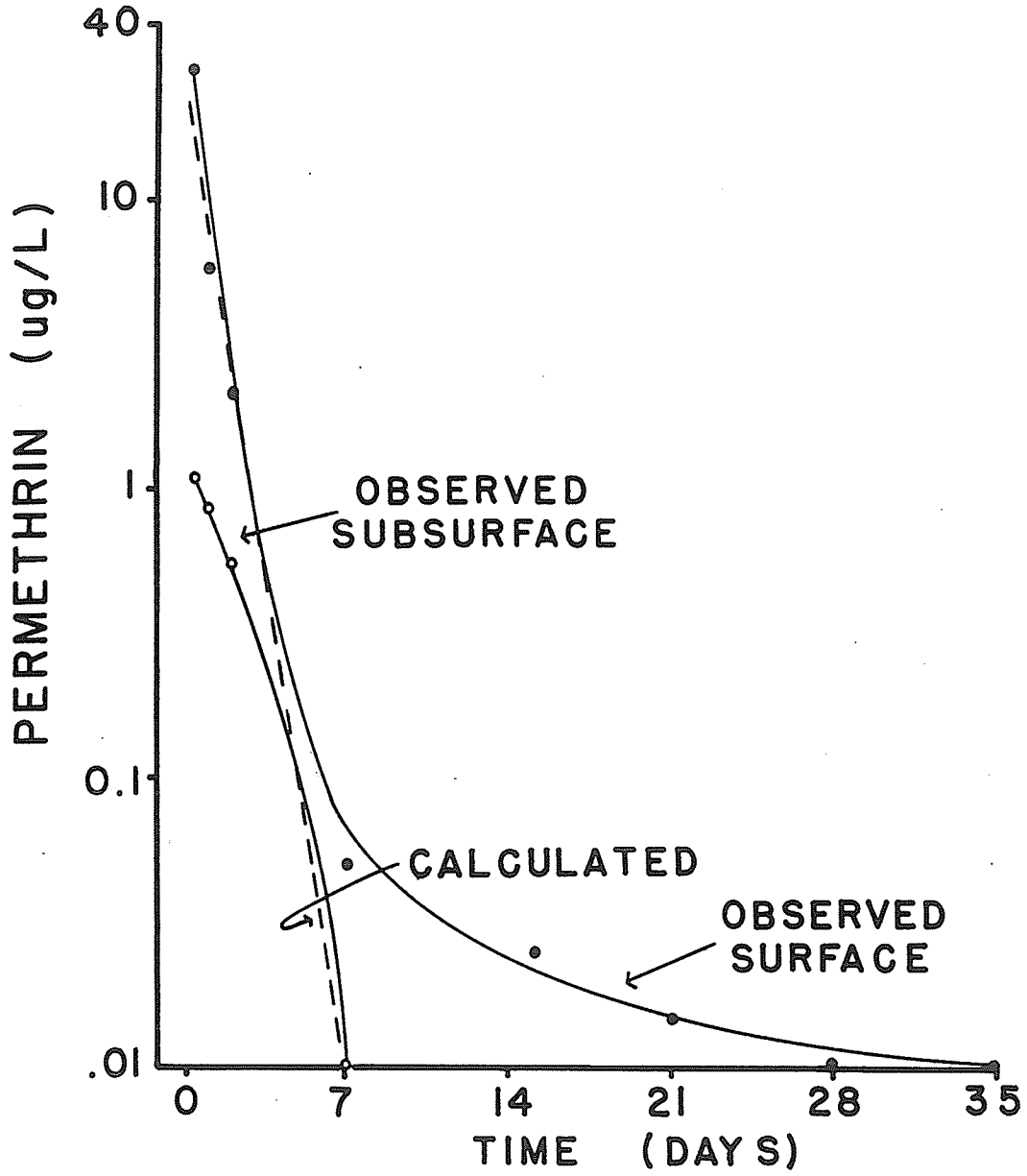


Figure 14: Permethrin concentration in surface and subsurface pond water, 1980

greater persistence than the observed values. The half-life for permethrin in the pond water from the observed data correspond to 8.5 h while from the model the half-life would be 15 h. The longer half-life determined by the model may be due to errors in estimating the rate constants k_{wo} and k_{wh}. k_{wo} was derived from the photolysis study in pyrex flasks and not in direct sunlight. k_{wh} was based on the uptake by soil in the sampling jars, and, not the pond bottom which had three times as much organic matter. Therefore, more accurate values for the constants might have predicted an increased loss of permethrin from the water and thus a half-life closer to the observed value.

In 1980, the permethrin concentration was determined in both surface and subsurface water samples (Figure 14). In the surface water the average initial concentration of permethrin in the water was 29.0 ug/L, which decreased very quickly to 2.2 ug/L by 48 h, 0.06 ug/L by one week, and 0.01 ug/L at 5 weeks post-treatment. The permethrin in the subsurface water sample was much lower in concentration and disappeared more quickly than the permethrin in the surface water of the pond. The initial and highest permethrin concentration reached was 1.1 ug/L in the subsurface water compared to the 29.0 ug/L in the surface water. The permethrin in the subsurface water of the ponds required only one week to decrease to 0.01 ug/L while the permethrin in the surface water did not decrease to this level until four weeks post-treatment. The results indicate that a surface film of the permethrin occurred on the water after application, with the surface water reaching and maintaining a higher permethrin concentration than the subsurface water.

The formation of a surface film of permethrin within the water column may be due to having used an oil based EC formulation, or to the very low water solubility of permethrin or both.

Maguire and Hale (1980) detected a surface film of fenitrothion after its application to ponds. The fenitrothion concentration in the surface microlayer was 1.5 mg/L at 40 minutes post-spraying which decreased to 2 ug/L at 49 h. In comparison, the fenitrothion levels in the subsurface water were 15 and 0.1 ug/L at 40 minutes and 49 h, respectively. Thus, it appears from these two studies that, in stagnant ponds in which very little mixing occurs, the concentration of the insecticide at the water surface is higher than in the subsurface water.

In Figure 14 (1980) it can be seen that the model did not predict the increased persistence of permethrin in the surface water over the 1979 data. Why permethrin was detected for 32 days longer in 1980 is not known. It is interesting to note that the model did correspond to the disappearance rate in the observed permethrin concentration in the subsurface water. Since the model is based on the initial surface water concentration which is much higher than the subsurface level, it would not be expected that the early part of the model would coincide with the subsurface concentrations. However, the data did suggest that the concentration in the subsurface water may be more meaningful since the hydrosoil-water interface rate constants are included in the model equation.

Corresponding to the decrease of permethrin levels in the water was an increase in the permethrin concentration in the hydrosoil, duckweed, and fathead minnows. The sorption of the permethrin by these substrates will be discussed in later sections.

4.4 TOTAL ^{14}C ACTIVITY IN THE POND WATER

As mentioned in the previous section, the permethrin residues disappeared very quickly from the pond water. However, even though permethrin was not present, ^{14}C activity, in the form of permethrin degradation products, was still detected in the pond water. Each summer one pond was treated with the ^{14}C label in the alcohol moiety and the other pond was treated with the ^{14}C label in the acid moiety; thus, even without identifying the actual degradation products present in the water, it is known that the ^{14}C activity in one pond was associated with permethrin degradation products containing only the alcohol moiety (methylene label) and that the ^{14}C activity in the other pond was associated with products containing only the acid moiety (cyclopropyl label). The ^{14}C activity would include not only the products from ester cleavage but also the hydroxylation or dechlorination products of the intact permethrin molecule. Thus, by monitoring the total ^{14}C activity, the fate of the methylene labelled permethrin molecule could be followed in one pond, and, the fate of the cyclopropyl labelled permethrin molecule could be followed in the other pond.

The total ^{14}C activity in the surface pond water for the 1979 treated ponds is shown in Figure 15. During the first day, the rapid loss in ^{14}C activity parellels the rapid decrease in permethrin concentration in the water. At approximately two days, however, the results show a marked variation: the pond treated with the cyclopropyl labelled permethrin showed a levelling off of ^{14}C activity and still contained more than 1 ug/L (permethrin equivalents) at 29 days post-treatment, whereas, the pond treated with methylene labelled permethrin showed a steady decline

in residual ^{14}C activity over the same 29 days. Therefore, the results indicate a different fate for the acid moiety and the alcohol moiety of the permethrin ester.

In 1980 the total ^{14}C activity in the surface pond water (Figure 16-A) also indicated different levels of persistence for the two moieties. Although the cyclopropyl labelled degradation products were present in a higher concentration than the methylene labelled degradation products, the differences were minimal and trends impossible to define. In the subsurface pond water (Figure 16-B) the trend for the cyclopropyl products to remain in the water and for the methylene products to disappear from the water was once again observed.

