



National Library of Canada
Collections Development Branch

Canadian Theses on
Microfiche Service

Bibliothèque nationale du Canada
Direction du développement des collections

Service des thèses canadiennes
sur microfiche

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

**THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED**

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

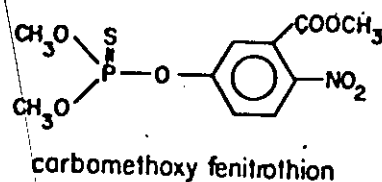
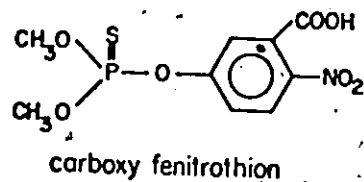
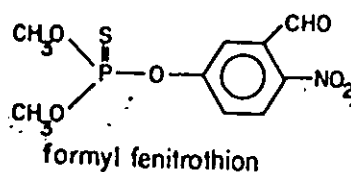
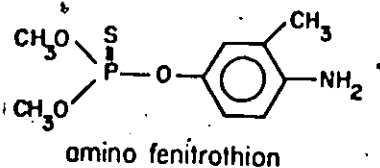
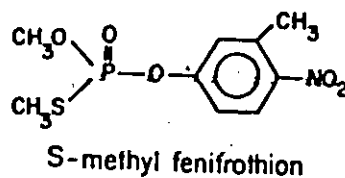
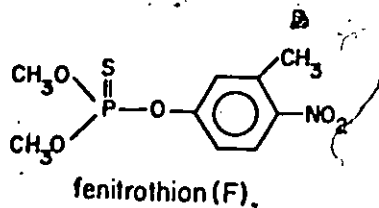
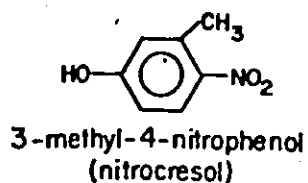
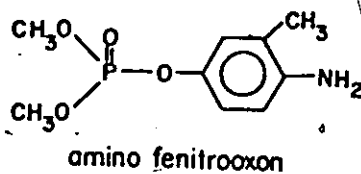
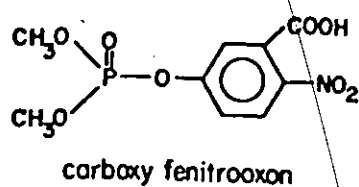
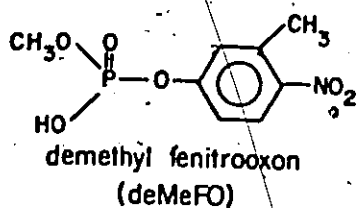
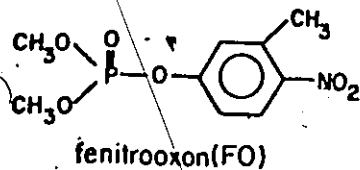
S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de mauvaise qualité.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

**LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE**



Fenitrothion and some metabolites.

Abstract

Fenitrothion, O,O-dimethyl-3-methyl 4-nitrophenyl-phosphorothioate, partitioned into sediment, water, aquatic plants, algae, and particulate matter in a fresh-water aquatic system.

Sediment and aquatic plants were found to be the major real or potential reservoirs for fenitrothion in a shallow (1 m.) aquatic model.

Significant pesticide degradation occurred in sediment, the major degradation products being amino-fenitrothion, fenitro-oxon, and S-methyl-fenitrothion.

Aquatic plants showed a high bioaccumulation of fenitrothion and some species contained significant levels of the degradation products, 3-methyl-4-nitrophenol, fenitro-oxon and S-methyl fenitrothion.

Mass balance calculations show that fenitrothion degraded readily in the sediment (est. $t_{1/2}$ = 6 days), water (est. $t_{1/2}$ = 46 hrs.) but much slower in some aquatic macrophytes (eg. Elodea sp. est. $t_{1/2}$ = 20-37 days).

This study and other work indicates that there is a small probability of fenitrothion persistence above the 0.01 ppm level in most of the aquatic fresh-water system except the plants.

The subject of pesticide persistence in aquatic macrophytes may require further study.

Résumé

Dans un système aquatique d'eau douce le fénitrothion, 0,0 diméthyl 3 méthyl 4 nitrophényl phosphorothioate se morcelle dans le sédiment, l'eau, les plantes aquatiques, les algues et les particules de matière.

Les principaux réservoirs réels ou latents de fénitrothion trouvés dans un modèle aquatique peu profond (1m) sont le sédiment et les plantes aquatiques.

Une dégradation significative du pesticide se produit dans le sédiment. Les principaux produits dégradés sont l' amino-fénitrothion, le fénitro-oxon et le S méthyl fénitrothion.

Les plantes aquatiques ont démontré une accumulation importante de fénitrothion. Certaines espèces possèdent des produits du pesticide dégradé tels que le méthyl 4 nitrophénol, le fénitro-oxon et le S méthyl fénitrothion.

Des calculs de masse totale ont démontré que le fénitrothion se décompose rapidement dans l'eau (est. $t_{1/2}$ = 46 hres), dans le sédiment (est. $t_{1/2}$ = 6 jours) mais beaucoup moins rapidement dans les plantes (est. $t_{1/2}$ = 20-37 jours).

Cette étude ainsi que d'autres travaux démontrent qu'il y a une petite probabilité de persistance du fénitrothion au dessus du niveau de 0.01 p.p.m. dans la plupart des systèmes aquatiques d'eau douce à l'exception des plantes aquatiques.

La persistance du pesticide dans les macrophytes aquatiques pourrait demander une étude plus approfondie.

Table of Contents

	<u>page no.</u>
A- <u>Introduction</u>	1
a) Water	2
b) Hydrolysis	4
c) Sediment	6
d) Chemical hydrolysis	8
e) Soil biota	9
f) Plants	13
g) Hypothesis	17
B- <u>Materials and Methods for Laboratory Study</u>	
a) Materials	
i) Chemicals	19
ii) Water, sediment, aquatic plants	20
b) Description of Laboratory Models	20
c) Sampling Schedule	22
d) Extraction Methodology	22
i) Water	22
ii) Plants	22
e) Analyses	
i) Scintillation counting	23
ii) Thin Layer Chromatography	24
iii) Autoradiography	24

	<u>page no.</u>
B2) <u>Materials and Methods for the Field Study</u>	
Description of Field Models	25
Materials	
a) Lake water	25
b) Sediment	26
c) Aquatic plants	26
1) Distribution of Samples	26
2) Fenitrothion Application	27
3) Sampling schedule	27
a) Water	27
b) Plants; c) Sediment	28
4) Extraction Methodology,	
a) Water	28
b) Plants; c) Sediment	29
5) Analysis	
a) Clean-up Procedure	30
b) Gas Liquid Chromatography	31
c) Thin Layer Chromatography	31
d) Physical Parameters of Lake Water	31
C- <u>Results</u>	
Check on Methodology	
Physical Parameters of Lake Water	33
Check on Clean-up Method	33
Check on Sediment Extraction	34
Minimal Detectable Levels TLC, Autorad.	34
Quench Curves	35

C- <u>Results</u>	<u>page no.</u>
Model One	35
Model Two	36
(Comparison of Models One and Two	38
Model Three	39
Model Four	42
Bioaccumulation Ratios	45
Field Model	46
Water Wash of the Sediment	49
Mass Balance	50
D- <u>Discussion</u>	
Model One	53
Model Two	55
Model Three	60
Model Four	65
Bioaccumulation Ratios	68
Field Model	70
Sediment	73
Mass Balance	82
Secondary Effects in the Aquatic System	84
E- <u>Summary and Conclusions</u>	89
F- <u>Suggestions for Further Research</u>	90
G- <u>Literature Cited</u>	92
H- <u>Tables, Figures and Plates</u>	107

List of Tables and Figures

	<u>page no.</u>
Table 1- Summary of Experimental Models	19-2
Table 2- Formulations	19-2
Figure 1: Method of Plant Extraction and Clean-up	23-2
Figure 2: Sample Distribution in the Field Model	27
Figure 3: Method of Soil Extraction	30-2
Table 3: Recoveries from the Clean-up Column	34-2
Table 4: Recoveries of Fenitrothion from Sediment	34-2
Figure 4: Distribution of C-14 Activity, Model 1	35-2
Table 5A: Fenitrothion in Water, Model 1	36-2
Table 5B: Fenitrothion in Water as a % Model 1	36-3
Figure 5: Distribution of C-14 Activity, Model 2	36-8
Figure 6: Fenitrothion in <u>E. densa</u> Model 2	37-2
Table 7A: Derivatives in <u>E. densa</u> , Model 2	37-7
Table 7B: Derivatives in <u>E. densa</u> , as a %, Model 2	37-8
Table 8A: Derivatives in the Water of Model 2	38-6
Table 8B: Derivatives in the Water of Model 2, as a %	38-7
Figure 7: Distribution of C-14 Activity in Model 3	39-2
Figure 8: Fenitrothion Uptake by <u>E. canadensis</u> , Model 3	40-4
Figure 9: Fenitrothion Concentration in <u>E. canadensis</u>	40-5
Table 9A: Fenitrothion in Water, Model 3	41-4
Table 9B: Fenitrothion in Water, as a % Model 3	41-5
Table 9C: Bioaccumulation Ratios in <u>E. canadensis</u>	41-6
Figure 10: Distribution of C-14 Activity in Model 4	42-2
Figure 11: Fenitrothion in Several Plants, Model 4	43-2
Table 10A: Derivatives in Several Plants, Model 4	43-5
Table 10B: Derivatives in Several Plants, as a %	43-6

List of Tables and Figures

	<u>page no.</u>
Table 11A: Derivatives in Water, Model 4	44-2
Table 11B: Derivatives in Water, as a %, Model 4	44-3
Table 12: Bioaccumulation Ratios in 3 Plants	45-2
Figure 12: Fenitrothion Distribution in the Field	46-2
Figure 13A: Fenitrothion in Water, Lighted Field Model	47-2
Figure 13B: Fenitrothion in Water, Dark Field Model	47-3
Figure 14: Fenitrothion in <u>E. canadensis</u> , Lighted Model	47-4
Figure 15: Fenitrothion in <u>E. canadensis</u> , Dark Model	48-2
Figure 16: Fenitrothion in Sediment, Field Model	48-5
Table 13: Derivatives in the Sediment, Field Model	48-10
Figure 17A: Derivatives in the Water Wash of Sediment	49-2
Figure 17B: Derivatives in the Water Wash of Sediment	49-3
Table 14: Derivatives in the Water Wash of Sediment	50-2
Table 15: Mass Balance in the Lighted Field Model, 2 days	51-2, 3
Table 16: Mass Balance in the Dark Field Model, 2 days	51-4, 5
Table 17: Mass Balance in the Lighted Field Model, 6 days	51-6, 7
Table 18: Mass Balance in the Dark Field Model, 6 days	51-8

List of Plates

page no.

Plate 1: Minimal Detectable Amounts of Fen. by TLC	24-2,3
Plate 2: Minimal Detectable Amounts of Fen; autoradiography	24-4,5
Plate 3: TLC of Water Extract, Model 1	36-4,5
Plate 4: Autoradiograph of Water Extract, Model 1	36-6,7
Plate 5: TLC of <u>E. densa</u> Extracts, Model 2	37-3,4
Plate 6: Autoradiograph of <u>E. densa</u> Extract Model 2	37-5,6
Plate 7: TLC of Water Extract, Model 2	38-2,3
Plate 8: Autoradiograph of Water Extract, Model 2	38-4,5
Plate 9: TLC of <u>E. canadensis</u> Extracts, Model 3	40-2,3
Plate 10: TLC of Water Extract, Model 3	41-2,3
Plate 12: Autoradiograph of Plant Extracts, Model 4	43-3,4
Plate 13: TLC of Water Extracts, Model 4	44-4,5
Plate 14: Autoradiograph of Water Extract, Model 4	44-6,7
Plate 15: TLC of <u>E. canadensis</u> Extract, Field Model	48-3,4
Plate 16: Tlc of Sediment Extracts, Field Model	48-6,7
Plate 17: TLC of Sediment Extracts, Field Model	48-8,9

Acknowledgements

I would like to express my gratitude to my supervisor, Dr. Pearl Weinberger, for her support, vision and patience throughout the period of this study.

I would also like to thank Dr. Roy Greenhalgh, Canada Department of Agriculture, for technical advice and constructive criticism in solving some difficult analytical problems, and Mrs. M. Wilson, Canada Department of Agriculture, for instruction in the use of the gas chromatograph.

Special thanks go to a laboratory colleague, Mr. R. Moody, for his assistance and helpful discussions in this work. A note of appreciation is given to Mr. J. Helies for his preparation of the figures in the text.

This thesis is dedicated to Miss C. Zufferey who offered much encouragement during this time.

Introduction

Fenitrothion, 0,0-dimethyl-3-methyl 4-nitrophenyl-phosphorothioate, is an organophosphorus pesticide used throughout the world for the protection of many commercial crops against the predation of a wide spectrum of insect pests. In Canada, large amounts are primarily used to protect Abies balsamea and Picea glauca against defoliation by the spruce budworm, Choristoneura formiferana.

A review of all environmental and other information concerning fenitrothion was undertaken at the Fenitrothion Symposium 1977 (Roberts et al) and the Panel Report following this Symposium identified "important areas of ignorance that precluded definitive answers" as to whether there was a link between Reye's Syndrome and the pesticide spray. One of the areas requiring further research was the fate of fenitrothion in the freshwater aquatic ecosystem. Limited data indicated that fenitrothion disappeared from the water in one to three days. Little information on the pesticide's eventual fate in the aquatic system was available. The Panel suggested that "it is still not clear whether the rapid disappearance of the insecticide from the water is associated with its sorption by the sediments or with degradative processes". (p.572, Roberts et al). The Panel identified a need for "a systemic examination of the quantitative rate relations which define the dynamics of the pesticide formulations within the terrestrial and aquatic ecosystems and hence in the micro-reservoirs". (p.576, Roberts et al, 1977).

It is suggested in this work that aquatic plants and sediment act as important micro- and macro-reservoirs for fenitrothion in a model aquatic system, and that both contribute to the degradation of the parent

compound to several derivatives, some of which are toxic. An attempt was made to determine a mass balance for the pesticide in the aquatic system to show the relative importance of each compartment in the binding and degradation of fenitrothion.

In an aquatic system, the partitioning of the pesticide may be an equilibrium involving the movement of the pesticide in the water, sediment, plants, algae, bacteria and particulate matter (Marshall and Roberts 1977). On the basis of mass and surface area, the major reservoirs of the pesticide should be the water, plants and sediment. A review of the existing information on the behaviour of the pesticide in each of these components in laboratory and field experiments would allow some qualitative predictions to be made about the fate of fenitrothion in a natural aquatic system.

Water

Fenitrothion has been shown to 'disappear' from:

- a) natural streams in New Brunswick at pH 6-8 with a half-life of two to eight hours; (Eidt and Sundaram 1975.)
- b) natural streams in Manitoba at pH 7.5 with an approximate half-life of 10 hours; (Moody et al 1977)
- c) shallow ponds in Ontario at pH 5.9 to 7.5 with a half-life of 0.75 to 4 days (Sundaram 1974).

The great variability in the persistence of fenitrothion in natural waters may be caused by differences in water movement, depth, turbidity, pH, plant coverage, inconsistency of spray deposit, and analytical problems.

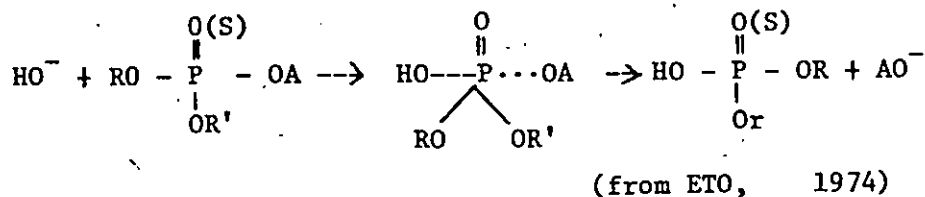
Two major processes are suggested to contribute to the 'disappearance' of fenitrothion from the water; photolysis and hydrolysis. Photolysis, the degradative action of certain wavelengths of natural light, was shown to similarly degrade fenitrothion deposited on chromatoplates or on the surface of bean leaves. In both cases, four major products, carboxy-fenitrothion, fenitro-oxon, 3-methyl-4-nitrophenol, 3-carboxy-4-nitrophenol and one minor product, carboxy-fenitro-oxon were detected. The pesticide degradation on bean leaves ($t_{1/2}$, 1.5 days) was shown to be much faster than chromatography plates ($t_{1/2}$, 6-12 days), possibly due to the protective action of the silica gel. (Ohkawa et al 1974). The degradation of the pesticide in aerated distilled water exposed to sunlight showed an even shorter half-life of eleven hours, but the same type of derivatives were formed. Faster pesticide degradation occurred in acetone irradiated with ultraviolet light with a pesticide half-life of 45 minutes (Ohkawa et al 1974). Similar results were obtained by other workers (Marshall and Greenhalgh 1976).

The vapour phase degradation of fenitrothion by light of 265 or 313 nm produced the same 4 products, carboxy-fenitrothion, fenitro-oxon, 3-methyl-4-nitrophenol and carboxyfenitro-oxon, (Brewer et al 1974). This evidence was used to support the idea that natural sunlight could degrade fenitrothion in the atmosphere in 10 hours to these same degradation products (Brewer et al 1974). The rate of pesticide degradation in water depends on pH, degree of aeration and intensity of irradiation. The similarity in degradation products in the distilled water, bean leaf and chromatograph study of Ohkawa (1974) indicates that photolysis may be an important mode of pesticide degradation in aquatic systems. Additional evidence was found in the comparison of fenitrothion treated water in light

and dark containers (Ohkawa et al 1974). The treated water in the dark containers showed much slower rates of pesticide degradation, with 97.9% of the activity remaining in the water after 28 hours as fenitrothion, compared to only 19.7% as fenitrothion in the sun-lit containers. However, the same types of derivatives were found in the water of illuminated and darkened models although the water of the darkened containers showed 10 to 250 times less of each degradation product than the water of the lighted container (Ohkawa et al 1974). In summary, fenitrothion may undergo three possible reactions when exposed to ultraviolet light or sunlight, a) oxidation of the P-S bond to a P=O bond, b) oxidation of the methyl group to a carboxyl group and c) cleavage of the P-O aryl bond to release the phenols (Ohkawa 1974).

Hydrolysis

Organophosphate pesticides are susceptible to hydrolysis at the P-O aryl bond in a reaction catalysed by the hydroxyl ion and similar to S_N2 displacement reaction at a carbon atom.



The rate of this reaction depends on water pH, temperature, the presence of catalytic reagents, and the chemical nature of the substituents on the molecule. Most OP pesticides are stable between pH 1 to 5 but at a pH greater than 7 the rate of hydrolysis increases by a factor of about 10 for each 1 unit rise in pH. The rate of hydrolysis also increases about 4 times for each 10°C rise in temperature (Etó, M. 1974).

Thus, the hydrolysis of fenitrothion in natural waters will vary significantly with the above properties of water, so that these properties must be measured in any study.

Laboratory studies using fenitrothion in buffered solutions of distilled water at various pH levels have shown the half-life to vary from 65, 85, 70, 47, 15, 16 and 22 days at pH 5, 6, 7, 7.5, 8, 9 and 10 resp. at 23°C (Greenhalgh et al 1979, in press). This study showed that the major decomposition product above pH 7 in phosphate buffer was 3-methyl-4-nitrophenol and DMPT due to base-catalysed hydrolysis. Below pH 7, in phosphate buffer, the major product was desmethyl-fenitrothion.

Few fenitrothion derivatives, apart from traces of amino-fenitrothion, were found in natural waters following an operational spray, (Zitko and Cunningham 1974; Moody et al 1977). Traces of S-methyl-fenitrothion were detected, briefly, at one hour, post spray in one study but these traces were likely found in the pesticide formulation before the spray (Moody et al 1977). The further derivatives, desmethyl-fenitrothion, desmethyl-amino-fenitrothion, and dimethyl-phosphorothioic acid were identified in water containing Bacillus subtilis (Miyamoto et al 1966). The reasons for the lack of information on fenitrothion derivatives in natural waters is unknown. Low pesticide concentrations in natural waters, late time of sampling, inadequate methodology, fast rates of hydrolysis of derivatives, and rapid partitioning of the pesticide into components other than water, may have contributed to the lack of substantive information on the fate of fenitrothion in natural waters.

A similar pesticide, parathion, showed complete hydrolysis

in three weeks in natural river water to produce p-nitrophenol and diethyl-phosphorothioate (Eichelberger and Lichtenberg 1971; Sethunathan et al 1977). The half life for parathion in a phosphate buffer solution at pH 6 was 43 hours, Thames river water at pH 8.0 was 65 hours, and Irthing river water at pH 7.5 was 68hours (Ruzuicka et al 1967). The accumulation of parathion by waterborne microorganisms was probably responsible for the persistence of low levels of parathion in the water (0.03 ppb at 40 days). The effects of pesticide sorption and desorption from the aquatic microorganisms should be considered when discussing pesticide persistence in natural waters. The long-term and low level persistence of parathion in water may be caused by the binding of the pesticide in the lipid component of organic material where it cannot undergo physical or biological degradation (Stewart et al 1971).

Sediment:

A suspected macro-reservoir for fenitrothion in an aquatic system is the sediment; which has been described as soil (Ponnamperuma 1977). Such soil sediments are formed of components of physical and organic origin and often of mixtures of the two.

The living organic components of the soil include plant roots, bacteria, algae, invertebrates and a host of other organisms which have the potential to absorb and degrade the pesticide.

The other organic component of the soil includes organic matter in the form of partially decomposed biological material, mainly plants and macro-algae, or advanced decomposition products such as humic and fulvic acids. Physical components of soil include clay

minerals, various oxides and hydroxides, organo-clay complexes and unconsolidated mineral matter. Some organic and inorganic components of the sediment have the potential to bind the pesticide and prevent access to physical and biological agents which could cause decomposition. The inorganic components can also catalyse hydrolysis of organophosphorous pesticides (Bailey and White 1970; Yaron 1975).

The soil characteristics, such as relative amounts and types of organic matter and clay minerals do influence pesticide absorption by the sediment. The properties of the clay mineral, such as surface area, cationic exchange capacity three dimensional structure, and surface charge density will influence pesticide adsorption and chemical hydrolysis (Bailey and White 1970; Sethunathan 1977).

The characteristics of the pesticide such as size, ionic character, surface charge density, shape and polarity may also influence pesticide absorption and chemical hydrolysis (Bailey and White 1970; Sethunathan 1977).

The bacterial, fungal and algal activity and the ability of the biota to grow in the sediment will influence the biotic degradation of the pesticide. These, together with the physical properties or processes affecting the aquatic environment such as pH, oxygen concentration, temperature, oxidation-reduction potential and the quality and intensity of light can affect pesticide decomposition (Sethunathan et al 1977, Bailey and White 1970, Bartsch, 1974, Bartha 1971, Poinke and Chesters 1973).

Generally, seven factors are known to influence the fate of a pesticide in a soil system. These include chemical, photochemical and microbial decomposition,

volatilisation, movement through the hydrosol, plant and organism uptake, and physical adsorption (Bailey and White 1970, Poinke and Chesters 1973). The adsorption of the pesticide to the sediment may be one of the most important factors in the aquatic system.

"The relationship between sediment and pesticide controls or influences the movement, distribution, toxicity and persistence of many pesticidal compounds which might appear in lakes or streams".
(Poinke and Chesters 1973).

Chemical Hydrolysis

Following physical adsorption to the soil four reactions may occur 1) photochemical reactions on the sediment surface 2) chemical hydrolysis 3) biotic degradation or 4) a pesticide adsorption protected from degradative processes. Photochemical reactions on sediment surfaces have not been well studied and may not be significant due to extreme attenuation of the ultraviolet spectrum in water (Yaron 1973). Biotic degradation of the pesticide has been shown to be the predominant degradative pathway in aquatic systems, but under some circumstances, as found for parathion, chemical hydrolysis may become important on the soil surface (Yaron 1975).

The chemical conversion of parathion on 14 sterile irradiated soils (pH 6.4-8.2) showed 3- 23% hydrolysis on air dried soils and <10% hydrolysis on moist samples after 130 days. Conversion was affected by clay and organic matter. The rate of hydrolysis decreased from kaolinite (1:1 mineral) to montmorillonite to organic matter, and this rate was

inversely related to adsorption affinity of the parathion to these substances. This phenomenon was explained by the hypothesis that surface catalysis was occurring at specific active surface sites. Adsorption to kaolinite is surface adsorption (due to 1:1 structure) whereas most adsorption to montmorillonite is internal adsorption between the 2:1 minerals layers where the pesticide may be unavailable for a particular type of catalysis (Yaron 1975).

Yaron (1975) suggested that the site of catalytic hydrolysis was the ligand water associated with the exchangeable cation (Ca^{2+} , Mg^{2+}) and not the soil itself. On a kaolinitic mineral, the ligand water of the cation is oriented so that parathion will cleave. In montmorillonite where the greatest surface area is internal, more highly polar and crowded with water and exchangeable cations, the correct orientation cannot be assumed and this catalysis does not occur.

Using 3 different irradiated soils with similar amounts of clay but varying in organic matter from 1.5% to 12%, parathion degradation was found to decrease with increasing soil organic matter content (Yaron 1975). It was suggested that organic matter coated the active sites to reduce this catalysis. Almost no parathion decomposition occurred on irradiated calcium peat soil (pH 6.5, 95% organic matter), so it was assumed that organic matter adsorbed the pesticide making it unavailable for surface catalytic activity.

Soil Biota

The soil biota, mainly yeasts, bacteria and some algae have been shown to be highly important in the degradation of organophosphorous

pesticides either in laboratory experiments with isolated species, or in the natural soil environment.

A yeast, Torulopsis utilis, and an algae, Chlorella pyrenoidosa oxidised Thimet (0,0-diethyl-S-(ethylsulfinyl)-methyl-phosphorodithioate) to the phosphorothioate sulfoxide form as well as hydrolysing the parent compound.

Chlorella pyrenoidosa further oxidised the phosphorodithioate sulfoxide derivative to the phosphorothiolate sulfoxide (0,0-diethyl-S-(ethyl sulfinyl) methyl-phosphorothiolate). (Almed and Cassida 1958). Further work showed that 2 bacteria, Thiobacillus thiooxidans and Pseudomonas fluorescens, hydrolysed 25% and 58% of Thimet respectively in 8 days but could not oxidise the pesticide.

Parathion, 0,0-diethyl-4-nitrophenyl phosphorothioate, was shown to degrade to p-nitrophenol and amino-parathion in submerged loam soils, (pH 6.3) but no amino-parathion was formed in autoclaved soil where parathion was most persistent. "The high stability of parathion in autoclaved wet soils indicates that the hydrolysis of parathion is not dependent on moisture alone but is interrelated with microbiological activities" (Lichtenstein and Schulz 1964).

Similar experiments with parathion in lake sediments showed that the major degradative products were amino-parathion, p-nitrophenol and some unidentified water-soluble product that may have been diethyl-phosphoric acid (Graet et al 1970, Katan et al 1976). In a non-sterile peptone-lake water model, complete parathion degradation occurred within 35 hours with amino-parathion and one unknown being the major products.

In a non-sterile peptone-lake water-sediment system the parathion degradation took longer (95% at 300 hours) because of parathion adsorption to the sediment, and amino-parathion was the major derivative.

Chemical hydrolysis in a sterilised soil-water system, both in water and sediment, totalled 0.56% of the initial parathion per day, giving complete hydrolysis in 180 days (Graetz et al 1970).

Bacteria, isolated from polluted water, inactivated the insecticidal activity of organophosphorous pesticides such as fenitrothion and methyl-parathion (Yasuno et al 1965). Later work, under aerobic conditions, showed that Bacillus subtilis could degrade fenitrothion to amino-fenitrothion, desmethyl-fenitrothion, desmethyl-amino-fenitrothion, and dimethylphosphorothioic acid, with amino-fenitrothion being the major product (65%) (Miyamoto et al 1966). Under anaerobic conditions, very small amounts of derivatives other than amino-fenitrothion were formed.

Further work in a water model, showed that both the fungi, Fusarium sp., and the B. subtilis produced not only the derivatives found by Miyamoto but the derivatives formylamino-fenitrothion and acetylamino-fenitrothion (Takimoto et al 1976). In several submerged soils (pH 5.2-7.6) the fenitrothion degraded to carbon dioxide (<10%), 3-methyl 4-nitrophenol, amino-fenitrothion, formylamino-fenitrothion, and acetylamino-fenitrothion. Also, large amounts of unknown water-soluble derivatives, up to 30% to 40% of the applied activity, were found in the soil-water system (Takimoto et al 1976). No oxidation products such as fenitro-oxon have been found in submerged soil-water systems, so that reduction,

hydrolysis and some acylation reactions are the expected degradative pathways.

The role of bacterial activity in the high rates of pesticide degradation observed in natural or near-natural conditions was reaffirmed by Miyamoto using sterile and non-sterile water or soil-water combinations. Under sterile conditions there was little pesticide degradation other than a slow hydrolysis (Miyamoto 1977; Takimoto et al 1976).

Bacterial enzymes have been implicated in the hydrolysis of parathion. An inducible enzyme, parathion hydrolase, was isolated from an adapted mixed cell culture, which could hydrolyse parathion at a rate of $416 \text{ n mol min}^{-1} \text{ mg}^{-1} \text{ protein}$. This rate of hydrolysis was 2450 times greater than the rate of chemical hydrolysis by 0.1N sodium hydroxide at 40C. This enzyme could hydrolyse 8 of 10 organophosphorous pesticides at rates ranging from 12 to 1360 n.mol/min/mg protein (Munnecke and Hsieh 1974; Munnecke 1976a).

Two strains of Pseudomonas sp. have been found to degrade p-nitrophenol to nitrite and phenol and the phenol was then utilised as a carbon source by the bacteria (Simpson and Evans 1953; Sethunathan 1977). It was then found that a Pseudomonas sp. could use p-nitrophenol as a sole carbon source (Siddaramappa 1973).

Thus it may be possible that bacteria could completely degrade organophosphorous pesticides to compounds that could be used by bacteria as a carbon source.

To this point the evidence indicates that the bacteria are the major biota responsible for the biotic degradation of pesticides in the

sediment. Since fungi are primarily aerobic, they would have little influence in anaerobic sediments. However, it is not known what proportion of the fenitrothion could be degraded by the fungi in the water where conditions are more favourable for growth.

It was found that rates of malathion degradation in water were circa 5000 times faster for bacteria, than for a fungus, Aspergillus oryzae, under similar conditions of biomass, malathion concentration, temperature and agitation rate. Even though both cultures were adapted to use malathion as a sole carbon source both degraded the pesticide to similar products. Thus it was hypothesised that bacteria are a more important source of organophosphate degradation in the water (Lewis et al 1975) than fungi.

It was shown that the synergistic microbial activity of Arthrobacter sp. and Streptomyces sp. was required to completely degrade diazinon to carbon dioxide. Each bacterial species could degrade diazinon and use it as a carbon source but complete degradation by each species alone did not occur (Gunner and Zuckerman 1968). This type of co-operative bacterial activity is likely to be found in the mixed populations of natural sediments and this may ensure complete degradation of most organophosphorous pesticides.

Plants

The uptake of organophosphorus pesticides by plants is an important macro-reservoir of pesticides in a land or aquatic system.

Corn treated with an emulsifiable concentrate of fenitrothion at a rate of 1 to 3 pounds per acre showed initial levels at zero time of

14 to 42 $\mu\text{g/g}$ of fenitrothion, 0.08 to 0.17 $\mu\text{g/g}$ of fenitro-oxon and 0.35 to 0.74 $\mu\text{g/g}$ of 3-methyl-4-nitrophenol. By 28 days residue levels had declined to the range of 0.63 to 0.011 $\mu\text{g/g}$ fenitrothion, 0.44 to 0.22 $\mu\text{g/g}$ of 3-methyl-4-nitrophenol and less than 0.002 $\mu\text{g/g}$ fenitro-oxon (Leuck and Bowman 1969).