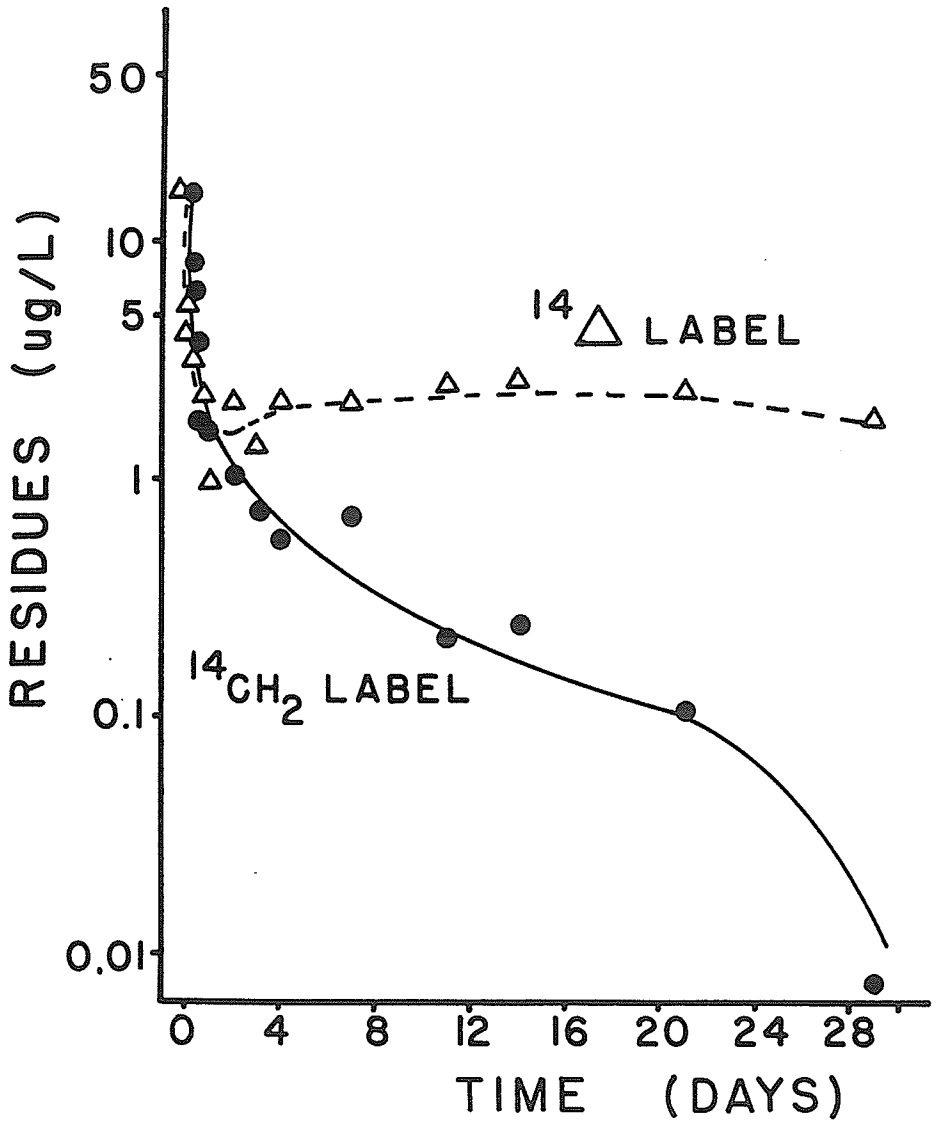


Figure 15: Total ^{14}C activity in surface pond water, 1979

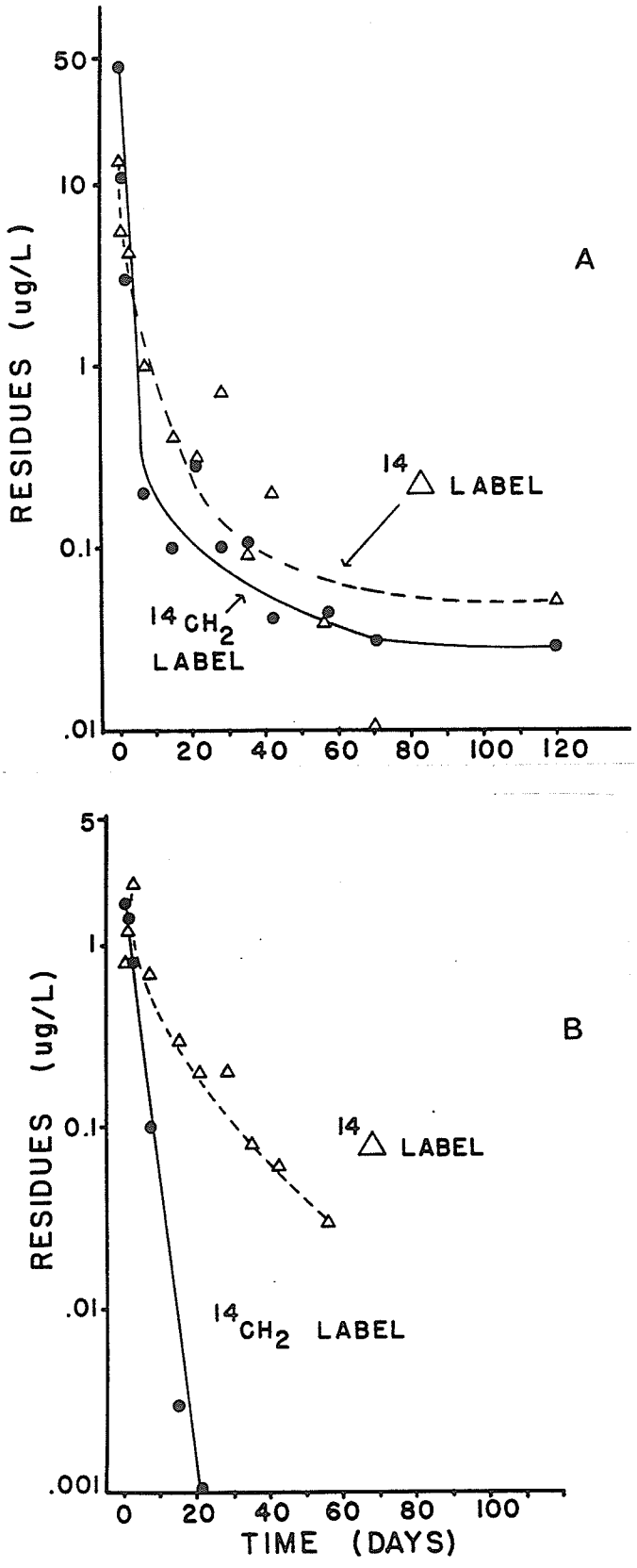


Figure 16: Total ^{14}C activity in surface (A) and Subsurface (B) pond water, 1980 (samples beyond 60 days in 16-B had <0.001 ug/L)

4.5 PERMETHRIN DEGRADATION PRODUCTS IN POND WATER

Results in the literature indicate that permethrin could be degraded in a pond environment by several pathways resulting in the production of a series of degradation products. Figure 17 indicates the major metabolites expected in a pond system due to photolysis and metabolism in plants, fish, and soil (Holmstead et al. 1978, Gaughan and Casida 1978, Glickman et al. 1979, and Kaufman et al. 1977, and Kaneko et al. 1978).

The detection of ^{14}C activity in the water beyond the time permethrin itself could be detected indicated the presence of degradation products in the water. TLC-autoradiography results confirmed that several degradation products had been generated.

Five permethrin degradation products, of which four have been identified, were detected for various lengths of time in the water. The identified products were the trans- and cis-cyclopropyl acids (Cl_2CA), phenoxybenzoic acid (PBacid), and phenoxybenzyl alcohol (PBalc) (Figure 18). These metabolites have also been identified in microsomal systems (Shono et al. 1979), in soil (Kaufman et al. 1977, Kaneko et al. 1978), and as the result of photolysis (Holmstead et al. 1978).

The rapid appearance of the degradation products in the water a few hours after application is consistent with a photochemical pathway. In such a short time, the sorption, metabolism and release of these products by the aquatic vegetation or the hydrosoil could be expected to be minimal.

The unknown degradation product was detected in both ponds, and therefore, since this product was detected independent of the position of the label in the starting material, it must be a non-cleaved product

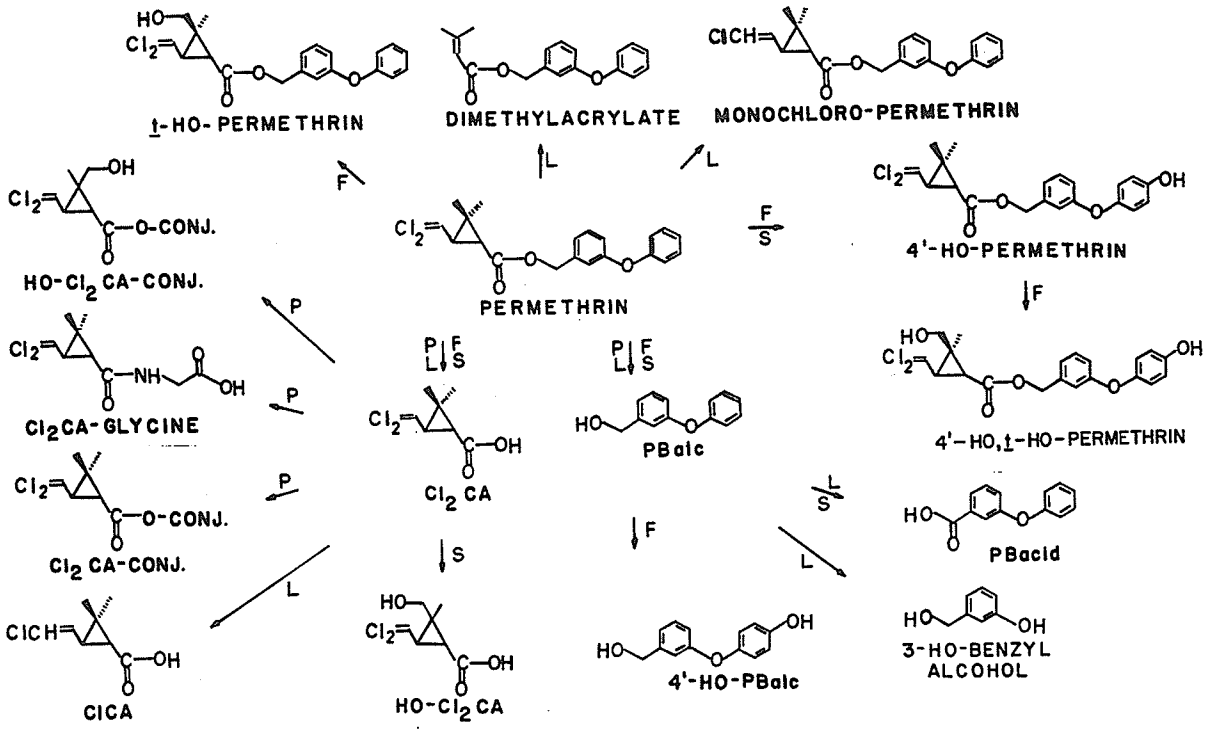


Figure 17: Major permethrin metabolites expected in a pond system (F = fish, L = photolysis, S = soil, P = plants)

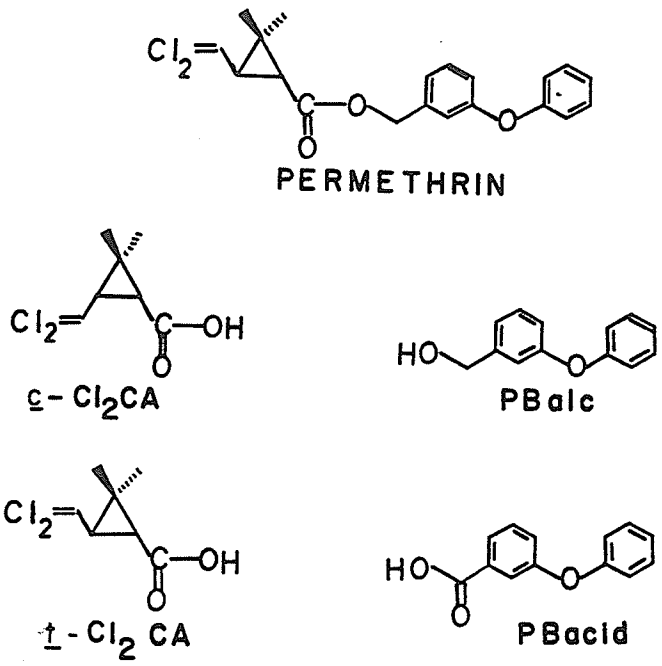


Figure 18: Permethrin degradation products identified in pond water: phenoxybenzoic acid (PBacid), phenoxybenzyl alcohol (PBalc), cis- and trans-cyclopropyl acid (Cl_2CA)

of the permethrin ester. As indicated in Figure 17, the following 4 non-cleaved products have been reported: dimethylacrylate and monochloro-permethrin by photolysis, trans-HO-permethrin as a metabolite in fish, and 4'-HO-permethrin from fish and soil. Since the fish were not present in the ponds at the time of application and because of the fast appearance of the unknown product, it is probably not the trans-HO-permethrin or 4'-HO-permethrin. Since the unknown appeared very quickly, coinciding with the reported rapid permethrin photolysis, the unknown could be the dimethylacrylate or the monochloro-permethrin. This was not confirmed since the amount of unknown metabolite available was too small for gas chromatography-mass spectrometry and the use of different solvent systems for TLC made it impossible to compare R_f values with literature results. In addition, in the preliminary photolysis experiments carried out in this study, unlabelled permethrin was used and thus metabolite production was not monitored.

The concentrations of the metabolites in the pond water were obtained by chromatographing the water extracts using two different TLC solvent systems, both of which separated the five degradation products, followed by autoradiography and LSC of the developed spots. Table 20 indicates the R_f values obtained in the different solvent systems.

In 1979 (Figure 19), the cis- and trans-cyclopropyl acids increased in concentration and remained fairly constant at 0.5 ug/L and 1.5 ug/L (permethrin equivalents) from 72 h to the end of the experiment at 29 days. Both the PBalc and PBacid, from the alcohol moiety were detected for shorter periods of time than the cyclopropyl acids. The PBalc was detected to only 48 h and the PBacid was undetectable beyond 21 days

TABLE 20

R_f Values of Permethrin Metabolites

Metabolite	R_f Value CFE solvent	CEaM solvent
Permethrin	0.55	0.68
PBalc	0.15	0.52
PBacid	0.23	0.29
cis-cyclopropyl acid	0.36	0.46
trans-cyclopropyl acid	0.29	0.39
Unknown	-	0.60

CFE= cyclohexane (saturated with formic acid)-ether (3:2).
CEaM= chloroform-ethyl acetate-methanol (6:3:1).

post-treatment. The unknown metabolite was detected in the water to 7 and 11 days in the two ponds.

At 24 h, a sudden drop in concentration of the cyclopropyl acids and PBacid occurred but was not observed for PBalc or the unknown. The reason for this decrease was not determined. However, it could be experimental error or the result of reduced photolysis during the night following the permethrin application.

Results of the 1980 study were similiar to the 1979 study in that the same five degradation products were detected in the pond water (Figure 20). The cyclopropyl acid isomers were still the most persistent but were not detectable beyond 35 days post-treatment. The non-cleaved product was also detected in both ponds but disappeared from the water by seven days post-treatment. As in the previous year, the PBalc was short-lived and was not detected beyond seven days, as was the PBacid.