Degradation of fenitrothion on bean leaf surfaces exposed to sunlight, showed the derivatives fenitro-oxon, 3-methyl 4-nitrophenol, 3-carboxy 4-nitrophenol, carboxy-fenitrothion and 2 unknowns. These products were identical to those formed on chromatoplates and indicates the photochemical degradation of the pesticide (Ohkawa et al 1974).

Quick adsorption and absorption of fenitrothion was seen in banana (Miyamoto et al 1965), rice (Miyamoto et al 1965b), and apple (Hosokawa et al 1974) with in situ metabolism and no translocation. Rice plants sprayed with a 50 $\mu\text{g/g}$ fenitrothion solution showed surface and internal deposits in the leaf with total concentrations of 11.79 $\mu\text{g/g}$ at zero time to 4.8 $\mu\text{g/g}$ by 10 days. Metabolites found in the leaf blade at 4 days included fenitro-oxon, (0.27 $\mu\text{g/g}$) dimethylphorothioc acid (1.76 $\mu\text{g/g}$) phosphorothioic acid or phosphoric acid (7.09 $\mu\text{g/g}$). At 2 days some desmethyl-fenitrothion was detected (0.45 $\mu\text{g/g}$). Thus fenitrothion was rapidly metabolised in the rice plants to water-soluble derivatives, and much of the phosphorus-32 label was in the form of "unextractables" in the plant tissue (Miyamoto et al 1965). Similar degradation products were found in apple, as well as p-nitrocresol, p-nitrocresol- β glucoside, S-methyl-fenitrothion and an unknown (Hosokawa et al 1974).

Absorption, translocation and some limited metabolism of fenitrothion has been shown in laboratory experiments with fir, Abies balsamea, and spruce, Picea glauca (Moody et al 1977). The disappearance of fenitrothion from the conifer needle surface was correlated with increasing internal pesticide concentration at the sub-cuticular level. Fenitrothion was translocated to new foliage and trace levels of an unknown water-soluble derivative were found (Moody et al 1977).

Birch, Betula alleghaniensis, pine, Pinus strobus and spruce, Picea glauca, seed embryos degraded fenitrothion to fenitro-oxon, desmethylfenitrothion, and S-methyl-fenitrothion (Hallett et al 1975). The desmethyl-fenitrothion in pine seed embryos was thought to be an intermediate in the formation of S-methyl-fenitrothion. Glutathione transferase dealkylated fenitrothion to form desmethyl-fenitrothion and S-methyl glutathione. The desmethyl-fenitrothion was then believed to be re-methylated to S-methyl-fenitrothion by fenitrothion, fenitro-oxon or by free methyl groups in the embryo (Hallett et al 1975).

Aquatic plants show absorption and metabolism of many pesticides. Myriophyllum brasiliense and Eichornia crassipes removed large amounts of diphenamid (N-N- dimethyl-2,2-diphenylacetamide) from the water and demethylated the largest portion to m-methyl,2,2-diphenylacetamide and several unknown minor products (Bingham and Shaver, 1977). Demethylation is a major means of detoxification of urea, methylamide and organophosphorus pesticides in plants and animals (Bingham and Shaver 1977).

One might expect that aquatic macrophytes possess the same enzymes to detoxify pesticides as terrestrial plants.

Elodea sp. bioconcentrated dieldrin by a factor of 1280 and metabolised it into one acetone-extractable unknown, one polar unknown, and an unextractable portion in the plant (Sanborn and Ching-Chieh Yu 1973).

By 14 days, Elodea nutalli absorbed 16.5% of the total activity of ^{14}C -activity from a soil-plant-water system to which phorate was applied to the soil at a $4.0 \mu\text{g/g}$ concentration. In E. nutalli, negligible amounts of benzene-soluble activity (0.2%), small amounts of water-soluble activity ($2.0 \pm 0.4\%$), and large amounts of bound residues ($14.3 \pm 2.9\%$) were found (Walter-Echols and Lichtenstein, 1978). The presence of E. nutalli in the water was believed to have caused the production of phorate sulfone as the major derivative in the water ($15.6 \pm 3.7\%$) whereas the water of the soil-water system did not show any phorate sulfone. It was suggested that the plants excreted oxygen to the water and this enabled the production of the further oxidation product, phorate sulfone, from the phorate sulfoxide.

The absorption of pesticides by submerged aquatic plants is likely an active process requiring metabolic energy. The uptake of ^{14}C -Diquat, a cationic herbicide, by E. canadensis was reduced 38% to 48% by treatment with the known respiratory inhibitors 0.01 M. sodium azide, 0.01M. potassium cyanide, or 0.001M. 2,4 dinitrophenol, and the uptake of Endothall was reduced 95% by the same inhibitors (Seaman and Thomas 1965).

Under field conditions after an operational fenitrothion spray, maximum levels of $4.19 \mu\text{g/g}$ fenitrothion were found in Lemna minor.

duckweed, at 10 hours and 0.15 $\mu\text{g/g}$ in Ceratophyllum demersum, hornwort, at 70 hours post-spray (Moody et al 1977). This represented a bioaccumulation of 95 and 130 times for L. minor and C. demersum over the fenitrothion concentration in the water at that time. No fenitrothion derivatives were detected in the plant samples (Moody et al 1977). Thus the aquatic plants would appear to be a reservoir for the fenitrothion in an aquatic system.

Up to 1977, fenitrothion had only been reported to disappear from natural waters and only occasional and incomplete reports indicated the fate of the pesticide in the field. To date no quantitative recovery of pesticide residue has been reported in a complete aquatic system with all the natural components of water, algae, plants, sediment, natural sunlight and other natural environmental conditions affecting the experiment. This study was initiated to fill this gap in knowledge. The time sequential partitioning and degradation of the pesticide in each of the components of a model system were determined and the fate of fenitrothion in an aquatic system was followed.

Hypothesis

The hypothesis which was formulated was that the aquatic plants and sediment are the major macro-reservoirs of the pesticide in shallow aquatic systems (less than 2 m. depth) and are the major foci of pesticide accumulation, persistence and degradation. Further, it was thought that laboratory models could imitate the processes occurring in the field and extrapolations could be made from laboratory to field models and hence to natural aquatic systems. Thus the laboratory models were scaled down versions of field models or of natural systems using

the same relative proportions of the same components. Four sets of laboratory models and one set of field models provided the systems on which the hypotheses were tested.

Materials and Methods

a) Materials For Laboratory Experiments

1) Chemicals

Purified (>99%) samples of fenitrothion and five other derivatives, S-methyl-fenitrothion, fenitro-oxon, amino-fenitrothion, 3-methyl-4-nitrophenol and fenitrothion aldehyde, were obtained from Agriculture Canada, Chemical and Biological Research Institute, Ottawa. Ring-labelled ^{14}C -labelled fenitrothion of specific activity of 5.54 m.Ci/mM was obtained from Sumitomo Chemical Company, Japan.

The purity of the unlabelled fenitrothion samples was determined by gas chromatography and thin-layer chromatography (Mendoza 1968). A qualitative estimate of the purity of labelled ^{14}C -fenitrothion was determined by scintillation counting of thin-layer chromatograms and by autoradiography. The unlabelled fenitrothion derivatives were used as reference TLC standards and their qualitative purity was shown on the thin-layer chromatograms (Mendoza 1968).

All solvents used for extraction were glass distilled (Caledon Laboratories, Georgetown, Ontario), and Scintiverse^R scintillation cocktail (Fisher Scientific) was used for counting sample extracts containing the ^{14}C -labelled fenitrothion.

Apart from the field experiment, the fenitrothion formulations contained a mixture of ^{14}C -labelled fenitrothion and unlabelled fenitrothion at a total fenitrothion concentration of 1 $\mu\text{g/g}$ or 10.0 $\mu\text{g/g}$ relative to the water, together with the adjuvants Aerotex 3470 (Texaco Ltd.) and Atlox 3409 (Texaco Ltd.), both at a concentration of 0.1 $\mu\text{g/g}$ or 1.0 $\mu\text{g/g}$

Table 1

Summary of Experimental Models

<u>Model</u>	<u>Amount</u> <u>of Water</u> <u>(ml)</u>	<u>Fenitro-</u> <u>-thion</u> <u>Mix</u>	<u>Plant</u> <u>Species</u>	<u>Plant</u> <u>Load</u> <u>(mg/l)</u>	<u>Other</u> <u>Compo-</u> <u>-nents</u>	<u>Initial</u> <u>Fenitrothion</u> <u>Concentration</u> <u>ug/g</u>
1	40.8	A	none	none	none	0.983
2	40.8	A	<u>E. densa</u>	464	none	0.983
3	40.8	A or B	<u>E. canadensis</u>	97, 176 563, 897	none or	0.983 9.832
4	1348.8	C	<u>E. densa</u> <u>S. graminea</u> <u>Myriophyllum sp.</u>	221	none	1.022
5	800 L	D	<u>E. canadensis</u>	2.9	sediment (22 kg.)	12.5

Table 2

Formulations

Fenitrothion formulations were added to give a final concentration in water:

	<u>Fenitrothion</u> <u>ug/g</u>	<u>Aerotex 3470</u> <u>ug/g</u>	<u>Atlox 3490</u> <u>ug/g</u>
A	0.983	0.098	0.098
B	9.83	0.983	0.983
C	1.022	0.099	0.099
D	12.51	1.251	1.251

relative to the water.

2) Water, sediment and plants

Water for the plant-water systems was obtained in October 1977 from Lac Bourgeois in Gatineau Park, Quebec. This water was kept in the laboratory in 8ℓ glass cylinders with aquatic plants and Lac Bourgeois sediment. Microscopic examination showed the water to have an active population of micro-biota. Unfiltered water from these holding tanks was placed in the experimental containers 2 hours prior to the start of any experiment.

The following 4 aquatic plants were used singly or in combination in this study, Elodea densa, Sagittaria graminea and Myriophyllum sp. (Boreal Laboratories, Mississauga, Ontario) and Elodea canadensis (Carolina Biological), Burlington, N.C. U.S.A.). The plants were equilibrated in 85ℓ holding tanks for at least one week prior to use at 22C.

b) Description of Laboratory Models

Four laboratory models and one field model were used in these studies and the field model is described in a later section. All laboratory models were enclosed in glass. One of these models consisted of lake water alone to which a fenitrothion formulation was added. The other three models contained lake water, fenitrothion formulation and one to several aquatic plants (table 1). Each model system was duplicated at least once for each of the 4 to 6 sampling times. At each sampling period, all components of each system were analysed for total pesticide and derivatives. The 4 laboratory models were used to study 4 different problems.

Model one was designed to show the degradation of fenitrothion in natural lake water in the laboratory. This model consisted of 40 ml. of lake water and a fenitrothion formulation which gave a final concentration of 0.983 $\mu\text{g/g}$ fenitrothion, 0.098 $\mu\text{g/g}$ Aerotex 3470 and 0.098 $\mu\text{g/g}$ Atlox 3409 relative to the water.

Model 2 was designed to identify the contribution of the aquatic plant to the degradation of the pesticide in the water. Model 2 was identical to model 1, with the addition of Elodea densa segments of length 2.5 cm to the water, to give a plant density equivalent to 464 mg. dry weight per litre of water.

Model 3 was designed to determine the effect of increasing plant densities on pesticide removal from the water. Model-3 was identical to model 1 with the addition of Elodea canadensis segments of sizes 1.2, 2.5, 7.6 or 10.2 cm. in length to give an equivalent plant density of 97,176, 563 or 897 mg/l. dry weight. The experiment was repeated at a higher fenitrothion concentration to give 9.83 $\mu\text{g/g}$ fenitrothion, 0.98 $\mu\text{g/g}$ Aerotex 3470 and 0.98 $\mu\text{g/g}$ Atlox 3409 relative to the water.

Model 4 was used to assess species diversity in relation to fenitrothion accumulation. Model 4 consisted of a 15 cm. plant or segment of each of the 3 plants, Elodea densa, Sagittaria graminea and Myriophyllum sp. floating in 1348 ml. of water. Together, the three plants in each container corresponded to a plant load equivalent to 221 mg dry weight per litre of water. The initial fenitrothion formulation was 1.022 $\mu\text{g/g}$ fenitrothion, and 0.0996 $\mu\text{g/g}$ each of Aerotex 3470 and Atlox 3409.

c) Sampling Schedule

In all experiments in the laboratory, plant and water samples in duplicate containers were analysed at 1, 5, 10 and 14 days after treatment. In models 1, 2 and 3, further sampling was carried out at 20 days and in model 1 the experiment was extended to 25 days. In all cases, all compartments of a model were analysed at each time period in duplicate.

d) Extraction Methodology

i) Water- In models 1, 2 and 3, the total 40.8 ml. of water were extracted 3 times each with 20 ml of chloroform. The chloroform was reduced on a Buchi evaporator to near dryness at 40°C and then the residue was brought up in 5 ml. of hexane. The hexane extracts were stored at -4°C prior to analysis. In model 4, the total 1348 ml. of water was filtered through a Buchner funnel with filter paper (Whatman #1) to remove plant debris. Five hundred ml of this water was extracted 3 times, each time with 100 ml. of chloroform. The total chloroform was again removed on a Buchi evaporator and the residue was brought up in 10 ml. of hexane for analysis.

In models 1, 2 and 4, 40 ml of the extracted water were frozen in four, fifty ml. test tubes at -70°C and then lyophilized on a Virtis 10-100 lyophilizer (Fisher). Each tube was rinsed 3 times each with 5 ml. of methanol and the total combined methanol was reduced to near dryness on the evaporator at 40°C. The residue was brought up in 5 ml. of methanol for analysis.

ii) Plants - Each plant sample was blot-dried and weighed prior

to extraction. Dry weights of untreated plant material were obtained by heating the plant material at 100°C for 24 hours, followed by equilibration at room temperature after which the dry weights were determined.

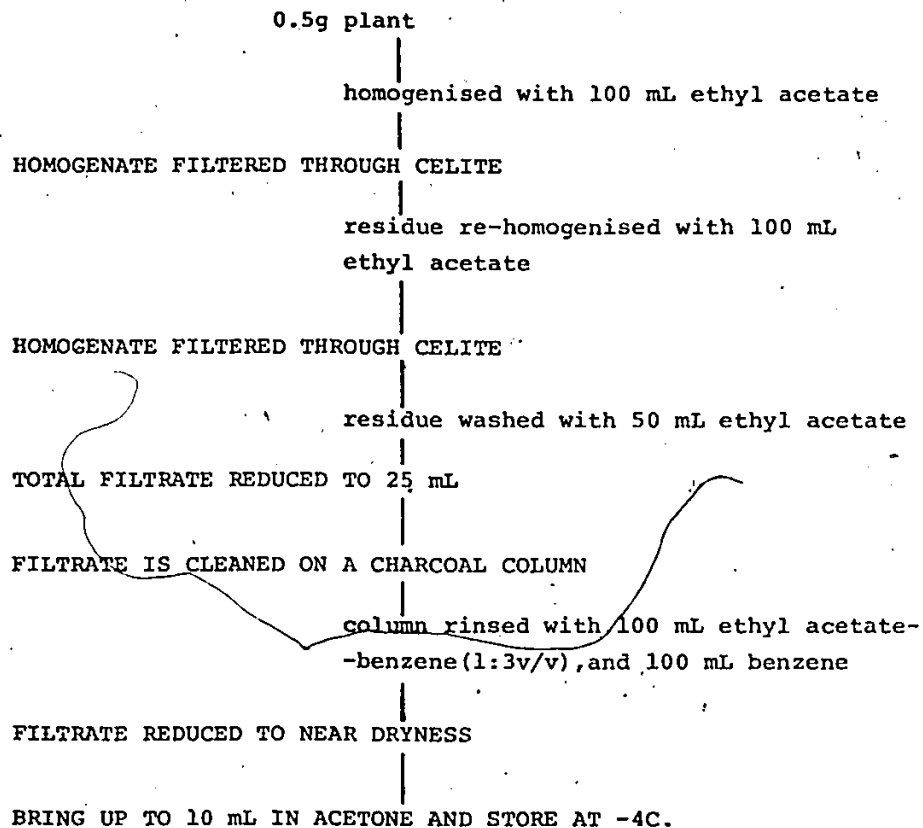
Duplicate plant samples were homogenized twice with 100 ml ethyl acetate in a Polytron sonicator at setting number 5 and the extracts were filtered through a 12 gram dry pre-rinsed Celite pad. The plant residue in the Celite pad was rinsed with 50 ml of ethyl acetate. The total extract was reduced to near-dryness on the evaporator at 40°C and then brought up in 5 ml of hexane for analysis (Figure 1)..

e) Analyses

1) Scintillation counting - All plant and water extracts were analysed with a Beckman LS-3133 P Scintillation counter. An SMI-Micro/Pector delivered 100 µl of sample extract into 10 ml of Scintiverse scintillation cocktail in counting vials and the ^{14}C activity in the extracts was determined using a Beckman counter. Control untreated plant, and water samples, as well as standard stock ^{14}C -fenitrothion solutions and external standards were counted in the same manner as the sample extracts.

Quench curves were obtained by determining the quench of control untreated plant samples on standard ^{14}C -fenitrothion solutions, and these values were used to correct sample counts where necessary. A quench curve was also obtained for the Silica gel G to permit quantification of the toxic TLC spots.

Figure 3: Method of Plant Extraction and Clean-up



ii) Thin Layer Chromatography - After scintillation counting to quantitate total ^{14}C activity, selected plant and water extracts were reduced in volume to 0.15 ml under a stream of nitrogen at 35°C . Following this, 50 μl of each selected sample were spotted onto each of 2 duplicate Silica Gel G chromatoplates (Fisher Scientific Co) and run in the solvent system ethyl acetate/cyclohexane (1:3 V/V). The beef-liver homogenate spray and colour reagent of Mendoza (1968) was used to develop the duplicate plates. Standards of purified fenitrothion and derivatives (Agriculture Canada, C.B.R.I. Ottawa) were run concurrently on the same plates to obtain relative R_F values. In the case of nitrophenol and fenitrothion aldehyde, these derivative standards were run on separate plates in the same solvent system to compare R_F values to unknown derivatives when the latter were present. Separate test plates were run to determine minimal detectable levels for fenitrothion, S-methyl-fenitrothion, fenitrothion and amino-fenitrothion (Plate 1,2). After measurement of the R_F values for all the derivatives, the toxic ^{14}C -labelled spots on one of the duplicate plates, were removed, placed in 5 ml of Scintiverse cocktail, and counted by the Beckman 3133 P scintillation counter.

iii) Autoradiography - In order to confirm whether the toxic spots shown on the TLC plate were truly C^{14} -labelled fenitrothion, or C^{14} -labelled derivatives, and to indicate the presence of those derivatives that would not show anticholinesterase inhibition, such as 3-methyl-4-nitrophenol, a duplicate TLC plate was developed for autoradiography. After the R_F values for the toxic derivatives were measured, the TLC plates were then covered with a Kodak No-Screen X ray film (NS-ZT) and left for 2 weeks in the dark at -70°C before film development.

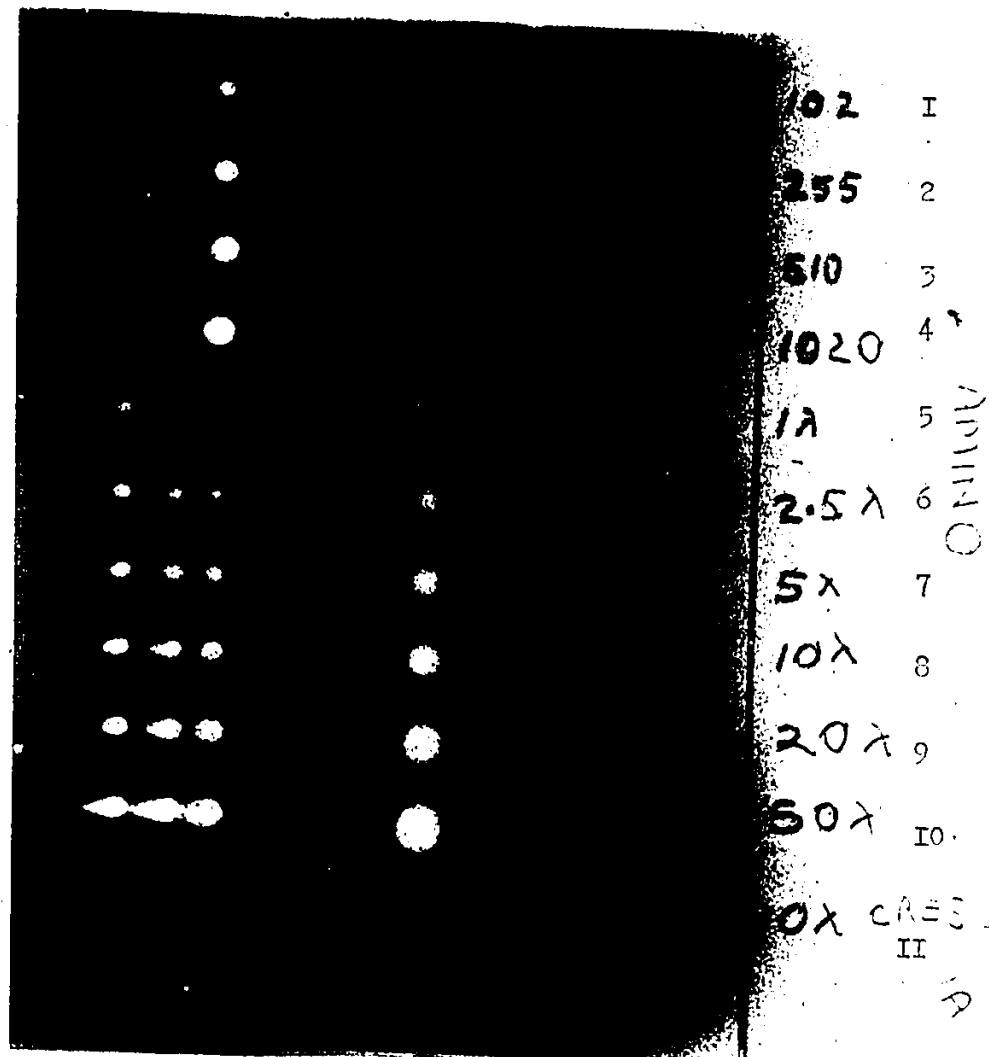


Plate I

#1

Plate 1. Minimal detectable amounts of fenitrothion and metabolites.

Aminofenitrothion Standards-#1-4, = 102, 255, 510, 1020 ng

Solution A-#5-10, 1 2.5, 5, 10, 20, 50 μ l.

Sol'n-A- 8.8 ng/ μ l fen., 16.4 ng/ μ l fenitro-oxon, 22.4 ng/ μ l

S methyl fenitrothion and 22.4 ng/ μ l aminofenitrothion.



I



2



3



4



5

6

5

7

Plate 2

#2

Plate 2. Minimal detectable amounts of ^{14}C -labelled fenitrothion as determined by autoradiography of T.L.C. plates.

Legend. #1 = 3000 CPM - 70.9 ng of ^{14}C fenitrothion
2 = 1200 CPM - 28.4 ng of ^{14}C fenitrothion
3 = 600 CPM - 14.2 ng of ^{14}C fenitrothion
4 = 300 CPM - 7.1 ng of ^{14}C fenitrothion
5 = 120 CPM - 2.89 ng of ^{14}C fenitrothion
6 = 60 CPM - 1.92 ng of ^{14}C fenitrothion
7 = 30 CPM - 0.71 ng of ^{14}C fenitrothion
8 = 12 CPM - 0.289ng of ^{14}C fenitrothion

1) Field Models

Model 5 - Three models and their duplicates were used to provide the limits of the microcosms used in Lac Bourgeois. The components and conditions of these are given in Table A, below:

Table A

Field Microcosms

<u>Components</u>	<u>Conditions</u>
1. Light model-pesticide formulation, lake water, sediment	Full sunlight
2. Dark model-pesticide formulation, lake water, sediment	No exposure to light
3. Control model-no pesticide formulation, lake water, sediment	Full sunlight

Six polyethylene cubes (1.14x1.14x0.91 M) encapsulated the models. They were positioned approximately 70 cm. apart, parallel to and approximately 11 m from the south shore of Lac Bourgeois in an east-west line. The dark model containers had removable top covers that were fabricated of opaque plastic to exclude light, while the other containers were translucent and uncovered to allow light penetration into the water. The containers were anchored to the bottom sediments by eight 6" steel posts which passed through external sleeves of each container.

a) Lake Water

Six days prior to spray day each container was filled with approximately 800 l of lake water. There was a submerged port on

each bag which was left open until spray operations began to permit equilibration between the surrounding lake water and the container water.

b) Sediment

Five days prior to the spraying operation, ten 600 ml pyrex beakers suspended by nylon fishing line on the bottom of each of these models.

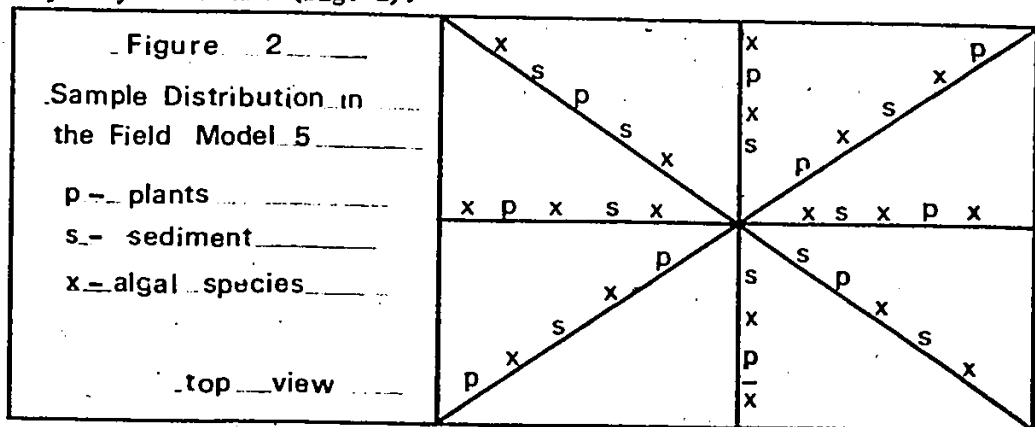
Lake sediment was dredged from the lake bottom within 2 meters of each container, and the large debris was sifted out by hand. Following this, approximately 22 kilograms of sediment (wet) were placed in each container to provide a 3-4 cm cover of sediment on the bottom. The dark model at a depth of 25 cm had 0.1% of the light intensity of the light model at the same depth.

c) Plants

Three to five plants of Elodea canadensis, previously equilibrated in holding tanks in the laboratory, were tied in bundles with nylon fishing line. Two plant bundles were attached separately to a fish line to permit one bundle to be suspended in the upper half of the water column (0-35 cm) and the other bundle in the lower half (35-60 cm). Two days before spraying ten of these lines were positioned in each container (Fig. 1) and anchored with lead weights.

i) Distribution of samples- All plant and sediment samples were suspended from one of four lines which spanned the top of each container. They were distributed as evenly as possible over the

surface area of the container and were maintained at the appropriate depth by fish line (Fig. 2).



ii) Fenitrothion Application- On spray day, 100 ml of the 10% fenitrothion formulation (10% fenitrothion, 1% Aerotex, 1% Atlox, 88% water (V/V)) were sprayed on the surface of each of the models to give an estimated initial water fenitrothion concentration of 12.5 µg/g in water. A polyethylene hand-pump sprayer enabled the delivery of the entire dose, plus three 30 ml water rinses, within 3 minutes.

A test run of water extraction methodology on day S-1 demonstrated that 1½ hours were required to extract all the water samples taken at one time from any one model.

To ensure comparable sampling times, the spray application was staggered on a 1½ hour basis to enable complete extraction of the water of each model at each sample time.

iii) Sampling Schedule -

a) Water- Water samples were taken from each container, twice daily for the first 3 days and then once on days 3, 6, 10, 14, and 28. At each sampling time, one liter of lake water was taken from both the

upper (0-10) and lower (11-50 cm) depths of each container with the aid of a nalgene hand pump. Water samples, collected in 12 ounce amber glass bottles, were extracted on the spot, commencing within 10 minutes of collection.

b) Plants - Following water collection, 1 fish line containing both upper and lower E. canadensis samples was collected at each sample time. These were withdrawn on a time schedule identical to that of the water sampling until completion of the experiment at 14 days. The plants were blot dried, sealed in Whirlpac bags and kept on ice in the dark until frozen for storage at -10°C (within 8 hours of collection).

c) Sediment - To avoid initial disturbance of the water column, sediment samples were not taken until two days post spray and then on 6, 10, 14 and 28. At each sample time, 2 beakers were carefully withdrawn from each model. Providing the sediment layer was undisturbed, all but 10 cm of the overlying water was decanted and discarded. The remaining slurry was transferred to 32 ounce Lab-Tek (Fisher Scientific Co.) polystyrene containers, where they were kept cool on ice in the dark until transported to a -20°C freezer within 8 hours of collection.

4) Extraction Methodology

a) Water

In the field, in order to remove the natural particulate matter and algae in the water, one litre of each lake water sample was filtered through a 40 x 2.2 (id.) cm scintered glass column packed with 2-3 grams of Celite pre-rinsed with water. Five hundred ml of the filtered lake water were partitioned in a 1000 ml separatory funnel

with 100 ml of chloroform. The procedure was repeated three times. The chloroform extract containing fenitrothion was capped in 12 ounce amber glass bottles with 3 grams of anhydrous sodium sulphate and was kept at -20°C until further analysis. The extracted water and chloroform extracts were comparably stored. The chloroform extracts were reduced to dryness at 40°C on the Buchi evaporator, brought up in 5 ml of acetone, and stored at -4°C prior to analysis. Some water samples were lyophilized by the same methods as were used in the laboratory experiments.

b) Plants

Elodea canadensis samples were weighed and extracted, as in the laboratory model, the only modification being that the ethyl acetate was reduced to 25 ml in volume and then transferred to the clean-up column prior to GC analysis. Plant samples were 'cleaned-up' for GC analysis by the method of Moody et al (1974).

c) Sediment

Sediment samples were thawed, stirred and filtered to remove excess water. This excess water was extracted by the standard chloroform method mentioned previously and this extract was labelled the "water wash" of the sediment. The sensitivity and % recovery of the procedure of Takimoto (Takimoto et al 1976) was determined by spiking control sediment samples with fenitrothion at 1 and 10 $\mu\text{g/g}$ for 2 hours. Following this, at each sampling period, seven 10g sub-samples were taken out of each 600 ml beaker withdrawn for analysis. From each of these five were placed in the oven at 100°C for 24 hours for dry weight determinations, the other two were extracted by the method of Takimoto

et al (1976 Figure 3).