Tables 21 and 22 indicate the distribution of radioactivity in pond water (1979 and 1980) of permethrin and its degradation products ex-

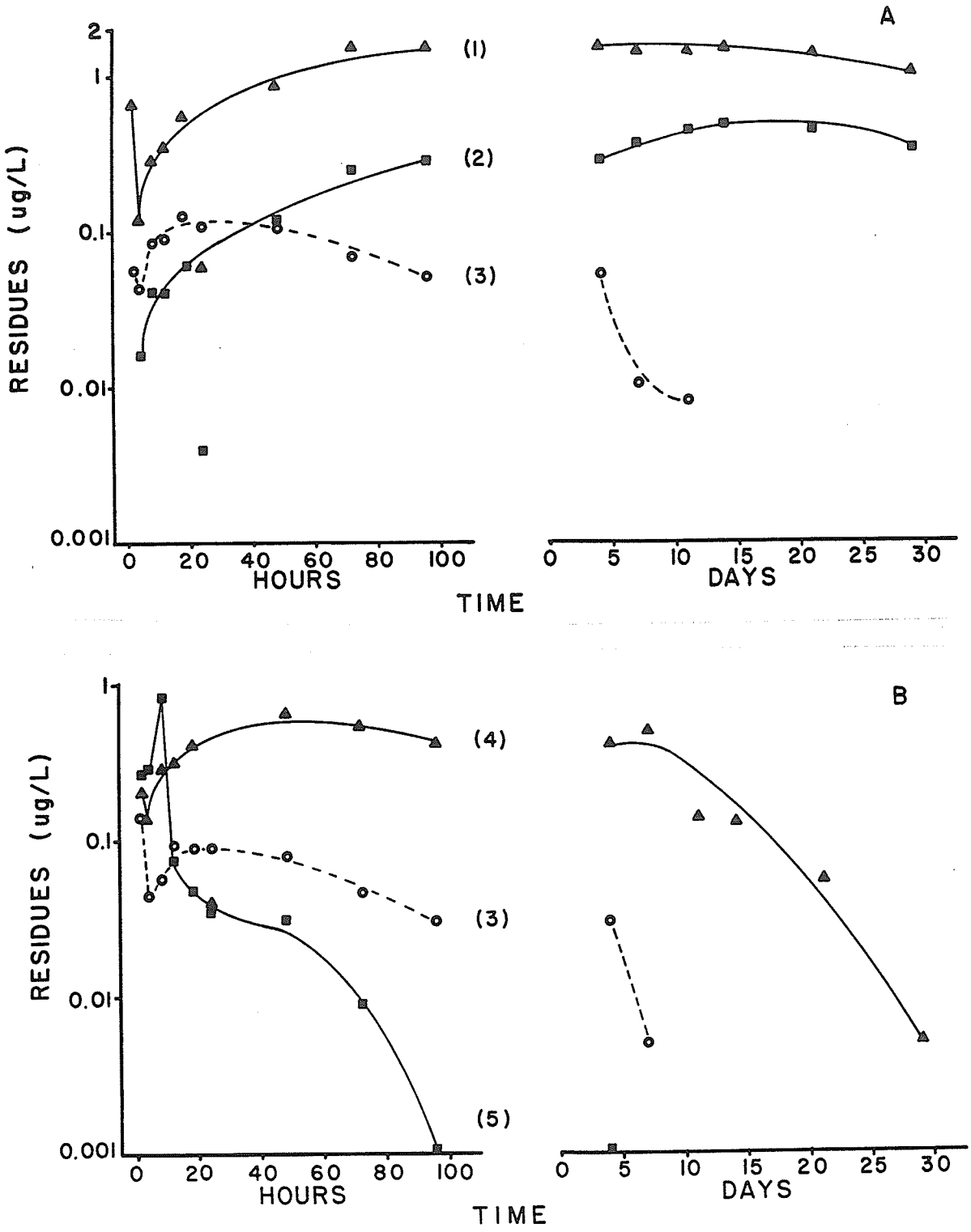


Figure 19: Concentration of permethrin degradation products in pond water of cyclopropyl (A) and methylene (B) labelled permethrin pond, 1979 (1 = t-Cl_2CA, 2 = c-Cl_2CA, 3 = non-cleaved product, 4 = PBacid, 5 = PBalc)

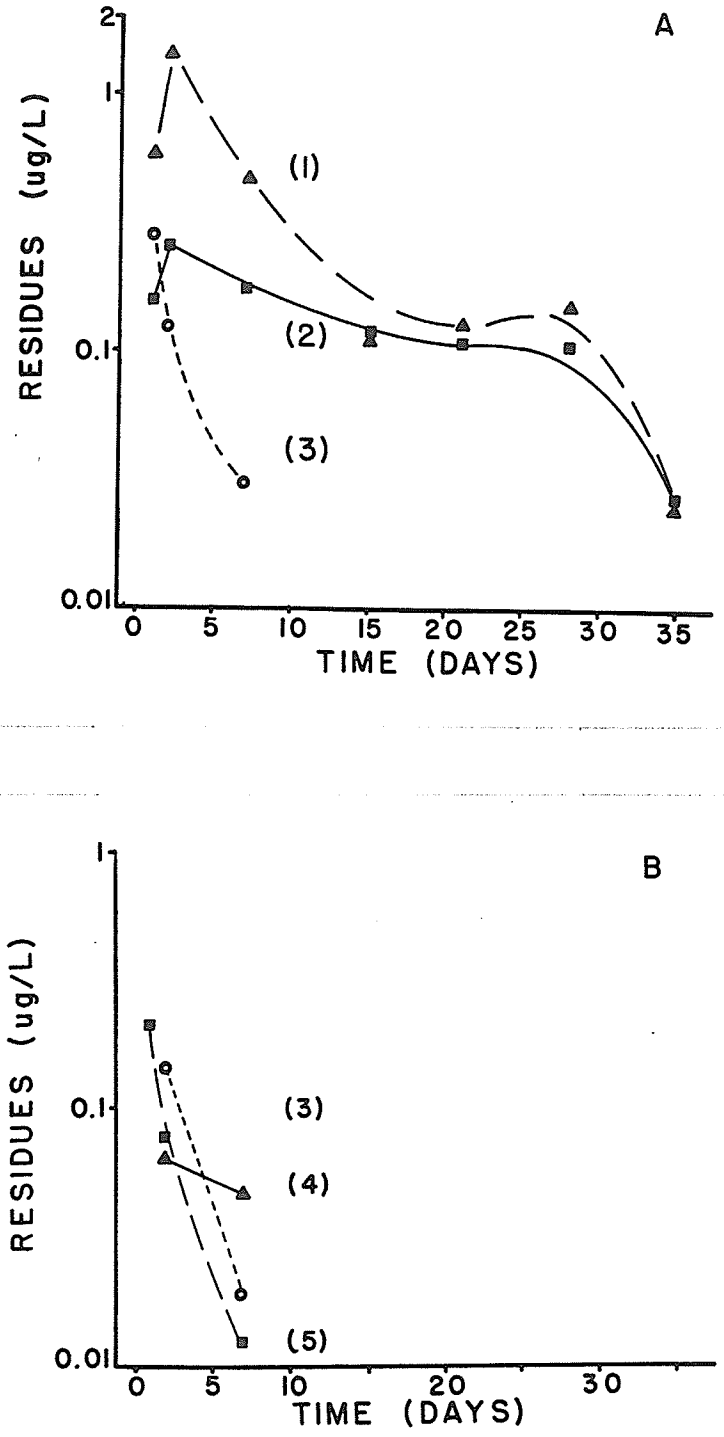


Figure 20: Concentration of permethrin degradation products in pond water of cyclopropyl (A) and methylene (B) labelled permethrin pond, 1980 (1 = t-Cl_2CA, 2 = c-Cl_2CA, 3 = non-cleaved product, 4 = PBacid, 5 = PBalc)

pressed as percent of the total activity present in the water. Table 21 shows the rapid degradation of permethrin in water. Up to 24 h post-treatment, very little degradation of the permethrin had occurred. However, by 48 h, the permethrin had degraded or partitioned into the sediment or vegetation so quickly that permethrin accounted for only 6.2% of the radioactivity in the cyclopropyl labelled permethrin pond, and 9.5% in the methylene labelled permethrin pond. That is, by 48 h, permethrin degradation products accounted for greater than 90% of the radioactivity in the pond water. By four days, none of the radioactivity in the water was associated with free permethrin.

In the cyclopropyl labelled pond, after four days post-treatment, the trans-cyclopropyl acid accounted for 70.4% of the activity; cis-cyclopropyl acid, 13.0% ; the non-cleaved product, 2.3%; with 14.4% being unaccounted for. During the remainder of the experiment, the percent activity that could not be accounted for increased from 14.4% to 24.3% which indicated that further degradation of the primary products had probably occurred at a slow rate.

Four days post-treatment, 75% of the radioactivity in the pond treated with methylene labelled permethrin was accounted for as PBacid; 0.2% as PBalc, and 5.4% as the non-cleaved product. From 11-29 days post-treatment, the only detected metabolite in the water was PBacid, accounting for 50-64% of the radioactivity. Even though the concentration of PBacid in the water decreased during this time period, the percentage of PBacid in the water, compared to the total activity, remained fairly constant. If the decrease in PBacid concentration in the water had been the result of further degradation, the percentage of PBacid should have

decreased, with a corresponding increase in percent of unknown activity. However, since this did not occur, the decrease in concentration of PBacid in the water could not have been due to degradation, but could have been the result of sorption to the hydrosoil and/or the vegetation.

The most significant difference in permethrin degradation in the water between 1979 and 1980 was the rapid disappearance of PBacid (7 days) in 1980 compared to 29 days in 1979. The data in Table 15 indicate that, at one week post-treatment, the PBacid accounted for 23.5% of the radioactivity in the water, while 46.0% was associated with unknown products. By 15 days, all the activity present in the water was due to unknown products, whereas in 1979, at 29 days, only 37.5 of the activity in the water could not be accounted for. Therefore, it appears that in 1980 the PBacid degraded to unknown products much more readily than had occurred in 1979. One explanation for the increased rate of PBacid degradation could be increased photolysis, since there are more hours of sunlight available in June (1980) compared to late August (1979).

TABLE 21

Distribution of Radioactivity Associated With Permethrin and its Degradation Products as Percent of Total Activity in the Water at Time Zero in Pond Water, 1979

Cyclopropyl labelled permethrin treated pond

Time	Percent of Total Activity at Time = t_0					
	Permethrin	t-Cl_2 CA	c-Cl_2 CA	Ester product	%	% other
2 h	94.1	4.1	--	0.4	98.6	1.4
4 h	94.2	2.1	0.4	0.7	97.4	2.6
8 h	101.1	7.0	1.0	2.2	111.2	---
12 h	86.0	9.6	1.1	2.5	99.2	0.8
18 h	75.2	25.2	2.7	5.9	109.0	---
24 h	113.3	6.1	0.4	11.2	130.6	---
48 h	6.2	42.4	5.7	5.2	59.5	40.5
72 h	3.6	110.9	18.2	5.1	138.0	---
96 h	0	70.4	13.0	2.3	85.6	14.4
7 d	0	65.9	16.8	0.5	83.2	16.8
11 d	0	58.2	18.0	0.3	76.6	23.4
14 d	0	55.1	18.0	0	73.0	27.0
21 d	0	55.6	18.3	0	73.9	26.1
29 d	0	57.3	18.4	0	75.7	24.3

Methylene labelled permethrin treated pond

Time	Permethrin	PB-acid	PB-alc	Ester product	%	% other
2 h	94.3	1.3	1.6	0.8	98.1	1.9
4 h	80.3	1.7	3.6	0.5	86.1	13.9
8 h	82.6	4.7	13.6	1.0	101.9	---
12 h	88.7	8.2	2.1	2.6	101.5	---
18 h	79.4	25.3	2.9	5.3	112.9	---
24 h	100.0	2.4	2.4	5.4	110.2	---
48 h	9.5	62.9	2.9	7.6	82.9	17.1
72 h	4.0	73.3	1.2	6.7	85.2	14.8
96 h	0	75.0	0.2	5.4	80.5	19.5
7 d	0	73.9	0	0.7	74.6	25.4
11 d	0	63.6	0	0	63.6	36.4
14 d	0	52.0	0	0	52.0	48.0
21 d	0	50.9	0	0	50.9	49.1
29 d	0	62.5	0	0	62.5	37.5

--- indicates that greater than 100% of the activity was accounted for.