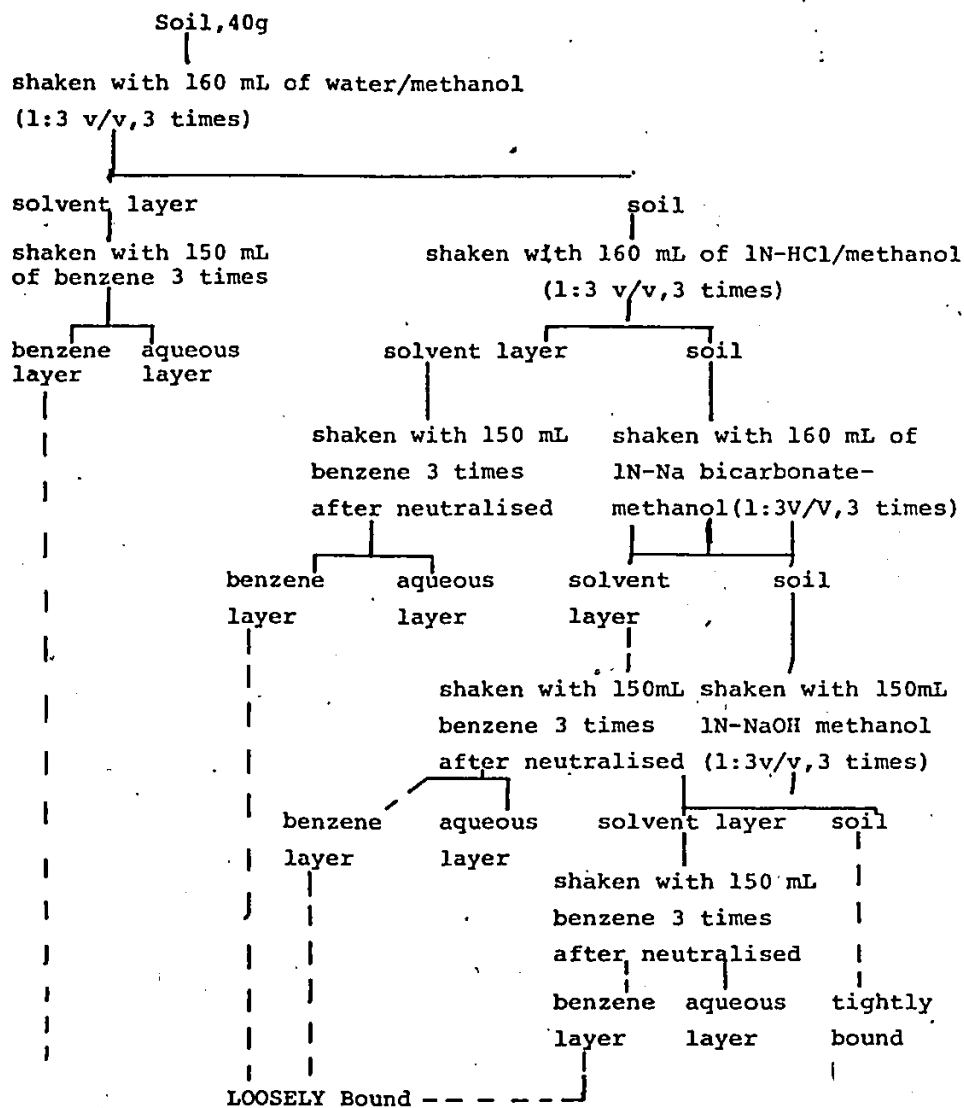
The sediment extracts obtained in this way were designated 'free' and 'loosely bound' (Takimoto et al 1976). The 'free' fraction was the benzene extract of the water methanol (1:3 V/V) extract of the sediment. The 'loosely bound' fraction was the combined benzene extracts of the other acidic and basic water/methanol extracts of the sediment. The benzene extracts were reduced to dryness on a Buchi evaporator at 40°C and then brought up to 25 ml in ethyl acetate. This extract was then run through a charcoal column clean-up procedure to remove interfering substances (Moody et al, 1974). This same clean-up procedure was used for plant extracts from the field model 5.

5) Analysis

a) Clean-up Procedure

The clean-up procedure was designed to remove plant pigments, waxes and resins which produced interfering peaks on the GC chromatogram (Moody et al (1974)). The clean-up column consisted of a 40 cm scintered glass disc column which was dry packed successively under vacuum with 2-3 g of Celite, 7 g of charcoal (Nuchar C-190 N, Fisher Scientific C-177): Celite mixture (1:6 charcoal/Celite w/w) and 2-3 grams of anhydrous sodium sulphate. The dry packed column was pre-rinsed under vacuum with 100 ml of hexane and the hexane was discarded. The wet column was then topped with 25 ml of plant or sediment extract in ethyl acetate. Following this, elution was carried out with 100 ml of 25% ethyl acetate in benzene, followed by 100 ml of benzene. The combined eluants were concentrated to near dryness on the Buchi evaporator at 40°C and brought up to 10 ml

Figure 3-Extraction of Soil



Separation procedures for decomposition products of
fenitrothion in soil. (from Takimoto et al, 1975)

in acetone for GC analysis.

b) Gas Liquid Chromatography (GC)

Samples were analysed with a Pye Model 104 gas-liquid chromatograph (GC) equipped with an alkali flame ionization detector and a rubidium chloride bead. The glass column (1.8 M x 4 mm (id.) contained 3% SE 30 Ultraphase on Chromosorb W-80-100 mesh. Column temperature was 215°C, gas flow was 40 ml/min of nitrogen and detector flow was 500 ml/min of air and 35 ml/min of hydrogen.

Peak areas of duplicate sample injections were compared with duplicate injections of fenitrothion standards; or fenitrothion plus derivative standards, to calculate the concentrations of fenitrothion and derivatives in the sample.

c) Thin Layer Chromatography

Sample extracts were run on silica gel G (Fisher Rediplates, Fisher Sci. Co.) TLC plates in a solvent system of 1:3 ethyl acetate/cyclohexane (Yule and Duffy 1972). The plates were then sprayed with beef liver homogenate and a chromogenic stain to detect anticholinesterase activity (Mendoza et al 1968).

d) Physical Parameters of Lake Water

Temperature, pH and oxygen were known to influence the rate of degradation of some organophosphorous pesticides in the water (Truchlik 1977; Ohkawa 1974; Sethunathan 1977) but it was not known whether other water parameters such as concentration of cations and carbon dioxide levels influenced pesticide degradation in the water.

For this reason, and also to evaluate the homogeneity of the field models compared to the natural lake, these parameters were measured in the test containers and surrounding lake water during the field experiment.

Results

1) Check on Methodology

A Hach Kit (Hach D1-E1 Portable Engineer's Water Testing Laboratory) was used to test the physical parameters of the lake water and the kit's reliability was determined in the laboratory. The kit was tested for its accuracy in pH determinations against standard buffer solutions (Fisher pH 3, 5, 7, 9) and against a laboratory pH meter (Beckman Zeromatic pH Meter). The test showed that the Hach Kit was accurate to within ± 0.1 pH units. The lake water pH (7.35) varied no more than ± 0.1 pH units between containers and the open lake at any one time. Analysis for oxygen showed fully saturated oxygen levels in waters of all the models. Measurements showed that temperature variation between upper (0-10 cm) and lower (11-60 cm) levels in the field models varied no more than $\pm 0.5^{\circ}\text{C}$ and the variation between containers was no more than $\pm 1^{\circ}\text{C}$ at any time.

Check on Clean-up Method

The charcoal column clean-up methodology was designed and tested for a variety of organophosphorus compounds and some of their oxygen analogues (Watts et al 1969) and the method had been tested for the recovery of fenitrothion and S-methyl-fenitrothion (Moody et al 1974). The purpose of sediment extraction was to quantify fenitrothion alone. Fenitrothion was expected to be at such low levels that derivatives, if present, would be at levels below our detection limits. The charcoal column clean-up method was checked for percent recoveries of the known derivative standards, S-methyl-fenitrothion, fenitro-oxon,

amino-fenitrothion as well as fenitrothion.

The percentage recovery of fenitrothion and derivatives from the clean-up column were performed using spiked samples at the 10 µg., 5 µg., 1 µg., and 0.1 µg level. The recovery of fenitrothion, fenitro-oxon and S-methyl-fenitrothion averaged $100.8 \pm 6.0\%$, $106.0 \pm 12.7\%$ and $99.0 \pm 15.3\%$ respectively and these were considered acceptable (Table 3). However, the recovery of amino-fenitrothion at $36.0 \pm 10.5\%$ was poor.

Sediment Extraction

The sediment extraction method (Takimoto et al 1976) was tested for the percentage recovery of fenitrothion from the sediment at the 1 µg/g and the 10 µg/g level after 2 hours incubation and the results are shown in Table 4. Percent recoveries ranged from 113.1% to 124.3% at the 1 µg/g level and 109.0% to 120.4% at the 10 µg/g level. This extraction method was considered acceptable.

Minimal Detectable Levels on TLC

The sensitivity of the TLC method (Mendoza et al 1968) was tested by spotting known amounts of fenitrothion and derivatives on the plate to determine minimal detectable amounts. Minimal detectable levels for fenitrothion, S-methyl-fenitrothion, fenitro-oxon, and amino-fenitrothion were 17.6 ng., 22.4 ng., 17.6 ng., and 56 ng respectively (Plate 1).

Minimal Detectable Levels for Autoradiography

The sensitivity of the autoradiograph method (Moody et al 1974)

Table 3

Percentage Recoveries from the Charcoal Column Clean-up

<u>Fenitrothion</u> <u>Level</u>	<u>Amino-</u> <u>Fenitrothion</u>	<u>Fenitrothion</u>	<u>Fenitrooxon</u>	<u>S-methyl-</u> <u>fenitrothion</u>
10 ug. A	27.0	99.8	100.7	76.0, 94.0
B	39.0	100.5	119.0	125
C	22.0	101.0	176	97.0, 102.0
5 ug. A	41.0	97.5	107.0	107.0
B	32.0	94.5	93.0	91.0
C	42.0	113.0	129.0	76.0
1 ug. A	25.0	94.0	92.0	109.0
B	54.0	109.0	110.0	<u>2</u>
0.1 ug. A	45.0	98.0	109.0	n/a ¹
Mean(S.D.)	36.0±10.5	100.8± 6.3	106.0±12.7	99.0±15.3

- 1) An off-scale interfering peak obscured the recording.
- 2) The one high value was assumed to be a contaminant and this was discarded.

Table 4

Percentage Recoveries of Fenitrothion from Sediment After
Two Hours Incubation at One p.p.m. and Ten p.p.m. Concentration

	<u>One p.p.m. level</u>		<u>Ten p.p.m. level</u>	
	<u>1</u>	<u>2</u>	<u>1</u>	<u>2</u>
Free Fraction:	87.2	91.2	116.3	88.5
Loosely Bound				
Fraction :	37.1	21.9	4.2	21.5
Total:	124.3	113.1	120.4	109.0

was tested using ^{14}C -labelled fenitrothion on TLC (Plate 2).

Quench Curves

Quench curves were obtained for control extracts of all 4 plant species. Quench was significant (above 3%) only in model four and the count was adjusted accordingly for the plant extracts.

Model One

Model one consisted of a fenitrothion formulation added to natural lake water.

The distribution of the total ^{14}C activity between the chloroform extract of water and the lyophilized water after chloroform extraction, together with the unrecovered ^{14}C -activity is shown in Figure 4. The total ^{14}C -activity in the chloroform extract declines from 104.9% at one day to 65.6% of the total initial deposit at 20 days. In the lyophilized sample, the total ^{14}C -activity increased from 2.0% to 18.3% over the same period. The unrecovered ^{14}C -activity increased from 0 to 16.1% of the initial deposit in the period of one to 20 days.

The chloroform extract of water contained fenitro-oxon, S-methyl-fenitrothion, 3-methyl-4-nitrophenol, fenitrothion and an unknown (R_f 0.38) as identified by TLC (see methods) and these are quantitated in tables 5a and 5b. The fenitrothion derivatives in the lyophilized fraction of the water were not identified except for small amounts of residual 3-methyl 4-nitrophenol (2.0%) and fenitrothion (1.4%) which were not completely extracted with chloroform.

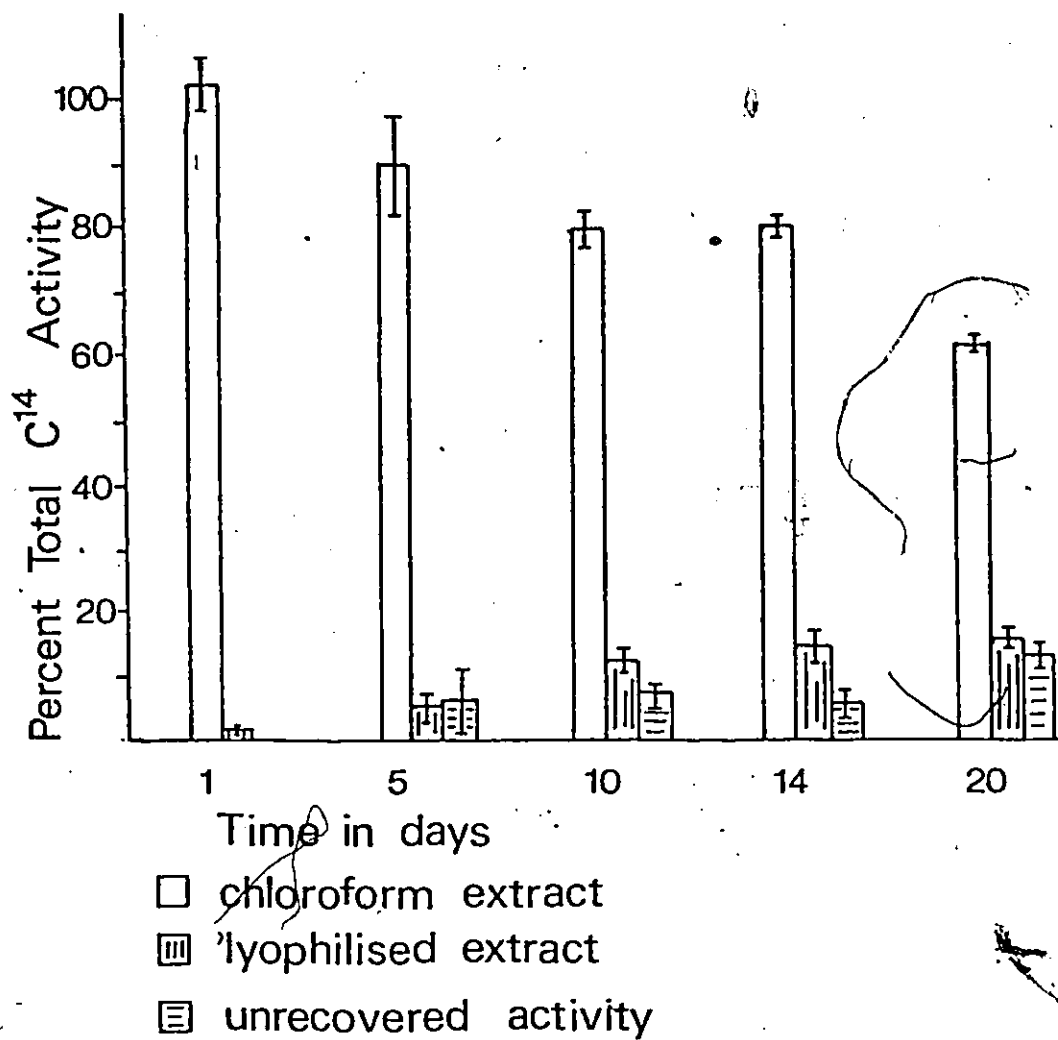


Figure 4: Distribution of C¹⁴ activity in model one.

However, the total ^{14}C -activity of the water soluble derivatives in the lyophilized fraction was quantitated (Table 5a and 5b) and shown to be at the origin of the TLC plate and autoradiograph (plate 3 and 4).

Of the total ^{14}C -activity in the total water, the chloroform and lyophilized extract of water, the percentage as fenitrothion declined from 98.6% at one day to 69.5% at 20 days (Table 5b)

The amount of recovered ^{14}C -activity as water soluble derivatives increased from 0.7% to 23.7% over the same period. The 3-methyl 4-nitrophenol increased gradually from 0.6% of the total recovered ^{14}C -activity to 6.1% in the period of one to 20 days. The small amounts of fenitro-oxon (0.2 to 3.1%), S-methyl fenitrothion (0.1% to 0.2%) and unknowns at Rf 0.38 (0.1% to 0.5%) did not increase to any large extent over this time period. These results gave a data base from which comparisons could be made with more complex models.

Models Two

This model consisted of natural lake water and an Elodea densa plant.

Figure 5 shows the distribution of ^{14}C -activity between the plant, the chloroform extract of the water, the lyophilised water, and the unrecovered portion.

A maximum absorption of 8.1% of the total ^{14}C -activity was present at 5 days in E. densa from a solution at an original concentration of 0.983 $\mu\text{g/g}$ fenitrothion in water. This plant load

Table 5A

Model One -Treated Lake Water

Concentration of Fenitrothion and Derivatives in Water ¹ (ng/g)					
Time (days)	Fenitro- -oxon	³ S-methyl fenitrothion	3-methyl- 4-nitro- phenol	Unknown	Fenitro- -thion
Rf					
0	0.05	0.11	0.26	0.38	0.48
1 W ² LW ³ (0.7%) ⁴	2.33	1.13	2.07 1.28		1025.3 6.3
5 W LW (3.5%)	3.73	0.95	4.29 1.16		925.9 11.7
10 W LW (11.4%)	8.63	0.98	9.71 5.02	0.78 2.76	727.5 4.9
14 W LW (9.4%)	4.95	1.47	13.68 6.3	nd 6.5	736.0 7.2
20 W LW (23.5%)	4.56	n/a	19.1 6.1	n/a 0.32	600.2 2.6

1- The initial fenitrothion concentration was 0.983 ug/g.

2- 'W' represents the chloroform extract of the water.

3- 'LW' represents the lyophilised water after chloroform extraction.

4- The number in brackets refers to the percent of the total recovered ¹⁴C-activity in the water which remained in the lyophilised extract at the origin of the TLC plate.

Table 5B

Model One -Treated Lake Water

Fenitrothion and Derivatives as a Percent of Total

¹⁴C-Activity Recovered from Water¹

<u>Time</u>	<u>Fenitro-</u> <u>-oxon</u>	<u>S-methyl</u> <u>fenitrothion</u>	<u>3-methyl-</u> <u>4-nitro-</u> <u>phenol</u>	<u>Unknown</u>	<u>Fenitro-</u> <u>-thion</u>
<u>Rf</u> <u>0</u>	<u>0.05</u>	<u>0.11</u>	<u>0.26</u>	<u>0.38</u>	<u>0.48</u>
1 W ² LW ³	0.23	0.11	0.40 0.17	nd	98.02 0.41
5 W LW 3.47	0.40	0.11	0.87 0.27		93.49 1.39
10 W LW 11.4	1.06	0.11	2.22 0.94	0.09 0.25	83.45 0.46
14 W 0.21 LW 9.42	0.60	0.16	3.12 0.98	0.49	84.44 0.57
20 W 0.20 LW 23.5	0.56		4.38 1.72	0.12	69.13 0.41

1-The initial fenitrothion concentration was 0.983 ug/g.

2-'W' represents the chloroform extract of the water.

3-'LW' represents the the lyophilised water after
chloroform extraction.

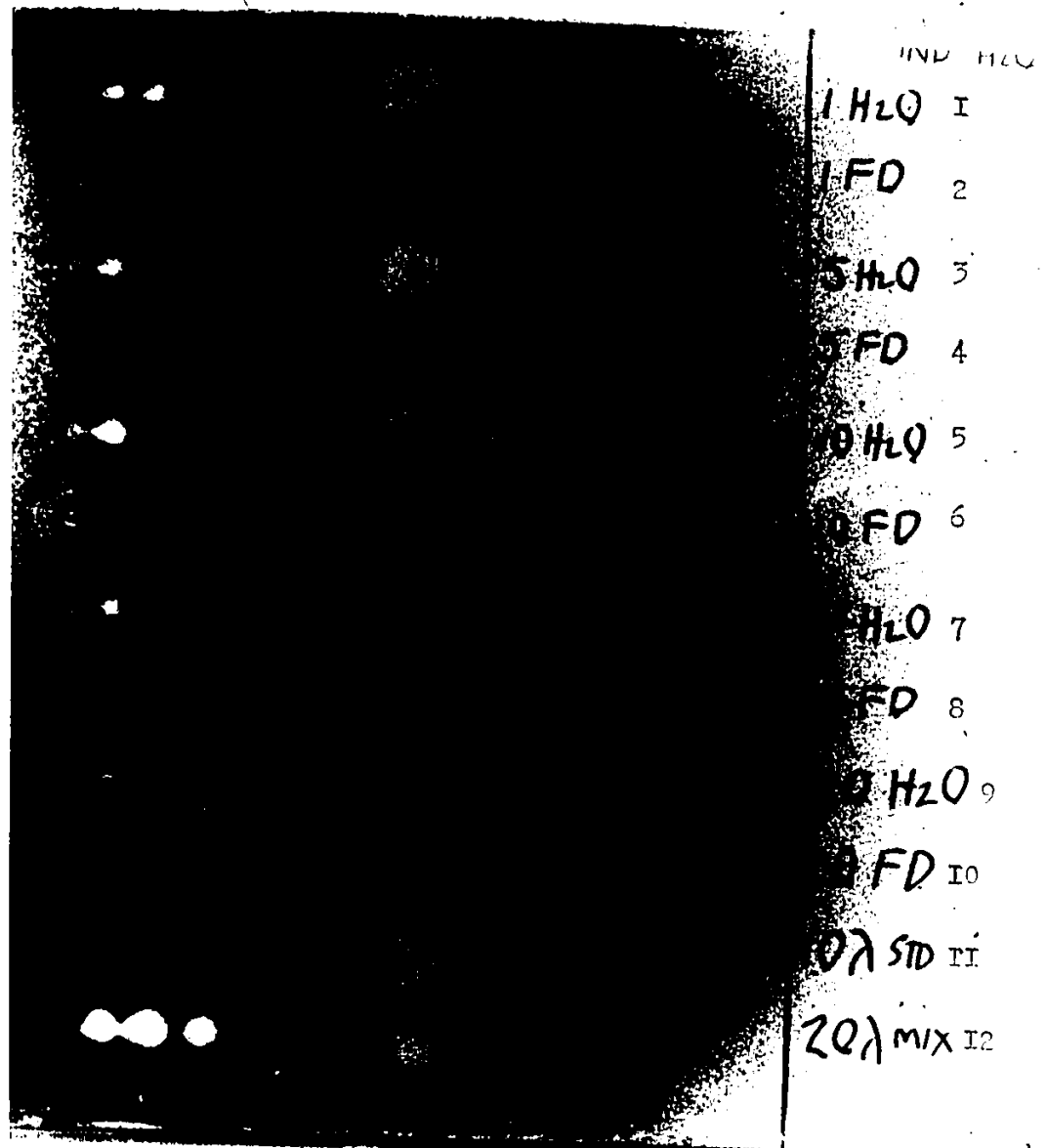


Plate 3

Plate 3. Model I: TLC of Lake water extracts

Legend: Chloroform extracts of water #1,3,5,7,9 -1,5,10,
19 and 20 day

Lyophilized extracts of water after chloroform extraction:

#2,4,6,8, #10 -1,5,10,14 and 20 day

¹⁴C labelled fenitrothion #11, 10 µl of stock

Mixed standards #12, 20 µl 8.8 NG/µl fenitrothion (RF=0.48),

16.4 NG/µl fenitro-oxon, 22.4 NG/µl S methyl fenitrothion
(RF=0.11) and 22.4 NG/µl aminofenitrothion (RF=0.16)

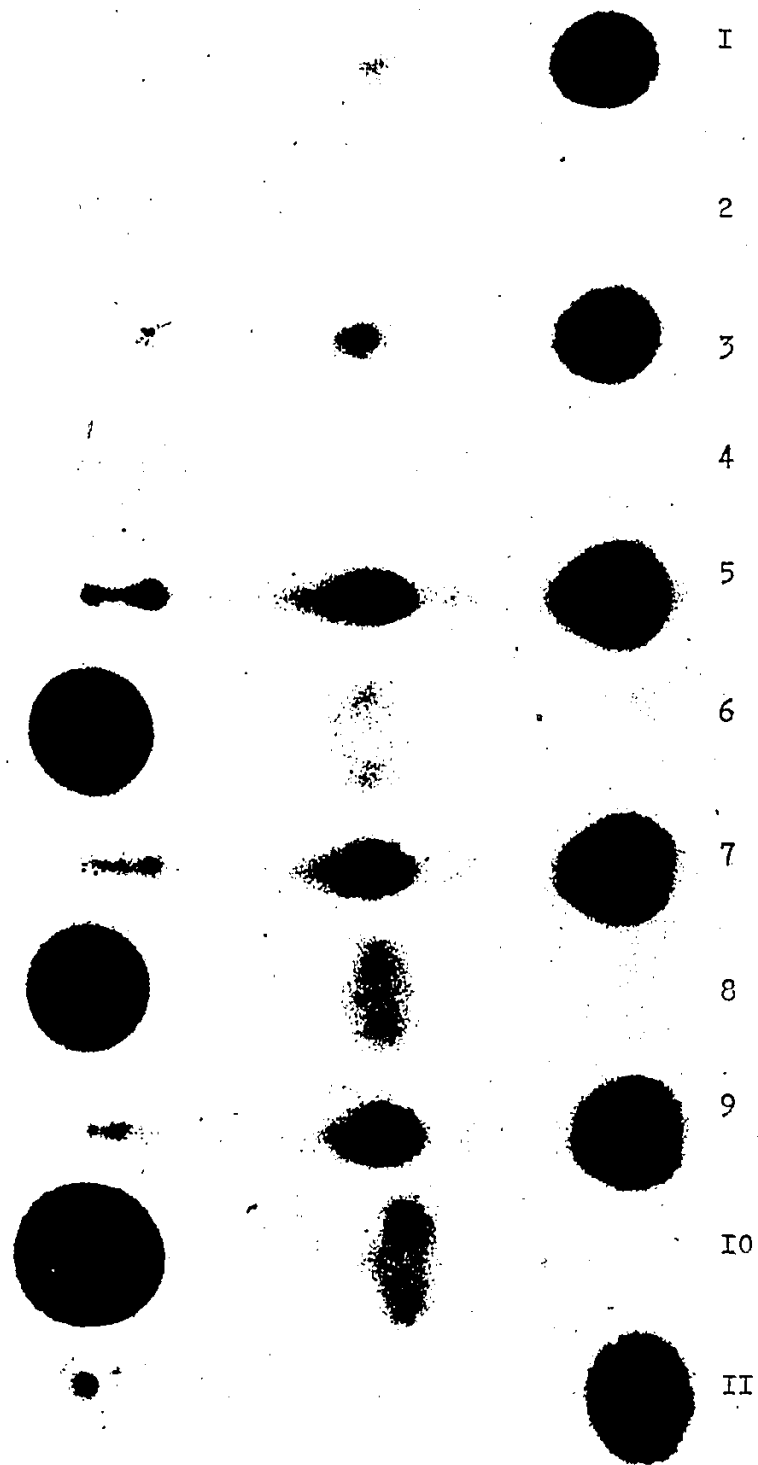


Plate 4

Plate 4. Model 1: Autoradiograph of lake water extracts.

Chloroform extracts of water: #1,3,5,7,9- 1,5,10,14 and
20 day samples.

Lyophilized extracts of water following chloroform
extraction: #2,4,6,8,10 - 1,5,10,14 and 20 day samples.

Stock ¹⁴C-labelled fenitrothion: #11- 10μl

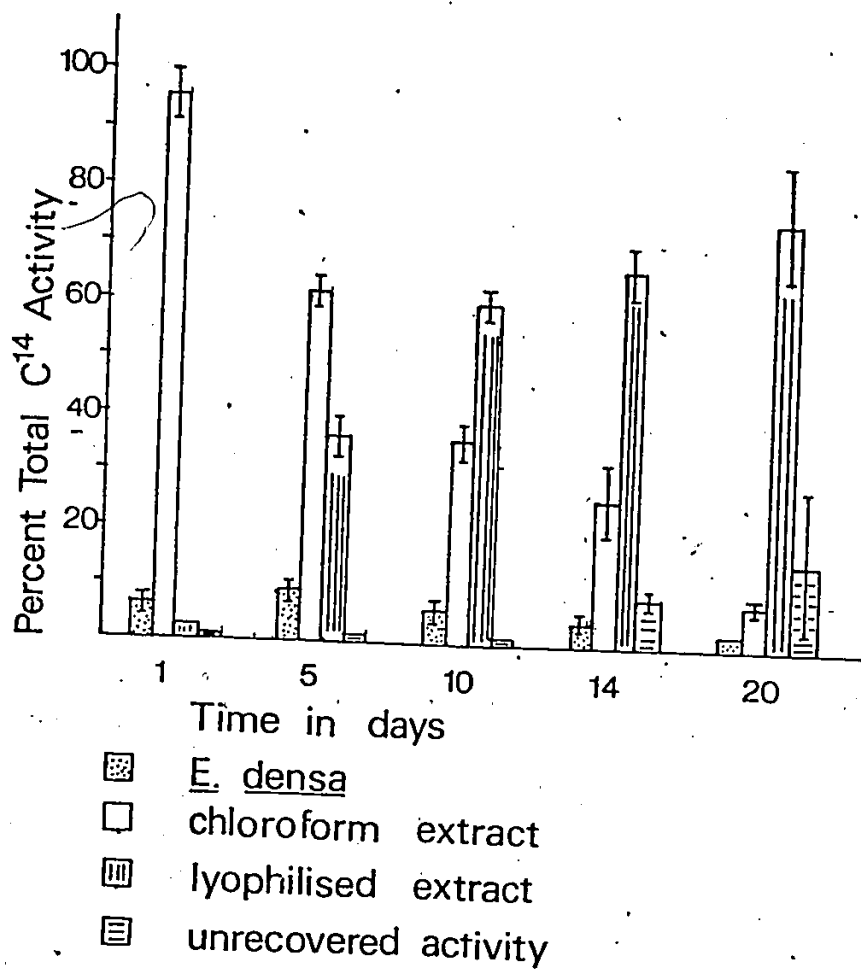


Figure 5: Distribution of C^{14} activity in model two.

represented the equivalent of 463 mg of dry weight plant per litre of water. This absorption peaked in 5 days at 137 µg/g of fenitrothion equivalent followed by a gradual decline to 27 µg/g at 25 days (Figure 6). Fenitrothion equivalent is the ^{14}C -activity recorded in the tissue extract converted into fenitrothion concentration, despite the fact that the total ^{14}C -activity is also comprised of several pesticide derivatives. This is necessary in the comparison of several plant species because each degrades fenitrothion in a slightly different way, and a true comparison in the total amount of pesticide absorbed can only be treated in this way.

A gradual decline in total ^{14}C -activity in the chloroform extract of water was also observed. This activity ranged from a high of 97.9% at one day to 25.8% at 14 days to 6.3% after 25 days (Figure 5).

A corresponding increase in total ^{14}C -activity occurred in the lyophilized water samples, from 0.7% at one day to 64.0% at 14 days to 69.8% at 25 days. The ^{14}C -activity in the lyophilized fraction represents the water-soluble derivatives not extracted by chloroform.

The unrecovered portion in model 2 increased from 0.2% at one day to 24% at 25 days post-treatment (Figure 5).

Thin-layer chromatography, autoradiography, and scintillation counting of E. densa extracts showed low levels of derivatives (Plates 5,6) and these are quantitated and shown in Table 7A and 7B.