TABLE 22

Distribution of Radioactivity Associated With Permethrin and its Degradation Products as Percent of Total Activity in the Water at Time Zero in Pond Water, 1980

Cyclopropyl labelled permthrin treated pond

Time	Percent of Total Activity at Time = t_0					
	Permethrin	t-Cl_2 CA	c-Cl_2 CA	Ester product	%	% other
2 h	94.1	0	0	0	94.1	5.9
24 h	35.2	11.0	2.9	5.2	54.3	45.7
48 h	42.9	34.5	6.1	3.0	86.3	13.7
7 d	8.0	47.0	17.6	3.1	75.7	24.3
14 d	12.5	27.5	30.3	0	70.3	29.7
21 d	10.0	43.0	36.3	0	89.3	10.7
28 d	2.9	21.9	15.4	0	40.1	59.9
35 d	22.2	27.8	30.0	0	80.0	20.0
42 d	0	0	0	0	0	100.0
56 d	0	0	0	0	0	100.0
70 d	0	0	0	0	0	100.0
84 d	0	0	0	0	0	100.0

Methylene labelled permethrin treated pond

Time	Permethrin	PB-acid	PB-alc	Ester product	%	% other
2 h	100.0	0	0	0	100.0	0
24 h	89.1	0	1.9	0	91.0	9.0
48 h	83.3	2.1	2.6	4.8	92.8	7.2
7 d	15.0	23.5	6.0	9.5	54.0	46.0
14 d	0	0	0	0	0	100.0
21 d	0	0	0	0	0	100.0
28 d	0	0	0	0	0	100.0
35 d	0	0	0	0	0	100.0
42 d	0	0	0	0	0	100.0
56 d	0	0	0	0	0	100.0
70 d	0	0	0	0	0	100.0

4.6 PERMETHRIN RESIDUES IN HYDROSOIL

In 1979 during the first two days post-treatment, the ^{14}C residues in the hydrosoil were similiar for both ponds. As in the case of the ^{14}C residues in the pond water, there was a marked difference in ^{14}C residue levels in the hydrosoil in the two ponds beyond the first two days (Figure 21-A). The pond treated with cyclopropyl labelled permethrin showed maximum ^{14}C residue at three to four days with a concentration equivalent to 160 ug permethrin/kg dry weight soil; whereas, the methylene labelled pond showed a maximum at 3 days with a concentration of 330 ug permethrin equivalents/kg dry weight soil. These results are complementary to those recorded for pond water and show that the more polar acid metabolites tend to remain in the water, while the less polar alcohol metabolites tend to associate with the hydrosoil. At a pH of approximately 7.5-8.5, the cyclopropyl acids would be present in their anionic form which would be repelled by the negative charges of the clay colloids in the hydrosoil.

In the summer of 1980 at 37, 52, and 60 weeks post-treatment, hydrosoil samples still contained ^{14}C activity. At 60 weeks, 16 ug permethrin equivalents/kg was detected in the hydrosoil of cyclopropyl labelled pond and 38 ug permethrin equivalents/kg in the methylene labelled pond.

Anomalous points in Figure 21-A are thought to be due to excessive quantities of detritus which collected on top of the soil substrate in the sample jars. The 0.5 cm sample taken for analysis from these jars would have been less dense, thus inflating the concentration values for the residues reported on a ug residue/kg dry weight basis.

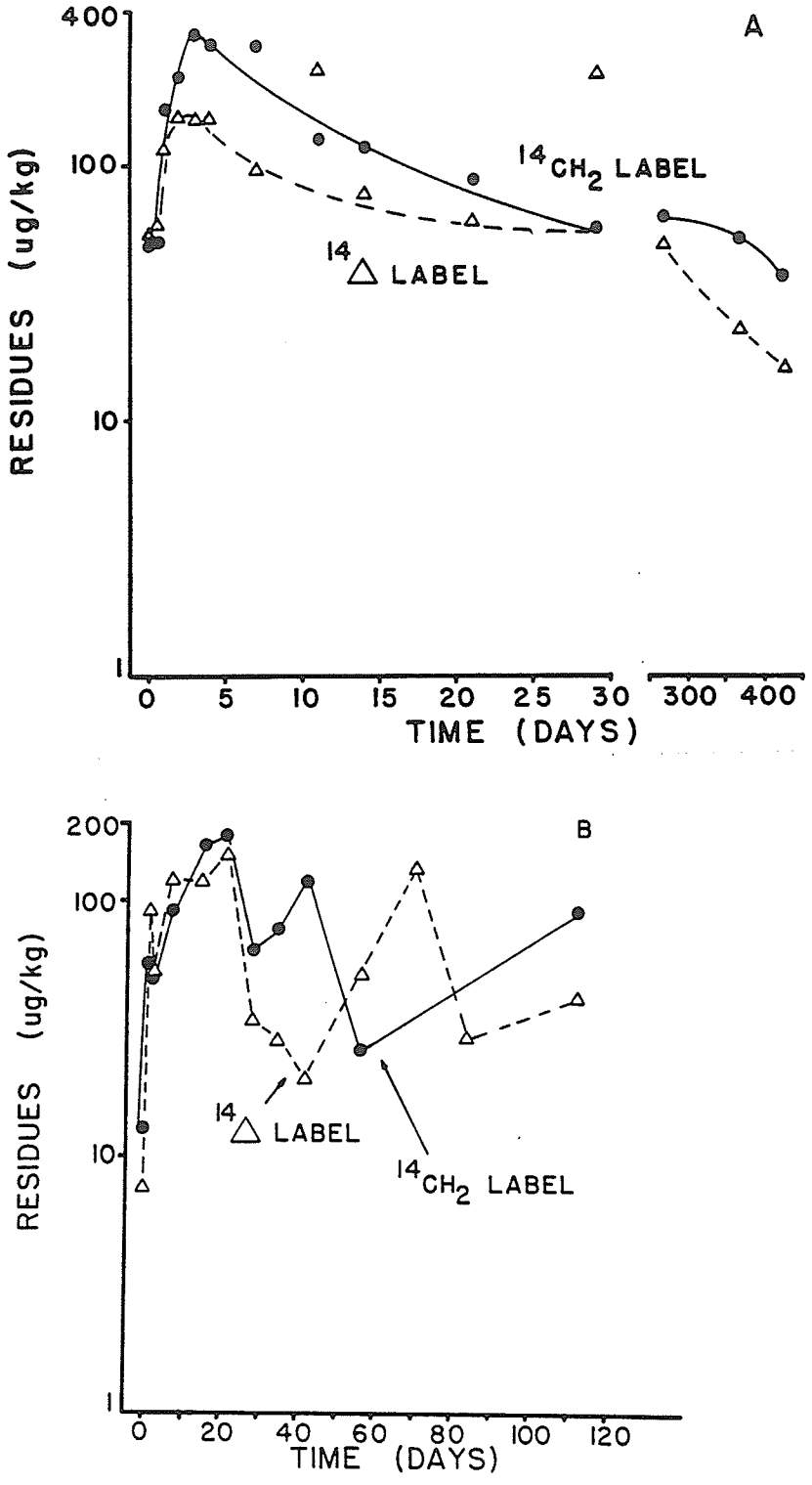


Figure 21: Total ^{14}C residues in hydrosoil in 1979 (A) and 1980 (B) (ug/kg dry weight)

Figure 21-B shows the total ^{14}C residues in the hydrosoil of the ponds treated in 1980. During most of the experiment, the total residues in the pond treated with cyclopropyl labelled permethrin were similar to the residues in the pond treated with the methylene labelled permethrin. However, at 56 and 70 days the ^{14}C cyclopropyl levels are higher. Why this occurred is not known, but a number of factors have been investigated. Weather records were checked to determine if a storm had occurred in the area of the ponds prior to sampling, but the results were negative. Also, permethrin residue levels were determined on a unit area basis rather than a weight basis. Data based on area would remove the sampling method as a source of error, assuming that the depth of the hydrosoil sample taken (0.5 cm) was sufficient to include all the permethrin, since the concentration would be based on total permethrin removed from the jars. The increase in permethrin concentration was still observed in the area based data, indicating that the sampling procedure itself was not responsible for the anomalous hydrosoil results.

Figure 22 indicates the permethrin residues in the hydrosoil from the 1979 and 1980 samples, respectively, as monitored by HPLC and LSC. The results shown are the averages of the two ponds treated each year; individual residue concentrations are listed in Appendix C. Also included in the graphs are the calculated values based on kinetic rates and following the simulation model reported by Marshall and Roberts (1977). The model is based on the following equation (calculations and term definitions shown in Appendix B):

$$V_h \, dC_h/dt = k_{wh} V_h C_w - k_{hw} C_h V_h - k_{ho} V_h C_h$$

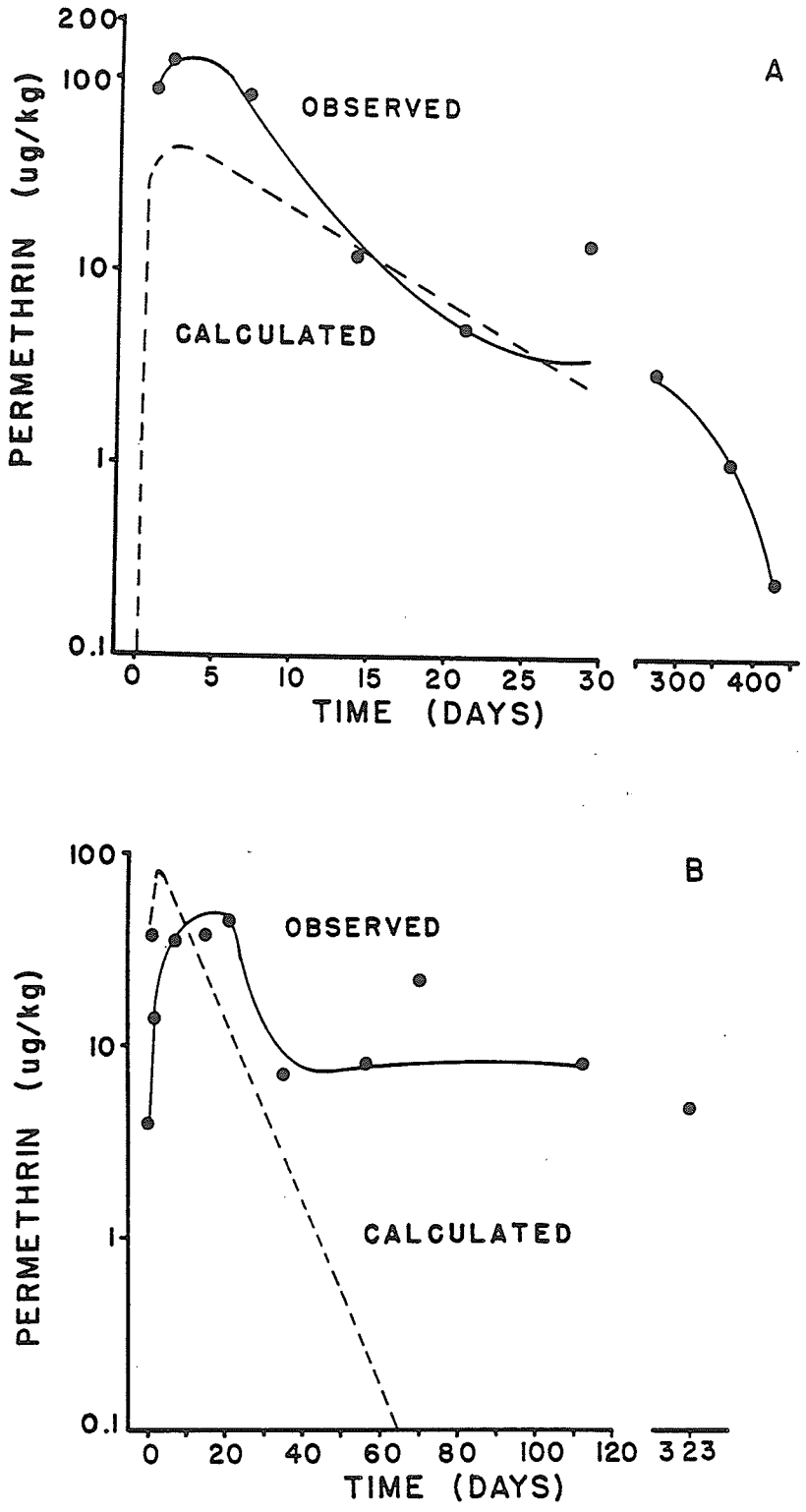


Figure 22: Permethrin concentration in hydrosoil in 1979 (A) and 1980 (B) (ug/kg dry weight)

As expected, the permethrin levels in the hydrosoil were much lower than the total ^{14}C activity which indicates that the hydrosoil contained permethrin degradation products either as a result of the sorption of the products from the water and/or the degradation of permethrin itself in the hydrosoil.