At 14 days, 90.0% of the ^{14}C -activity in E. densa remained as fenitrothion, 10.3% as 3-methyl 4-nitrophenol, and minor amounts

-37-2-

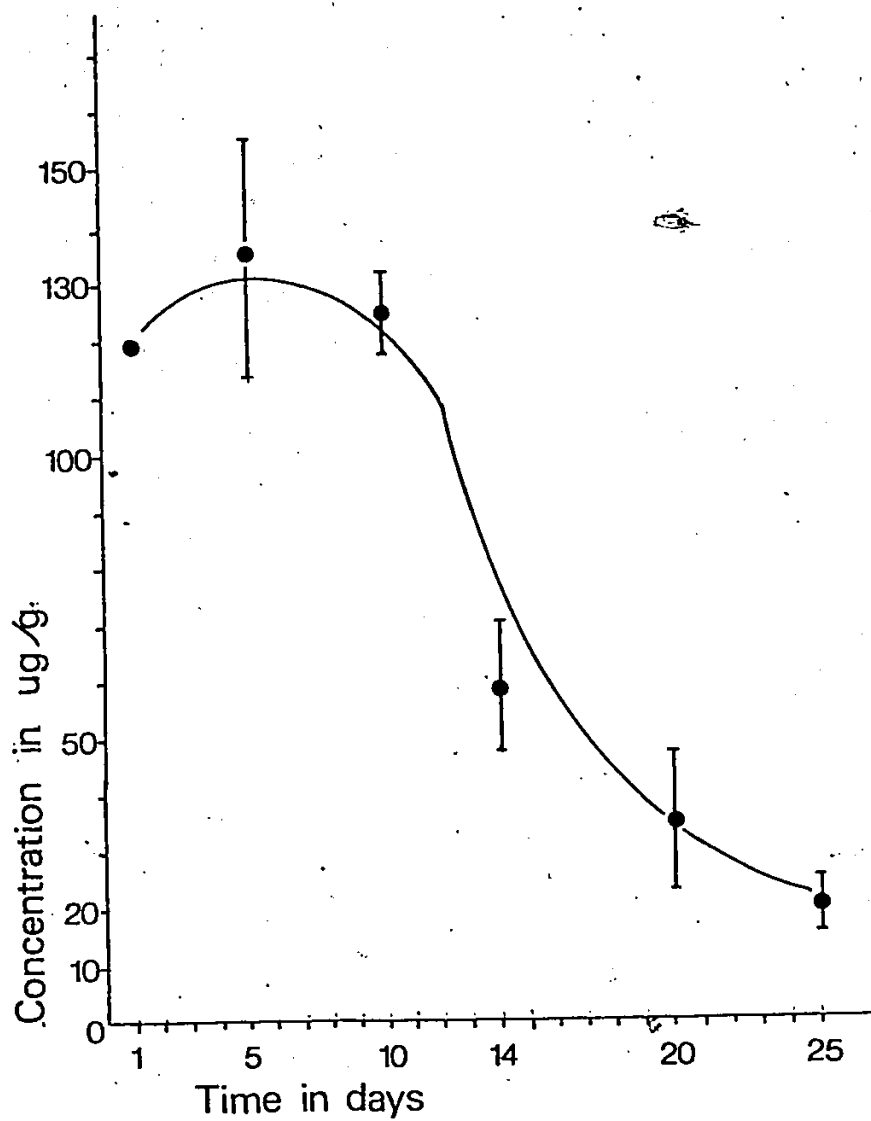
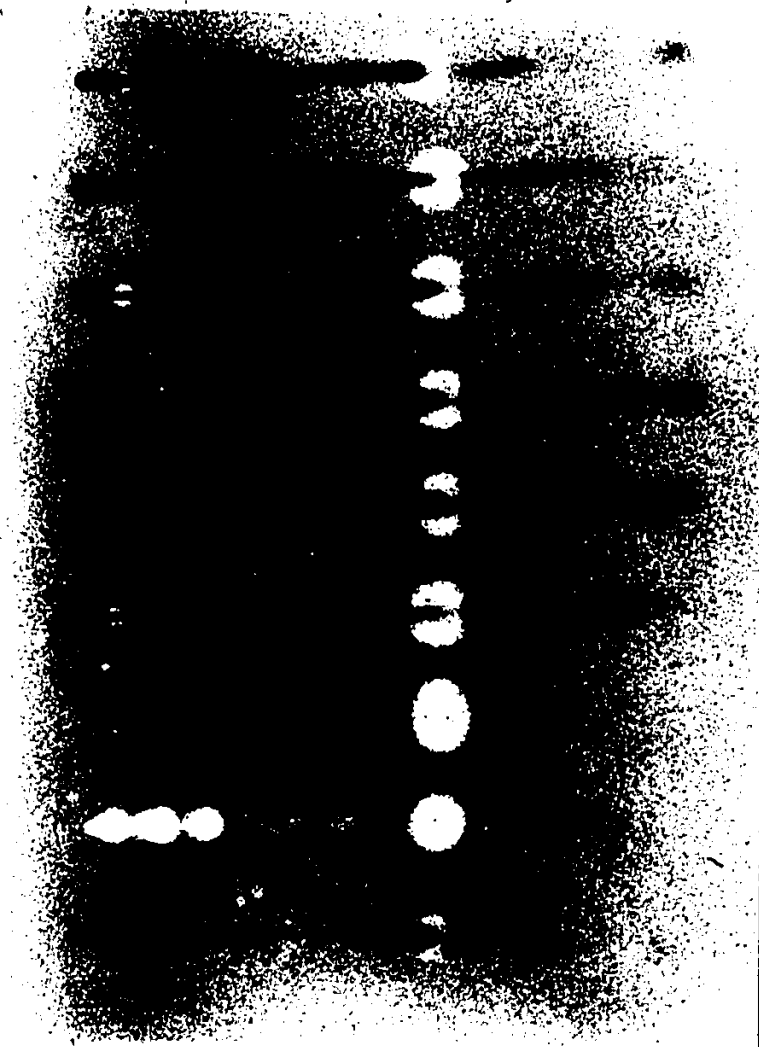


Figure 6: Penitrothion concentration in *E. densa*
in model two.



1 I
6 2
10 3
14 4
20 5
25 6
STD 70
MIX 840
CONT 9

Plate 5

#5

Plate 5. Model 2: T.L.C. of E. densa plant extracts.

E. Densa: #1,2,3,4,5,6- 1,5,10,14,20 and 25 day samples.

¹⁴C-labelled stock fenitrothion #7, 10µl

Mixed standard solution #8, 20µl of 16.4 NG/µl fenitro-oxon

(RF=0.05), 22.4 NG/µl S methyl fenitrothion (RF=0.11),

22.4 NG/µl aminofenitrothion (RF=0.16 and 8.8 NG/µl

fenitrothion (RF=0.48)).

Control plant-#9



Plate 6

16

Plate 6. Model 2: Autoradiograph of T.L.C. of E. densa plant extracts

Plants: #1,2,3,4,5,6 1,5,10,19,20 and 25 day samples.

¹⁴C-labelled stock fenitrothion: #7, 10µl

Table 7A

Model 2- E. densa

Concentration of Fenitrothion and Derivatives in E. densa

ug/g. dry weight

<u>Time</u> <u>(days)</u>	<u>Fenitro-oxon</u>	<u>S-methyl-</u> <u>fenitrothion</u>	<u>3-methyl-</u> <u>4-nitrophenol</u>	<u>Fenitrothion</u>
<u>Rf</u>	<u>0.05</u>	<u>0.11</u>	<u>0.29</u>	<u>0.52</u>
1	0.51		1.57	116.7
5	0.08		0.53	135.4
10	0.63		1.70	120.2
14	0.44		3.53	55.8
20	0.42	0.38	0.99	36.5
25	1.34	0.13	3.61	19.1

Table 7B

Model 2-E. densa

Fenitrothion and Derivatives as a Percent of Total

¹⁴C-Activity in E. densa

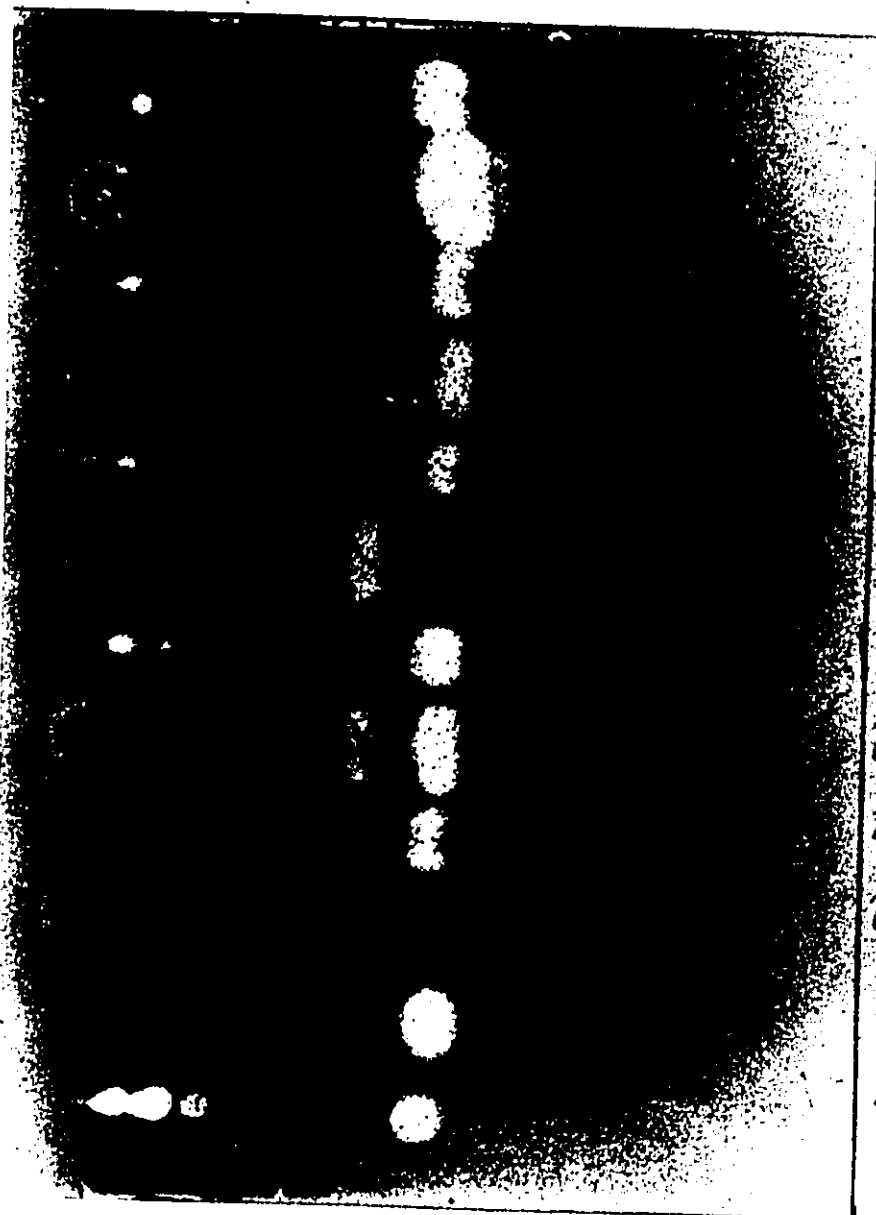
<u>Time</u> <u>(days)</u>	<u>Fenitro-oxon</u>	<u>S-methyl</u> <u>fenitrothion</u>	<u>3-methyl</u> <u>4-nitrophenol</u>	<u>Fenitrothion</u>
<u>Rf</u>	<u>0.05</u>	<u>0.11</u>	<u>0.28</u>	<u>0.52</u>
1	0.45		0.29	97.26
5	0.06		0.69	99.25
10	0.54		2.49	96.97
14	0.75		10.32	89.96
20	1.13	0.98	4.62	93.27
25	5.21	0.48	24.1	70.22

of fenitro-oxon (0.8%). At 20 and 25 days, low levels of S-methyl-fenitrothion (<1.0%) were detected. By 25 days, 70.2% of the ^{14}C -activity in the plant extract was fenitrothion, 24.1% was 3-methyl 4-nitrophenol and 5.2% was fenitro-oxon (Table 7B).

Thin-layer chromatography, autoradiography, and scintillation counting of the chloroform extracts of water and the lyophilised water showed low levels of chloroform-extractable derivatives, but large quantities of water soluble derivatives, (Plate 7 and 8) and these are quantitated in Tables 8A and 8B. Of the total recovered ^{14}C -activity in the total water at 5 days, 57.9% was fenitrothion, 4.9% was 3-methyl 4-nitrophenol and 36.8% were the water soluble derivatives. At 25 days 69.9% of the total recovered ^{14}C -activity in the water were water-soluble derivatives and 3-methyl 4-nitrophenol and fenitrothion were present in amounts of 23.3% and 3.3% respectively (Tables 8A, 8B).

Comparison of Fenitrothion and Derivatives in the Water of Models 1 and 2

The contribution of the plant to degradation of the pesticide in the water was revealed by the comparison of the amounts of fenitrothion and the amounts and types of derivatives found in the water of models one and two. The water of the plant-water system (Model 2) showed a greater increase in water soluble derivatives from 5 to 20 days. In model one (water alone) the water soluble derivatives totalled 3.5% after 5 days compared to 36.8% in model 2 (plant-water system) after the same time. The difference was even more marked at 20 days with 23.5% and 82.9% of the total ^{14}C -activity as water-soluble derivatives in model one and two, respectively. The maximum amounts of unknowns (R_f 0.38) at 2.6% and the



E 027 115
 5 H₂O I
 5 FD 2
 10 H₂O 3
 10 FD 4
 14 H₂O 5
 14 FD 6
 20 H₂O 7
 20 FD 8
 25 H₂O 9
 25 FD 10
 5 TO (K) II
 5 TO 12
 PVA

Plate 7

Plate 7. Model 2: T.L.C. of extracts of water from E. densa model

! Chloroform extracts of water: #1,3,5,7,9- 5,10,14,20 and
25 day samples.

Lyophilized extracts of water after chloroform extraction:

#2,4,6,8,10 - 5,10,14, 20 and 25 day samples.

¹⁴C-labelled fenitrothion -#11

Mixed standard #12, 20µl containing 8.8 NG/µl fenitrothion

(RF=0.48), 16.4 NG/µl fenitro-oxon (RF=0.06), 22.4 NG/µl

S methyl fenitrothion (RF=0.11) and 22.4 NG/µl aminofenitro-
thion (RF=0.16).

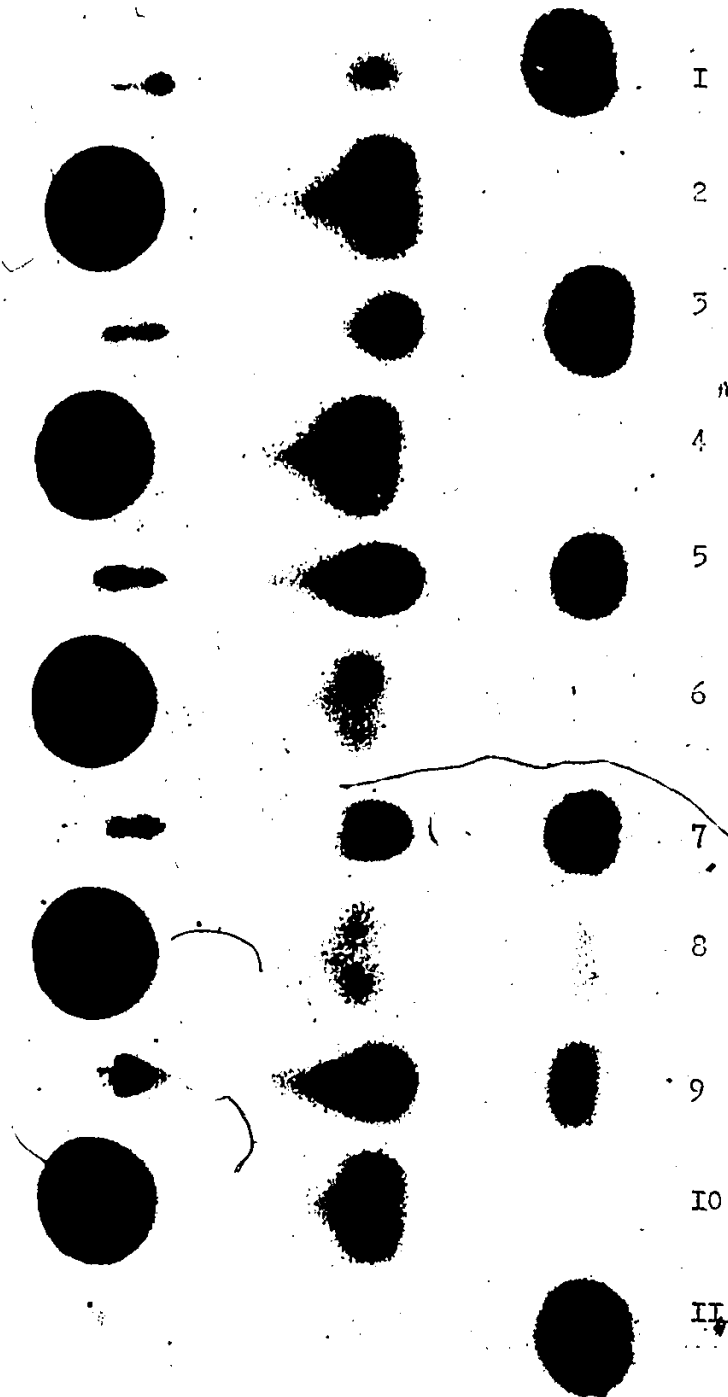


Plate 8

Plate 8. Model 2: Autoradiograph of T.L.C. Extracts of water from

E. densa model.

Chloroform extracts of water: #1,3,5,7,9-5,10,14,20,25 day.

Lyophilized water extracts after chloroform extraction

#2,4,6,8,10-represents 5,10,14,20,25 samples.

Stock C-14 fenitrothion, #11-10 uL

Table 8A

Model 2- E. densa Study

Concentration of Fenitrothion and Derivatives in Water (ng/q.)