In 1979 the permethrin concentration in the hydrosoil (Figure 22-A) increased very quickly, followed by a gradual decrease in concentration. From a maximum permethrin concentration of approximately 120 ug/kg at two days, the concentration decreased to 2.8 ug/kg by the following spring (259 days). By 420 days, the permethrin concentration was 0.25 ug/kg.

In 1980 (Figure 22-B), the permethrin was again very readily sorbed by the hydrosoil and maintained a concentration of ca. 40 ug/kg until at least 21 days. By 35 days, the level dropped to 7 ug/kg where it remained fairly constant to 112 days. The following spring, at 323 days post-treatment, permethrin was still detected in the hydrosoil at a concentration of 4.9 ug/kg. Therefore, in both the 1979 and 1980 permethrin treated ponds, permethrin in the hydrosoil was detected the following spring at levels between 2.8 and 4.9 ug/kg.

Rettich (1980) found that many aquatic invertebrates were severely affected by permethrin concentrations in the water of 2-5 ug/L. The effect of 2.8-4.9 ug/kg permethrin in the hydrosoil on benthic organisms is not known. The bioavailability of this permethrin may be cause for concern.

The simulation model failed to follow the observed data in two areas. The first error was in the maximum concentration (120 ug/kg observed in

1979 vs. 45 ug/kg calculated). The second error was that the model did not predict the persistence of permethrin in the hydrosoil that was observed in the actual data.

In the short term, up to 29 days, the model and observed rates of permethrin degradation in the hydrosoil were similar. From two to 29 days, the observed data yielded a half-life of 6.9 days compared to the model's predicted half-life of 6.7 days of permethrin in the hydrosoil. Both of these half-life values agree with the 6-12 day half-life reported by Kaneko et al. (1978) for permethrin in dry soil. However, beyond 29 days the rate of permethrin degradation decreased such that at 420 days, permethrin was still detectable in the hydrosoil corresponding to a half-life of ca. 80 days in the hydrosoil. The model predicted a value of 1.3 ug/kg hydrosoil at 35 days; it was not until the following summer (365 days) that the observed concentration reached 1.0 ug/kg.

The error in predicting permethrin persistence is better exemplified in Figure 22-B. For the first 30 days the model and the observed data are reasonably similiar, but, beyond that time, the model predicted a decrease to a level of 0.1 ug/kg at 64 days, while the observed data remained fairly constant at 7-8 ug/kg to at least 112 days and decreased to ca. 5 ug/kg at 323 days.

A curve similiar to the observed data in Figure 22-B was described by Goring et al. (1975) as a general degradation curve for pesticides in soil as the result of microbial degradation. Immediately after the application of the pesticide in their study, very little degradation occurred during the induction period for the microbes. As the microbes adapted and multiplied, the rate of degradation increased (less than

first order), and the pesticide concentration decreased. Goring et al. (1975) proposed that eventually the pesticide in the soil became entrapped in the soil organic matter which prevented microbial degradation, and, thus, the degradation rate decreased and very little change in concentration occurred. The combination of degradation and entrapment resulted in a net reaction order in excess of first order. Since microbial degradation of permethrin in the soil occurs (Kaufman et al. 1977), a model that allowed for changes in reaction order, unlike the first order model of Marshall and Roberts (1977) may result in a better prediction of pesticide persistence in the hydrosoil.

The Marshall and Roberts (1977) model is based on the rate of degradation, k, in the hydrosoil which remains constant over time. Hamaker and Goring (1976) found that pesticides in the soil are either in a mobile state and available for degradation, or in an immobile or bound state and unavailable for degradation. Therefore, k is not constant, but changes in relationship to the fraction of the pesticide in the mobile state. By using first order kinetics with k, k_1 (rate of entry into bound state), and k_{-1} (rate of leaving the bound condition), the calculated curve was found to fit the observed curves for persistent pesticides, including data that was not strictly first order.

Following solvent extraction, the hydrosoil samples were combusted to determine the amount of non-extractable residues remaining in the hydrosoil. Table 23 indicates the ^{14}C levels that were extracted and non-extractable. The amount of non-extractable residues in the hydrosoil ranged from 0 to 136 ng/g (dry weight). According to Kaufman et al. (1977) most of the radioactivity would have been associated with the fulvic acid and humin fractions of the hydrosoil.

TABLE 23

Extracted and non-extractable ^{14}C residues in hydrosoil (ng permethrin equivalents/g dry weight)

Sample	^{14}C Extracted	^{14}C Non-Extractable	% Permethrin
1979, Methylene labelled			
24 h	118	5	73
48 h	202	39	58
7 d	226	103	43
14 d	53	54	13
21 d	49	57	8
29 d	21	24	4
420 d	--	37	-
Cyclopropyl labelled			
24 h	117	10	69
48 h	171	47	46
7 d	57	23	26
14 d	47	21	15
21 d	33	18	4
29 d	108	50	16
420 d	---	14	--
1980, Methylene labelled			
1 d	52	0	75
2 d	26	4	63
7 d	51	24	36
14 d	64	64	27
21 d	81	80	30
35 d	22	36	19
56 d	7	11	17
70 d	33	48	15
112 d	24	136	8
Cyclopropyl labelled			
1 d	48	4	71
2 d	16	19	26
7 d	72	29	42
14 d	54	63	35
21 d	50	99	28
35 d	5	32	5
56 d	25	32	23
70 d	39	56	33
112 d	7	12	16

% permethrin calculated as % in ^{14}C extracted plus non-extractable residues.

HPLC analysis of the hydrosoil extract allowed the monitoring of the cis- and trans- ratio of the permethrin isomers (Table 16). The results indicate that the trans-isomer degrades more readily than the cis-isomer. Similar results in soil were reported by Kaufman et al. (1977). The faster degradation rate of the trans-isomer was also reported by Shono et al. (1979) for microsomal enzymes, Bigley and Plapp (1978) for insects, and by Gaughan and Casida (1978) for plants.

TABLE 24

Cis/Trans-Permethrin Ratio in Hydrosoil

	Cis/Trans Ratio			
	1979		1980	
Time	3	7	2	9
0 h (WATER)	45/55	34/66	35/65	44/56
2 h			30/70	42/58
1 d	52/48	37/63	37/63	50/50
2 d	58/42	43/57	42/58	56/44
7 d	72/28	59/41	57/43	62/38
14 d	61/39	63/37		
15 d			63/37	63/37
21 d	77/23	79/21	61/39	72/28
29 d	87/13	100/0		
35 d			64/36	84/16
56 d			59/41	74/26
70 d			68/32	84/16
112 d			62/38	83/17
259 d	100/0	66/34		
323 d			70/30	100/0
365 d	--	100/0		
420 d	--	100/0		

It is interesting to note that in the 1980 hydrosoil sample, the cis to trans ratios were fairly constant beyond 35 days. If degradation of

permethrin had been occurring, it would have been expected that the cis and trans ratio would have changed with an increasing percentage of cis-isomer, since it degrades more slowly than the trans-isomer. Since the ratio did not change, it appears that permethrin degradation did not occur very quickly, which would explain why the permethrin concentration in the hydrosoil remained fairly constant from 35-112 days and decreased only slightly by 323 days (Figure 22-B). This explanation would agree with the idea of Hamaker and Goring (1976) that the pesticide becomes unavailable for degradation over time.

Permethrin in a pond system is characterized by a rapid decrease in permethrin concentration in water concurrent with a rapid increase in hydrosoil residues, followed by a slow decrease in concentration in both substrates.

Malis and Muir (1980) observed a similiar pattern for fenitrothion distribution in Glenlea ponds. The insecticide disappeared very quickly from the water, reaching the detectable limit at 5 days post-treatment. Total ^{14}C residues in the sediment peaked at ca. 5 days and subsequently declined. Rapid loss from the water and rapid sorption by the sediment was also observed for phosphate esters in the Glenlea ponds (Muir et al. 1980).

The herbicide, fluridone, when applied to the Glenlea ponds (Muir et al. 1980 b), resulted in a distribution pattern very different from that of permethrin. Fluridone levels in the pond water treated at 70 ug/L did not drop below 2 ug/L until 12 weeks after application. Unlike the permethrin, the fluridone residues took a long time (10 weeks) to reach maximum concentration in the hydrosoil with very little decrease in concentration up to 45 weeks. Muir postulated that the grasses and cat-

tails in the pond were responsible for sorption of the herbicide and the observed rapid decrease in fluridone concentration in the water.

4.7 PERMETHRIN RESIDUES IN DUCKWEED

The duckweed, like the hydrosoil, was analyzed for residues of total ^{14}C activity (Figure 23). Figure 23-A (1979) indicates that the residue levels for ponds treated with both the methylene and cyclopropyl labelled permethrin were similar. On a dry weight basis, the residue levels reached 60-70 ug permethrin equivalent/g compared to an initial concentration of permethrin in the water of 0.015 ug/mL. ^{14}C labelled material was clearly concentrated by the duckweed.

The maximum total ^{14}C residues in the duckweed in 1980 (Figure 23-B) were approximately 90-100 ug permethrin equivalents/g. Until 15 days post-treatment, the residue levels were similar in both ponds. Beyond 15 days, residues in the duckweed were higher in the pond treated with methylene labelled permethrin. At 112 days, residues (0.2-0.8 ug permethrin equivalent/g) were still detectable in the duckweed but had declined to greater than 1% of the maximum level.

In both 1979 and 1980, at one week post-treatment, the total ^{14}C residue in the duckweed had decreased to 5-10% of maximum concentration. This rate of decrease was much faster than that reported by Gaughan and Casida (1978) who found 70% of the ^{14}C remaining in cotton leaves in the field one week after permethrin application.

Figure 24 indicates the average permethrin residues in the vegetation as detected by HPLC-LSC analyses. Individual results are shown in Appendix D. The results indicate that permethrin was readily sorbed by

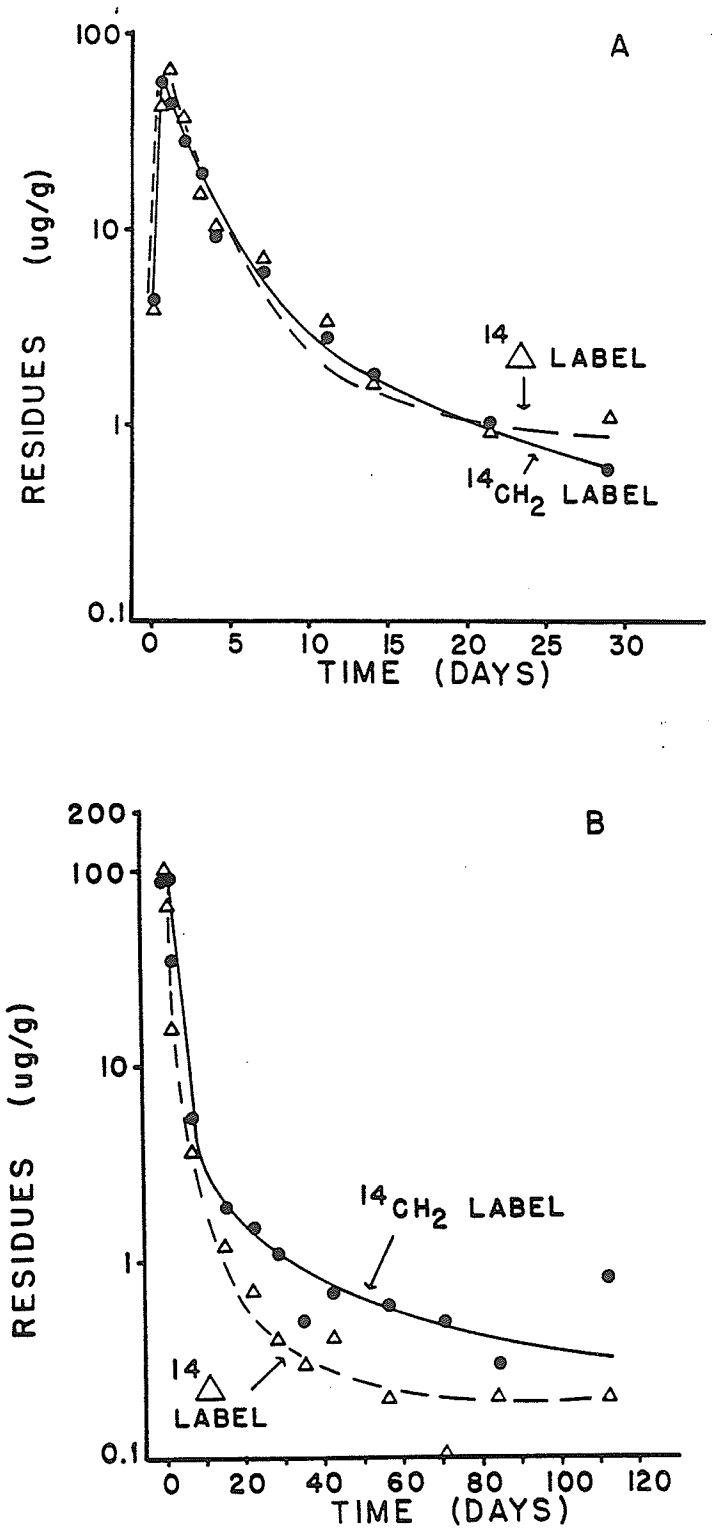


Figure 23: Total ^{14}C residues in duckweed in 1979 (A) and 1980 (B) (ug/g dry weight)

the vegetation. By 24 h the permethrin concentration in the duckweed (on a dry weight basis) was 30 ug/g in 1979 and 55 ug/g in 1980, compared to an initial water concentration of 15 ug/L. The permethrin levels in the duckweed decreased very quickly, reaching less than 0.1 ug/g by 15 and 21 days in 1979 and 1980, respectively.

The high sorption values for permethrin would have been expected for two reasons. The first is that permethrin is a highly lipophilic compound, and, as a result, has an increased affinity for leaf waxes, and, thus, the duckweed would show a high sorption rate. Secondly, the permethrin formulation used was oil based, and, thus, a surface film would have occurred maintaining a higher permethrin concentration in the surface water as shown in Figure 14, and, thus, would have resulted in the increased uptake of permethrin.

Based on the concentration of permethrin in the water and the duckweed at 24 h, the uptake by duckweed represents a concentration factor of 9300-21500 (two year average). Fluridone, which is more persistent in the water than permethrin, had a concentration factor of only 19-85 for duckweed in Glenlea ponds (Muir et al. 1980). Malis and Muir (1980) found that the duckweed concentration factor for fenitrothion was 1500-2000. These results indicate that permethrin is very lipophilic, and may result in very high levels of permethrin in aquatic plants, compared to other pesticides.

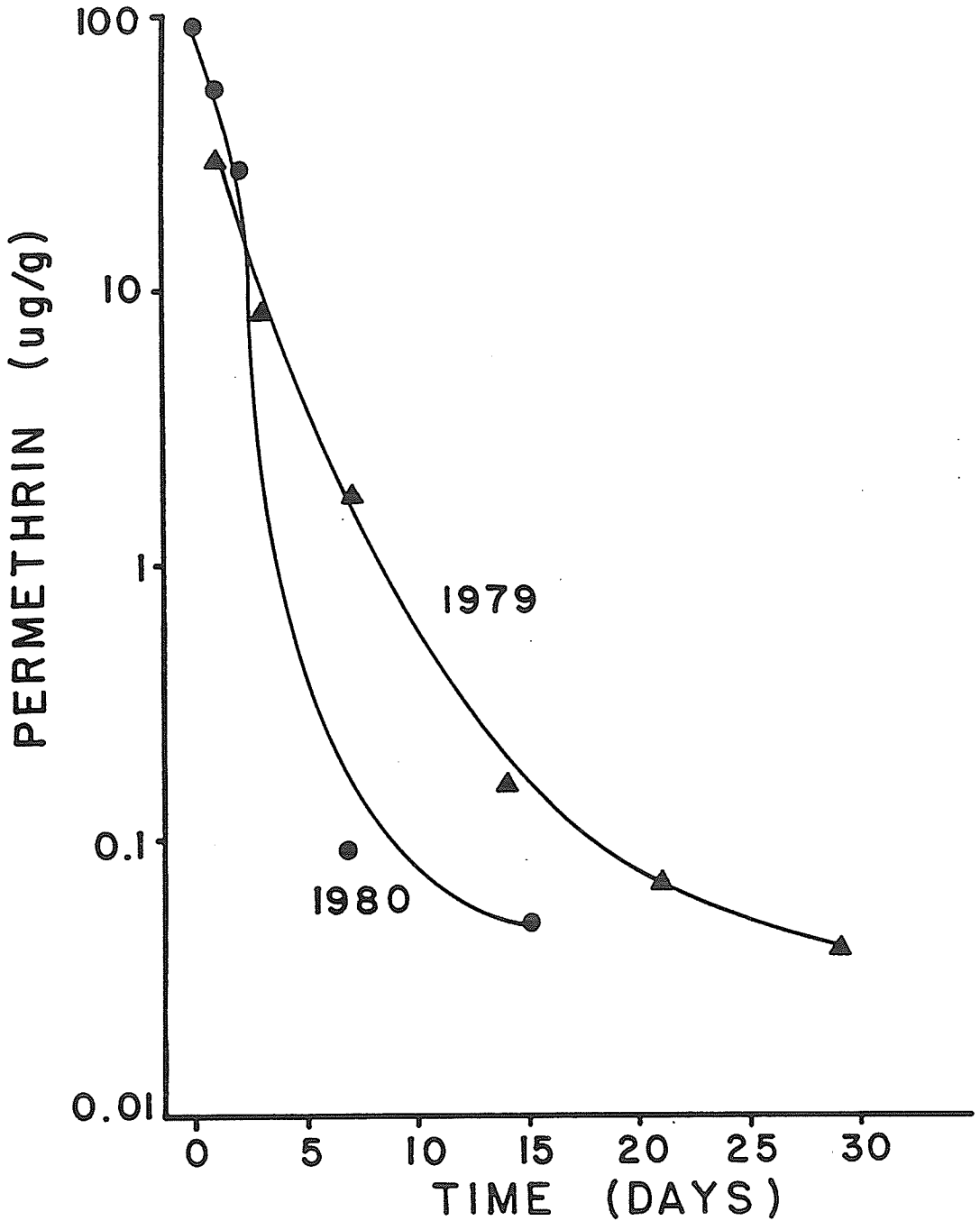


Figure 24: Permethrin concentration in duckweed in 1979 and 1980 (ug/g dry weight)

4.7.1 Growth Inhibition in Duckweed

In both 1979 and 1980, qualitative observation of the two treated ponds indicated a growth inhibition of the duckweed compared to the growth on non-treated ponds. Figures 25-28 illustrate this effect in the 1980 ponds. In both 1979 and 1980, prior to the permethrin application, excess duckweed was scooped from both the contol and treatment ponds (Figures 25 and 26). In both years the duckweed population in the non-treated pond increased rapidly so that within three weeks a thick mat of duckweed covered the entire pond (Figure 27). However, in the permethrin treated ponds, the duckweed growth was inhibited so that at the end of three weeks only a monolayer of fronds along the pond edges was observed (Figure 28).

Even though each pond had been treated with ^{14}C-permethrin labelled in different positions, the ponds were biological replicates. The permethrin in the two treated ponds was identical, as a pesticide. Therefore, the growth inhibition of the duckweed following permethrin application occurred in replicate ponds for two years in a row which supports the hypothesis that the permethrin application did affect the duckweed growth.

The reason for the growth inhibition is not known, but since permethrin disappears very quickly from the water and vegetation, the inhibition may be due to formulation components or to the permethrin metabolites. Stratton et al. (1980) noted that the permethrin degradation products PBaldehyde, PBacid, and PBalc were 3-50 times more toxic than permethrin towards photosynthesis and acetylene reduction in algae. The reduction of these processes by the degradation products could explain

Figure 25: Non-treated pond at time of application

Figure 27: Non-treated pond at three weeks post-treatment

Figure 28: Treated pond at three weeks post-treatment

the growth inhibition of the duckweed. As well, the diphenyl ether metabolites of the permethrin alcohol moiety resemble the structure of several herbicides such as nitrofen and diclofop-methyl and may be the cause of the duckweed growth inhibition.

4.8 PERMETHRIN RESIDUES IN FATHEAD MINNOWS

In 1979, only the total ^{14}C residue in the fish was monitored. Within 24 h of the introduction of the fathead minnows to the treated ponds (3 days post-treatment), considerable uptake of ^{14}C labelled compound had occurred (Table 25).

TABLE 25

Uptake of ^{14}C Label by Fathead Minnows in Ponds (ug permethrin equivalent/kg wet weight fish)

Time	^{14}C-cyclopropyl		^{14}C-methylene	
	ug/kg	CF	ug/kg	CF
3 d	----------Fish		Added----------	
4 d	326	181	97	139
11 d	57	29	107	178
14 d	93	42	125	250
21 d	176	80	158	316
29 d	231	105		

The concentration factor is based on the total ^{14}C concentration in fish divided by the average of the total ^{14}C concentration in the water during exposure from day three until the fish sample was collected. The concentration factors indicate that the methylene label was taken up by the minnows to a greater extent than the cyclopropyl label, as occurred with the hydrosoil results, consistent with the polarity of the respec-

tive metabolites; i.e., the cyclopropyl products (carboxylic acids), being more polar than the methylene labelled products, are not taken up to the same extent by the fish.

In 1980, permethrin uptake by the fathead minnows was monitored by HPLC-LSC (Table 26). The results indicate that, by 2 h, the fish had sorbed considerable amounts of permethrin. Even though the subsurface water contained only 1.1 ug/L permethrin at 2 h, the fish contained 1.06 mg permethrin/kg (wet weight). The permethrin residues in the fish decreased very quickly, so that by four weeks post-treatment, the fish contained no detectable permethrin. Even though the fish contained no permethrin, the analyses of the fish extract indicated the presence of ^{14}C residues. When the last fish samples were caught, the fish in the cyclopropyl labelled pond, at 10 weeks, contained 41 ug permethrin equivalents/kg and at 12 weeks the fish in the methylene labelled pond contained 263 ug permethrin equivalents/kg.

The rapid uptake and elimination of permethrin in the fathead minnows was similiar to that observed in rainbow trout by Glickman et al. (1981). In the trout, the major degradation pathway was the elimination of polar metabolites in the bile.

Even though the permethrin level in the fathead minnows reached 1.06 mg/kg by 2 hours post-treatment, no fish mortality was observed even though the 96 h LC_{50} for fathead minnows is 2.6 ug/L (Chipman Chemicals 1978). The lack of mortality was probably due to the rapid decrease in permethrin concentration in the water and the rapid elimination of permethrin from the fish which resulted in a short exposure period and, on the average over 96 hours, a low permethrin concentration in the water.

TABLE 26

Permethrin Uptake by Fathead Minnows (ug/kg wet weight)

Time	Cyclopropyl Pond		Methylene Pond	
	Permethrin	^{14}C	Permethrin	^{14}C
2 h	1063	1479		
24 h	88	150		
48 h	89	213		
72 h	----------	Fish	Added	----------
96 h	16	92	36	187
7 d	ND	30	4	68
15 d	ND	8	4	108
28 d	ND	49	ND	124
35 d	--	61	ND	197
42 d	--	--	ND	195
56 d	ND	104	--	---
70 d	--	41	--	---
84 d	--	--	--	263

Kenaga and Goring (1980) reported a relationship between water solubility and chemical concentration in biota for terrestial-aquatic ecosystems, based on the equation

$$\log BCF = 2.183 - 0.629 \text{ (log water solubility (mg/L))}$$

The resulting BCF for permethrin was calculated to be 419. Based on the subsurface cyclopropyl labelled permethrin concentration, the concentration factor at 2, 24, and 48 h for permethrin by the fat-head minnows were 1500, 125, and 125, respectively. Kenaga and Goring's model failed to predict the high concentration factor that occurred after application or account for the rapid drop in values at 24 and 48 hours.

4.9 PERMETHRIN VOLATILITY

Volatilization of permethrin from the pond water was not monitored. In order to have some idea of the importance of permethrin volatilization as a loss mechanism from the water, the rate of permethrin volatilization was predicted by the method of Mackay and Leinonen (1975). The overall liquid mass transfer coefficient ($K/_L$) for permethrin was calculated from the following equation:

$$1/K_L = 1/k_L + RT/Hk_G$$

$$= 2122 \text{ m/h}$$

$$K_L = 4.7 \times 10^{-4} \text{ m/h}$$ where:

k_L = liquid phase mass transfer coefficient (m/h) for permethrin.

$= 6.7 \times 10^{-2}$ m/h

k_G = vapour phase mass transfer coefficient (m/h) for permethrin.

$= 6.4$ m/h

R = gas constant (8.2×10^{-5} m^3atm/mol K)

H = Henry's constant for permethrin

$= 1.75 \times 10^{-6}$ atm m^3/mol

T = absolute temperature (K)

Equations for obtaining k_L, k_G, and H are shown in Appendix E.

The permethrin K_L value of 4.7×10^{-4} m/h was in the range of other low water solubility insecticides such as DDT, lindane, and dieldrin (Mackay and Leinonen 1975).

The relative magnitude of resistance in the liquid phase was found to be 0.71% indicating that permethrin volatilization was vapour phase controlled which also agreed with the results for DDT, lindane, and dieldrin.

From the K_L value, the permethrin half-life in the pond due to volatility was calculated.

Half-life (h) = $(1/K_L)$ (0.69) (Depth (m))

= 440 h

The permethrin volatility half-life of 440 h was very long and loss through volatilization could be considered to be negligible when compared to the half-lives for loss in water due to photolysis, hydrolysis, or microbial action of 18.1 h and 4.2 h due to hydrosoil sorption.

The following equation was used to predict the permethrin concentration in the water due to loss solely through volatilization, at a given time post-treatment:

$$C_i = C_o \exp(-K_L\ t/\text{depth})$$

For example, in the methylene labelled pond (1979) the equation predicted a decrease in the initial permethrin concentration in the water from 15.6 ug/L to 15.3 ug/L at 24 h due solely to volatilization. However, the actual concentration at 24 h was 1.7 ug/L, not 15.3 ug/L, which further indicates that permethrin volatility was not a very important loss mechanism.

4.10 MASS BALANCE

From the 24 h data of the 1979 pond treated with methylene labelled permethrin, a mass balance was constructed to see how much of the applied radioactivity could be accounted for. The results for the water, duckweed, and hydrosoil residues are based on the total ^{14}C activity detected. The volatility loss is based on the calculations in the previous chapter. The value for the surface grass is an assumption based on

the area/weight relationship of duckweed and its rate of uptake. Calculations for the mass balance are shown in Appendix F.

Table 27 shows the mass balance at 12, 24, 48 h, and 14 days post-treatment for the pond treated with methylene labelled permethrin (1979). At 12 h, the largest percentage of ^{14}C activity (25.1%) was accounted for in the water. However, by 24 h the hydrosoil accounted for 30.5% of the residue and 40.9% by 48 h. The data clearly illustrates that the hydrosoil was the major sink for the permethrin in the pond system. It is also clear that although total ^{14}C activity was being monitored, only 60.1-76.3% of the activity could be accounted for during the first few days of the experiment and only 24.6% by 14 days post-treatment.

A number of factors may have resulted in the low recovery of activity. The most important factor may be the differences in the pond sediment and the hydrosoil added for sampling purposes. The hydrosoil, which was the major permethrin sink, had an organic matter content of 3-4% compared to approximately 12% in the sediment. Therefore, the sediment, with its higher organic matter content, may sorb the permethrin to a greater extent than the hydrosoil and thus account for more of the residue than estimated in the mass balance calculations.

Sampling of the hydrosoil in jars compared to core sampling of the sediment was preferred since it did not destroy the pond bottom or polyethylene liner, the samples were more consistent, and the permethrin concentration was not diluted by taking large core samples with a small surface area. An improvement in the sampling procedure might be the use of sediment in the jars.

TABLE 27

Mass balance of ^{14}C activity in pond treated with methylene labelled permethrin, 1979

Component	% of Initial Permethrin Added			
	12 h	24 h	48 h	14 days
Water	25.1	10.7	6.8	1.6
Hydrosoil	9.4	30.5	40.9	22.2
Duckweed	15.3	13.4	8.7	0.6
Surface grass*	6.3	5.5	3.5	0.2
Volatility	4.0	6.0	16.4	---
Total accounted for	60.1	66.1	76.3	24.6
Unaccounted for	39.9	33.9	23.7	75.4

--- Volatility value at 14 days was not calculated since there was no permethrin in the water beyond 72 h.
* Grass along edge of pond that is partially submerged.

The surface application of permethrin to the pond may also have affected the mass balance. Scott et al. (1981) reported higher residues in the water if the pesticide was introduced from under the water surface compared to surface application. It is possible that some permethrin was lost during application.

Another source of error in the mass balance calculations are the many assumptions made. For example, the volume of sediment, duckweed, and surface grass. As well, permethrin volatility was based on physical parameters and not quantitatively measured.

Even though the volatility measurement was based on permethrin itself, loss of ^{14}C activity due to volatilization of degradation products would also be limited since the acid products would be in their anionic form at pH 7.5 - 9.0 and thus not very volatile. Thus, degradation product volatility would have contributed very little error to the mass balance.

Even with the shortcomings mentioned, the artificial outdoor ponds provide a very useful research tool. More precise models could be established in the laboratory, but the principle advantage of the ponds is that they provide controlled replicated experimental sites which resemble, as closely as possible, conditions in the natural environment.

Chapter V

CONCLUSIONS

At the application rate of 15 ug/L, permethrin was found to be an effective larvicide against Aedes larvae with the permethrin quickly disappearing from the water with less than 1% of the applied concentration detected 5-6 days post-treatment. The five degradation products detected in the water were more persistent in the water than the permethrin, with the cyclopropyl acid isomers being detected up to five weeks post-treatment.

Duckweed readily sorbed the permethrin from the water resulting in a concentration factor of 9300-21500. By 29 days post-treatment, the permethrin levels had decreased to levels less than 0.1 ug/g. The high residue levels in the duckweed may explain the growth inhibition that was observed in the duckweed population in the permethrin treated ponds.

Fathead minnows also readily sorbed the permethrin from the water, but the permethrin levels in the fish decreased very quickly probably through either metabolism or excretion of the permethrin.

Permethrin persistence was found to be longest in the hydrosoil. In the 1979 and 1980 treated ponds, permethrin was still detected in the hydrosoil at 420 and 323 days, respectively, at 0.25 and 4.9 ug/kg.

In view of permethrin's efficacy as an insecticide and its low mammalian toxicity, permethrin appears to have the potential as a replacement for some of the organophosphate insecticides used today. However, its

persistence in the hydrosoil greatly reduces its suitability as an insecticide. Before widespread use of permethrin in the environment occurs, further studies investigating permethrin's persistence in the hydrosoil should be undertaken. Such studies should include the identification of the degradation products in the hydrosoil, bioavailability of hydrosoil residues, and the effect of various sediment types on permethrin sorption.

Appendix A

MAJOR/MINOR PERMETHRIN METABOLITES DETECTED IN VARIOUS SYSTEMS

Major Permethrin Metabolites	Soil	Photo	Cock-roach	Plant	Mam-mals	Fish Micro-somes
t-HO-Per					6	
4'-HO-Per	2					8
4'-HO,t-HO-per					7	
PBalc	1,2	3			6,7	8
4'-HO-PBalc						8
PBalc-Glyc				5		
PBalc-conj (plant)				5		
3-HO-Benzyl alcohol		3				
PBacid	1,2	3	4		7	
PBacid-gly					6,7	
PBacid-gluc					7	
PBacid-glutamic acid			4		6	
PBacid-glutamine			4			
4'-HO-PBacid			4			
2'-HO-PBacid-sulfate					7	
4'-HO-PBacid-sulfate					7	
4'-HO-PBacid-gluc			4			
Benzoic acid		3				
Cl_2CA	1,2	3	4		7	8
c-HO-Cl_2CA	2		4		6,7	
t-HO-Cl_2CA	2		4			
c-HOCl_2CA-lactone					6,7	
HO-Cl_2CA-conj (plant)				5		
Cl_2CA-gluc			4		6 7	
Cl_2CA-glycine			4	5		
Cl_2CA-glutamic acid			4			
Cl_2CA-glutamine			4			
Cl_2CA-conj (plant)				5		
Carbon dioxide	1					

1= Kaufman et al. (1977).
2= Kaneko et al. (1978).
3= Holmstead et al. (1978).
4= Shono et al. (1978).
5= Gaughan and Casida (1978).
6= Gaughan et al. (1978) (cows).
7= Gaughan et al. (1977). (rats).
8= Glickman et al. (1979).

Appendix A (continued)

Minor Permethrin Metabolites	Soil	Photo	Cock-roach	Plant	Mam-mals	Fish Micro-somes
t-HO-Per			4	5	7	8
c-HO-Per			4		6,7	8
t-HO-per-gluc					6	
c-HO-per-gluc					6	
monochloro-per		3				
2′-HO-Per				5	7	
4′-HO-Per			4	5	6,7	
4′-HO-c-per-gluc			4			
4′-HO-t-HO-c-per			4			8
4′-HO-t-HO-c-per-gluc			4			
4′-HO-t-HO-per				5	6	8
Dimethylacrylate		3				
PBalc			4	5		
PBalc-gluc					6	
4′-HO-PBalc			4			
PBald		3				8
Benzyl alcohol		3				
Benzaldehyde		3				
PBacid						8
3-HO benzoic acid		3				
PBacid-gluc					6	
4′-HO-PBacid-sulfate					6	
ClCA		3				
Cl_2CA				5	6	
Cl_2CA-glut					6	
c,t-HO-Cl_2CA				5	6,7	8
c-HO-Cl_2CA-lactone				5		8
c-HO-c-Cl_2CA-lactone			4			

1= Kaufman et al. (1977).
2= Kaneko et al. (1978).
3= Holmstead et al. (1978).
4= Shono et al. (1978).
5= Gaughan and Casida (1978).
6= Gaughan et al. (1978) (cows).
7= Gaughan et al. (1977) (rats).
8= Glickman et al. (1979).

Appendix B

SIMULATION MODEL FOR PESTICIDE DISTRIBUTION IN POND

Marshall and Roberts (1977) based their simulation model on the following equations.

$$V_w \, d\, C_w/dt = k_{hw}V_hC_h - k_{wo}V_wC_w - k_{wh}V_hC_w$$

$$V_h \, d\, C_h/dt = k_{wh}V_hC_w - k_{hw}C_hV_h - k_{ho}V_hC_h$$

C_w = concentration of insecticide in water (ug/g).

C_h = concentration of insecticide in hydrosoil (ug/g).

k_{ho} = hydrosoil dissipation rate constant (day^{-1}).

k_{hw} = hydrosoil desorption rate constant (day^{-1}).

k_{wh} = hydrosoil sorption rate constant (g water/g sediment/day).

k_{wo} = water dissipation rate constant (day^{-1}).

V_w = effective size of water (g).

V_h = effective size of hydrosoil (g).

The rate constants were determined in the following manner.

1. k_{wo} was derived from the slope of a semi-ln plot of the permethrin concentration in the water of the photolysis experiment vs time.
2. k_{wh} was obtained from a plot of total ^{14}C uptake in the hydrosoil during the first two days. The slope of the plot = (k)(initial concentration in water).
3. k_{hw} was obtained using the following equation of $k_{hw} = (1/(k_{OC} \times OC)(S) + 1)k_{wh}$. Where K_{OC} = 10600, OC = 3.4%, and S = solids/total weight = 0.5.

4. k_{ho} was derived from a plot of ln C_h vs time.

The solutions of the above equations are known to be the following (Cho 1981).

$$K_1 = 1/V_w \ (k_{wo}V_w + k_{wh}V_h)$$

$$K_2 = 1/V_w \ (k_{hw}V_h)$$

$$K_3 = 1/V_h \ (k_{hw}V_h + k_{ho}V_h)$$

$$K_4 = 1/V_h \ (k_{wh}V_h)$$

$$r' = -1/2 \ (K_1 + K_3) + 1/2 \ [(K_1 + K_3)^2 - 4(K_1K_3 - K_2K_4)]^{1/2}$$

$$r'' = -1/2 \ (K_1 + K_3) - 1/2 \ [(K_1 + K_3)^2 - 4(K_1K_3 - K_2K_4)]^{1/2}$$

Therefore, the equations used to predict the concentration of permethrin in the hydrosoil and water are the following.

$$C_h = K_4C^o/(r'-r'') \ [e^{r't} - e^{r''t}]$$

$$C_w = C^o/(r''-r') \ [(K_1 + r'') \ e^{r't} - (K_1 + r') \ e^{r''t}]$$

Where C^o is the initial concentration in the water.

Appendix C

PERMETHRIN LEVELS IN HYDROSOIL, 1979 AND 1980

Time (days)	Permethrin Concentration (ug/kg)			
Pond	3	7	2	9
.08			4	3
1	87	90	39	37
2	100	140	19	9
7	21	140	27	42
14	10	14	35	41
21	2	8	48	41
28	25	2		
35			11	2
56			3	13
70			12	31
112			12	3
259	0.6	5		
323			9	0.8
365	0	2		
420	0	0.5		

Appendix D

PERMETHRIN RESIDUES IN DUCKWEED (UG/G DRY WEIGHT)

Time (days)	Permethrin Concentration	
	Methylene labelled	Cyclopropyl labelled
1979		
1	31	28
3	9	8
7	1	2
14	0.2	0.1
21	0.1	0.03
29	0.06	0.02
1980		
.08	118	74
1	78	31
2	29	26
7	0.9	1
15	ND	0.1

Appendix E

VOLATILITY CALCULATIONS

Henry's constant was derived from the following equation.

$$k' = P/m$$

Where,

k' = Henry's constant

P = Vapour pressure of permethrin = 3.4×10^{-7} mm Hg.

m = Molal concentration = 2.56×10^{-4} moles/m^3.

$k' = 1.75 \times 10^{-6}$ atm m^3/mole.

The liquid phase and vapour phase mass transfer coefficients were determined as follows.

k_L = (liquid phase mass transfer rate)(mol wt CO_2/mol wt permethrin)$^{1/2}$.

= (0.2 m/h)(44/391)$^{1/2}$.

= 6.7×10^{-2} m/h.

k_G = (Vapour phase mass transfer rate)(mol wt H_2O/mol wt permethrin)$^{1/2}$.

= (30 m/h)(18/391)$^{1/2}$.

= 6.4 m/h.

Appendix F

MASS BALANCE CALCULATIONS FOR METHYLENE LABELLED POND, 1979

Sediment

- Total sediment surface area (bottom + sides) = 145000 cm^2
- Total volume of soil = (145000 cm^2)(0.5 cm) = 72500 cm^3
- Weight of soil = (72500 cm^3)(0.8 g/cm^3) = 58000 g

Duckweed

- 0.00703 g dry weight/cm^2
- surface area of pond = 66430 cm^2
- with 20% coverage of pond = 93.4 g duckweed (dry weight)

Surface grass along pond edge (5 cm strip)

- area = 5470 cm^2
- 0.00703 g dry weight/cm^2 (from duckweed)
- weight of surface grass = 38.5 g

Component		Weight Permethrin (mg)	%
Water (1.66 ug/L)(2000 L)		3.32	10.7
Sediment (163.4 ug/kg)(58000 g)		9.48	30.5
Duckweed (44618 ug/kg)(93.4 g)		4.16	13.4
Surface grass (44618 ug/kg)(38.5 g)		1.72	5.5
Volatility		1.87	6.0
	Total	20.55	66.1
	Remainder	10.49	33.9
	Added	31.04	

Appendix G

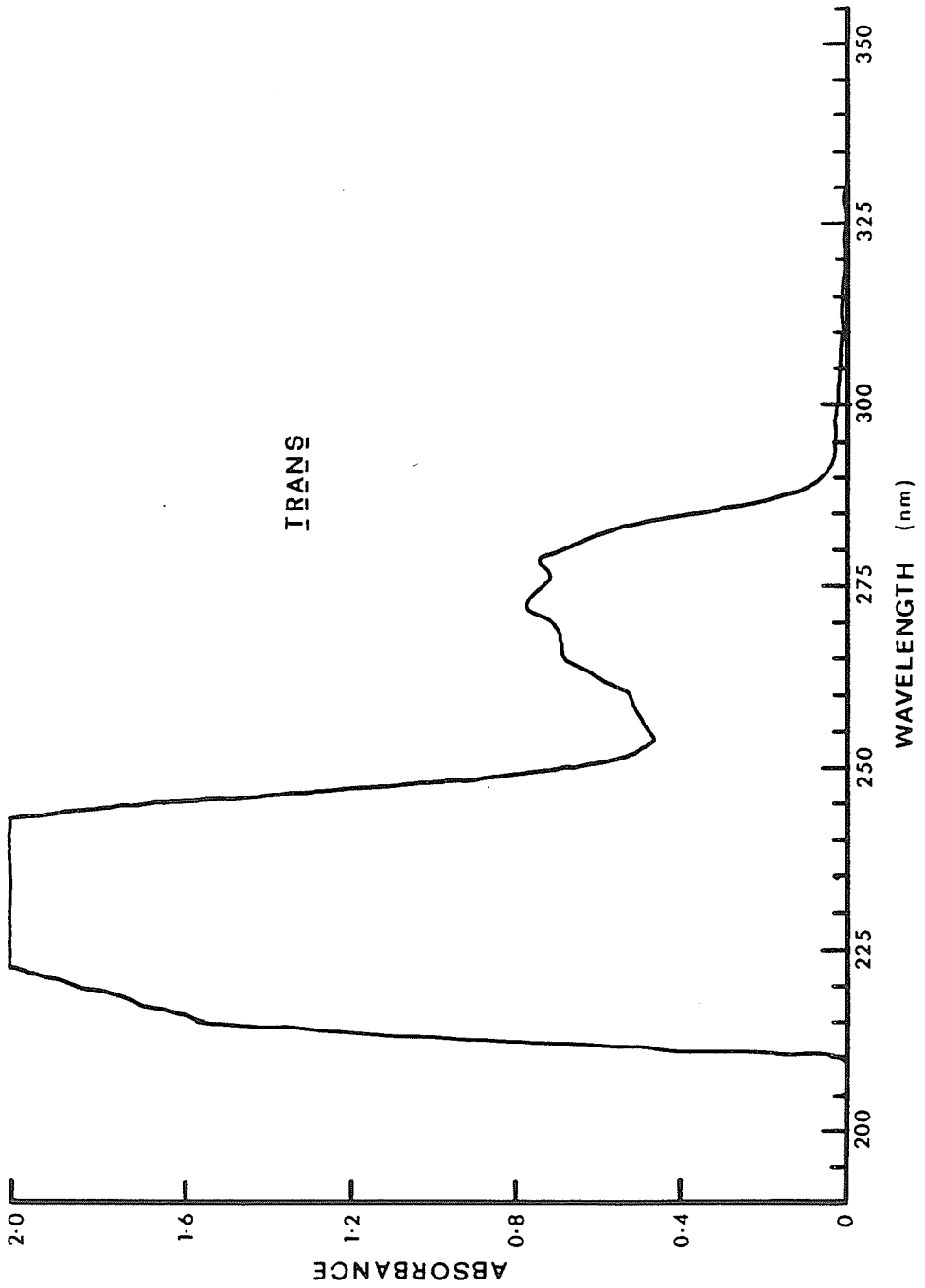
POND TREATMENTS

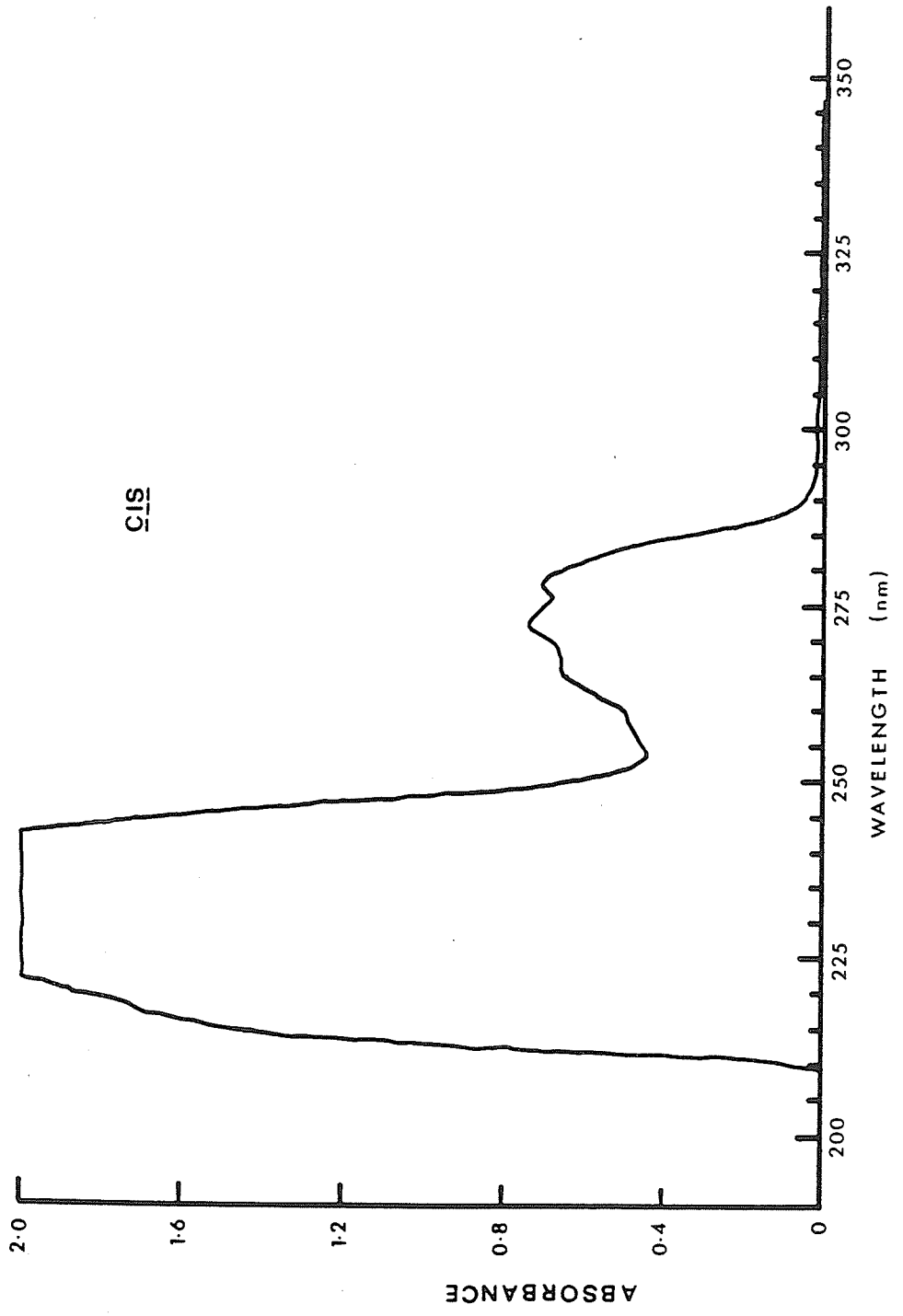
	LABEL		CONTROL
	Cyclopropyl (88 uCi)	Methylene (69 uCi)	
1979	3	7	2
1980	9	2	5

Appendix H

UV SPECTRA OF PERMETHRIN ISOMERS

The results of the spectra analysis indicate that permethrin absorbs very little UV light above the 290 nm wavelength found in sunlight at the earth's surface. Therefore, it is postulated that photosensitizers, such as humic acids in the pond water, maybe be responsible for initating the photoisomerization reaction. Sensitized photoisomerization of cis- to trans-1, 3-pentadiene in humic acid solutions has been described by Zepp et al. (1981).


TRANS
ABSORBANCE
2·0
1·6
1·2
0·8
0·4
0
200
225
250
275
300
325
350
WAVELENGTH (nm)


CIS
ABSORBANCE
2·0
1·6
1·2
0·8
0·4
0
200
225
250
275
300
325
350
WAVELENGTH (nm)

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