<u>Time</u> <u>(days)</u>	<u>Fenitro-</u> <u>-oxon</u>	<u>S-methyl-</u> <u>fenitrothion</u>	<u>3-methyl</u> <u>4-nitro-</u> <u>-phenol</u>	<u>Unknown</u>	<u>Fenitro-</u> <u>-thion</u>
<u>Rf</u> <u>0</u>	<u>0.05</u>	<u>0.11</u>	<u>0.29</u>	<u>0.42</u>	<u>0.52</u>
5 W ² LW ³ (36.7%) ⁴	3.46	0.78	4.51 19.19	nd nd	587.80 8.38
10 W LW (42.8%)	3.43	1.00	15.54 30.88	nd nd	327.33 3.60
14 W (0.45%) LW (64.4%)	5.47	1.40	42.94 9.68	5.7×10^{-2} 2.4×10^{-2}	162.11 5.54
20 W LW (82.9%)	2.70	1.15	18.60 7.16	nd 2.02	51.32 9.98
25 W (0.57%) LW (69.9%)	3.73	0.69	23.16 35.37	nd nd	8.90 n/a

1-The initial fenitrothion concentration was 0.983 ug/g.

2-'W' represents the chloroform extract of the water.

3-'LW' represents the lyophilised water after chloroform extraction.

4- The number in brackets refers to the percent of the total recovered ¹⁴C-activity in the water which remained in the lyophilised extract at the origin of the TLC plate.

Table 8B

Model 2-E. densa Study

Fenitrothion and Derivatives as a Percent of Total ¹⁴C-Activity
Recovered from the Water¹

Time (days)	ng/g				
	Fenitro- -oxon	S-methyl- fenitrothion	3-methyl- 4-nitro- phenol	Unknown	Fenitro- -thion
<u>Rf</u> <u>0</u>	0.05	0.11	0.29	0.42	0.52
5 ² W ₃ LW ₃ (36.7%)	0.36	0.08 4.11	0.80	nd	56.9 0.99
10 W LW (42.8)	0.53	0.15	4.17 4.57	nd nd	47.53 0.23
14 W 0.45 LW 64.4	0.70	0.17	9.42 1.94	0.06 2.61	19.53 0.62
20 W LW 82.9	0.38	0.15	4.50 1.61	nd 2.44	6.81 1.24
25 W 0.57 LW 69.9	1.47	0.26	15.74 7.23	nd	3.32 nd

1-The initial fenitrothion concentration was 0.983 ug/g.

2- 'W' represents the chloroform extract of the water.

3- 'LW' represents the lyophilised water after chloroform extraction.

3-methyl 4-nitrophenol at 11.4% of the total ^{14}C -activity were greater in the plant-water system (model 2) than that of the water system (model 1), with 0.5% as the unknown (Rf 0.38) and 6.1% as 3-methyl 4-nitrophenol after 20 days. Amounts of fenitro-oxon and S-methyl fenitrothion were similar in both models. The results of this comparison would indicate that the presence of the plant in a water system increased the fenitrothion degradation to certain chloroform extractable derivatives and water-soluble derivatives.

Model three

Model 3 was designed to show the accumulation of fenitrothion by Elodea canadensis in a model with plant loads which could occur in natural shallow pond systems (less than 2m).

At each sample time, the total ^{14}C -activity in the plants, water and unrecovered portion is shown in Figure 7. The unrecovered ^{14}C -activity shown in Figure 7 represents the difference between the total amount of ^{14}C -activity added to the system and the total ^{14}C -activity recovered from the total plant-water system.

A gradual decline in the total ^{14}C -activity in the water, as represented by the chloroform extract of the water, was noted. At 1 day, 95% of the total ^{14}C -activity added at zero time, was still present in the water as chloroform extractables. At 14 days, only 71.0% of the total ^{14}C -activity added to the system was present in the water as chloroform extractables.

About 5.0% to 13.5% of the total ^{14}C -activity was sequestered

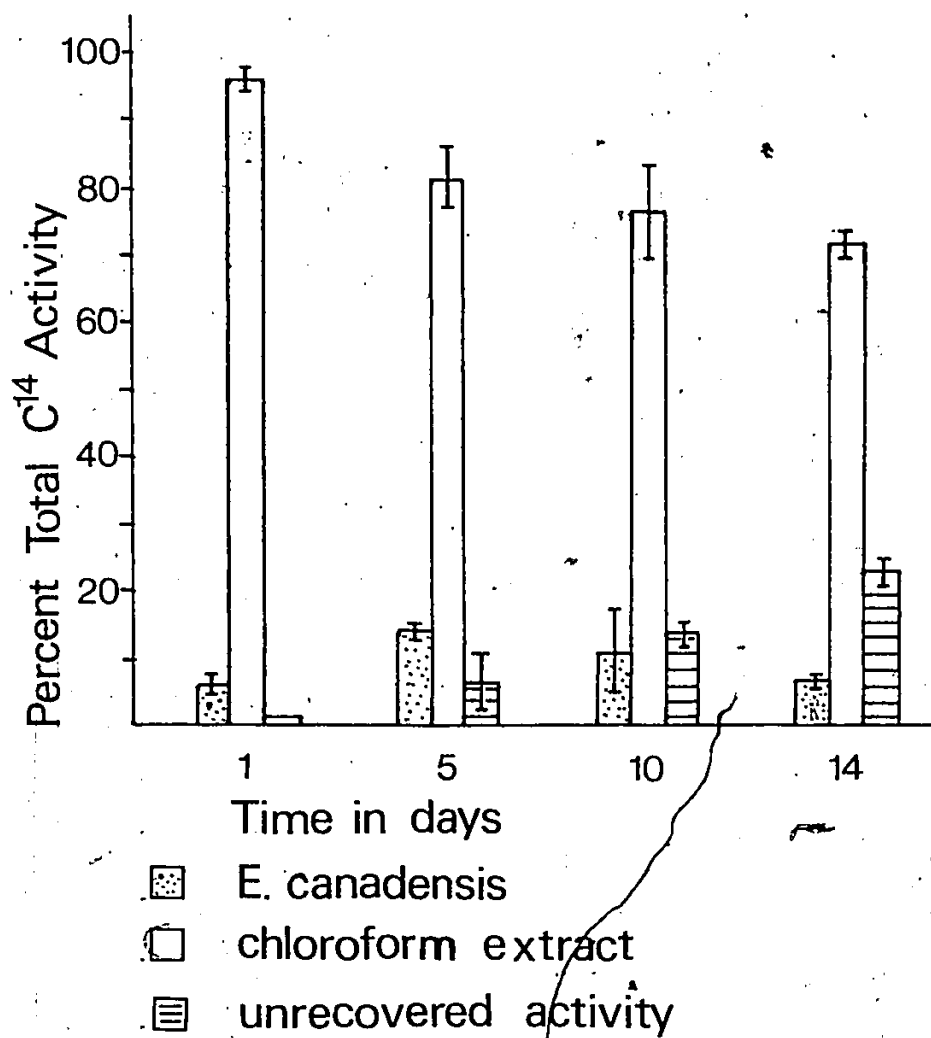


Figure 7: Distribution of C¹⁴ activity in model three.

in E. canadensis (177 mg. dry weight per litre of water) over the period of one to fourteen days, with the peak absorption at 5 days. At 14 days, the amount of unrecovered ^{14}C -activity increased to 23.0% of the total applied activity.

Thin layer chromatography (Plate 9) and direct counting of the ^{14}C -activity of the TLC plate gel of the plant extracts, revealed that more than 98.7% of the ^{14}C -activity was in the form of fenitrothion.

The total fenitrothion accumulated in E. canadensis tissue at all plant load levels at each of the 2 fenitrothion concentrations is shown in Figure 8. Accumulation in the Elodea fell into 2 groups according to segment size. There was very little difference in accumulated fenitrothion between segments of sizes 1.2 and 2.5 centimeters or between the 7.6 and 10.2 cm segments.

Interestingly, under a 10 $\mu\text{g/g}$ fenitrothion load in the water, the 10.2 cm segments (897 mg/l) of E. canadensis absorbed 180 μg of fenitrothion, about 45% of the total activity applied to the system, whereas under a 1 $\mu\text{g/g}$ fenitrothion concentration the same-sized segments absorbed 11.5 μg of fenitrothion, or about 29% of the total applied pesticide.

In Figure 9 the actual concentration of fenitrothion in E. canadensis is shown, this being a transformation of Figure 8. The rate of absorption of fenitrothion and the absolute amounts present depended on the concentration of pesticide in surrounding water. Using the 1.2 cm segments as an example, a peak absorption of $47,207 \pm 1207 \mu\text{g/g}$ of fenitrothion in Elodea was found in the 10 $\mu\text{g/g}$ fenitrothion solution compared to a maximum of $1435 \pm 236 \mu\text{g/g}$ in the 1.0 $\mu\text{g/g}$

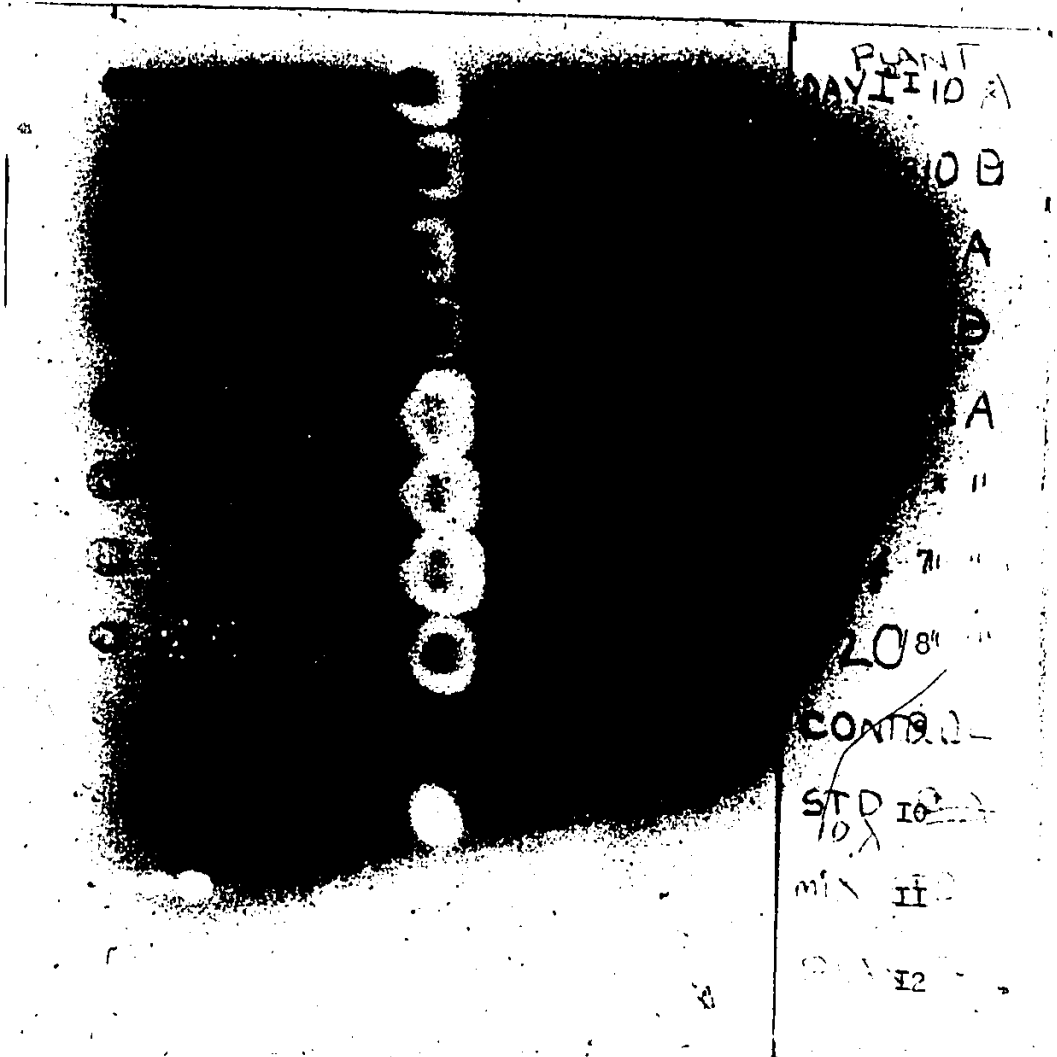


Plate 9

Plate 9. Model 3: T.L.C. of E. canadensis plant extracts

One day samples #1,2, from 10 µg/g fenitrothion solutions

One day samples #3,4 from 1 µg/g fenitrothion solutions

Plant extract #5,6,7,8 - 5,8,14 and 20 day samples from
10 µg/g fenitrothion solution

¹⁴C labelled fenitrothion #10, 10µl stock

Control plant sample #12

Mixed standard #11, 20 µl containing 8.8 NG/ µl fenitrothion
(RF=0.48)

16.4 NG/µl fenitro-oxon (RF=0.06) 22.4 NG/µl S methyl
fenitrothion (RF=0.11), and 22.4 NG/µl aminofenitrothion
(RF=0.16).

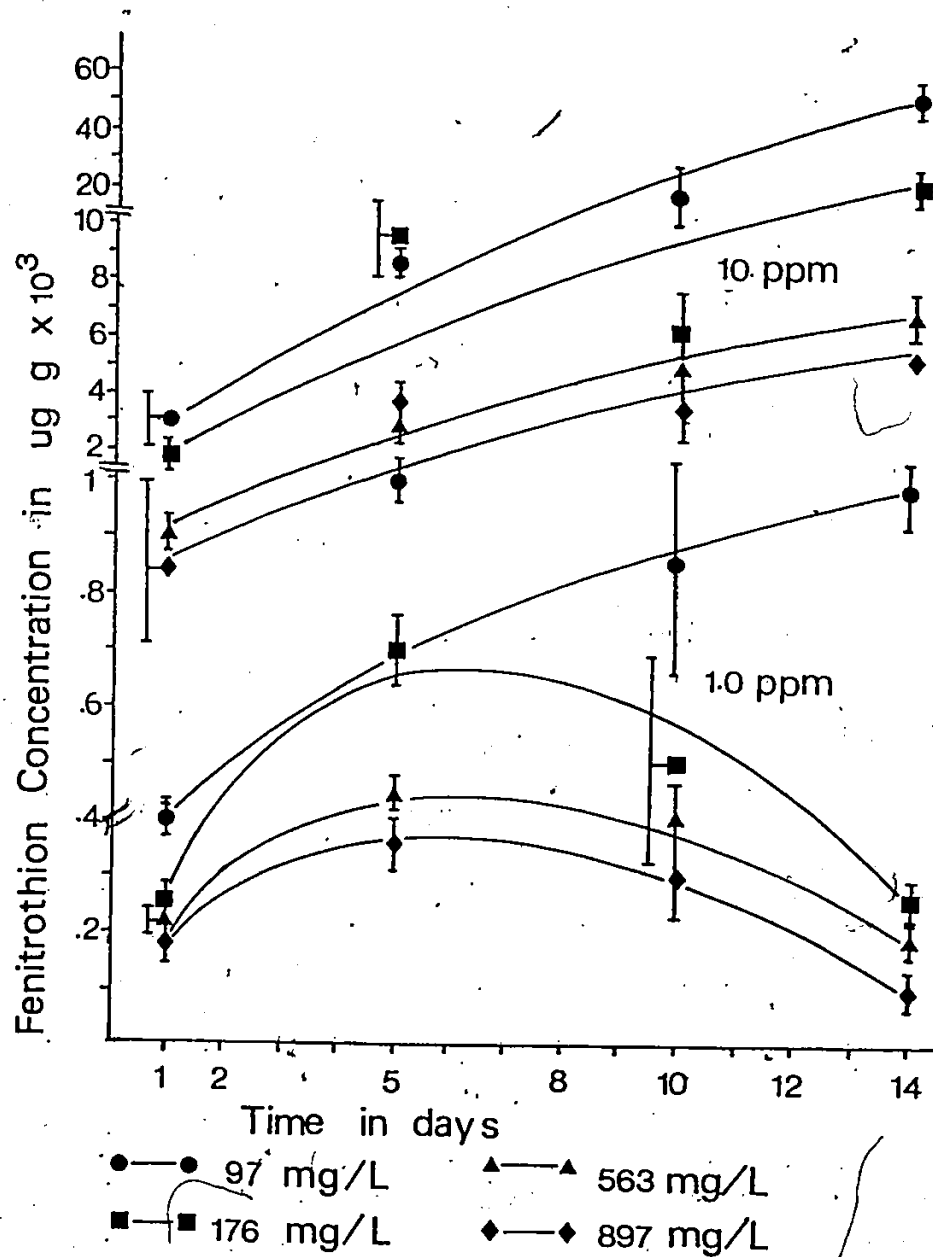


Figure 8: Total fenitrothion uptake by *E. canadensis* in model three.

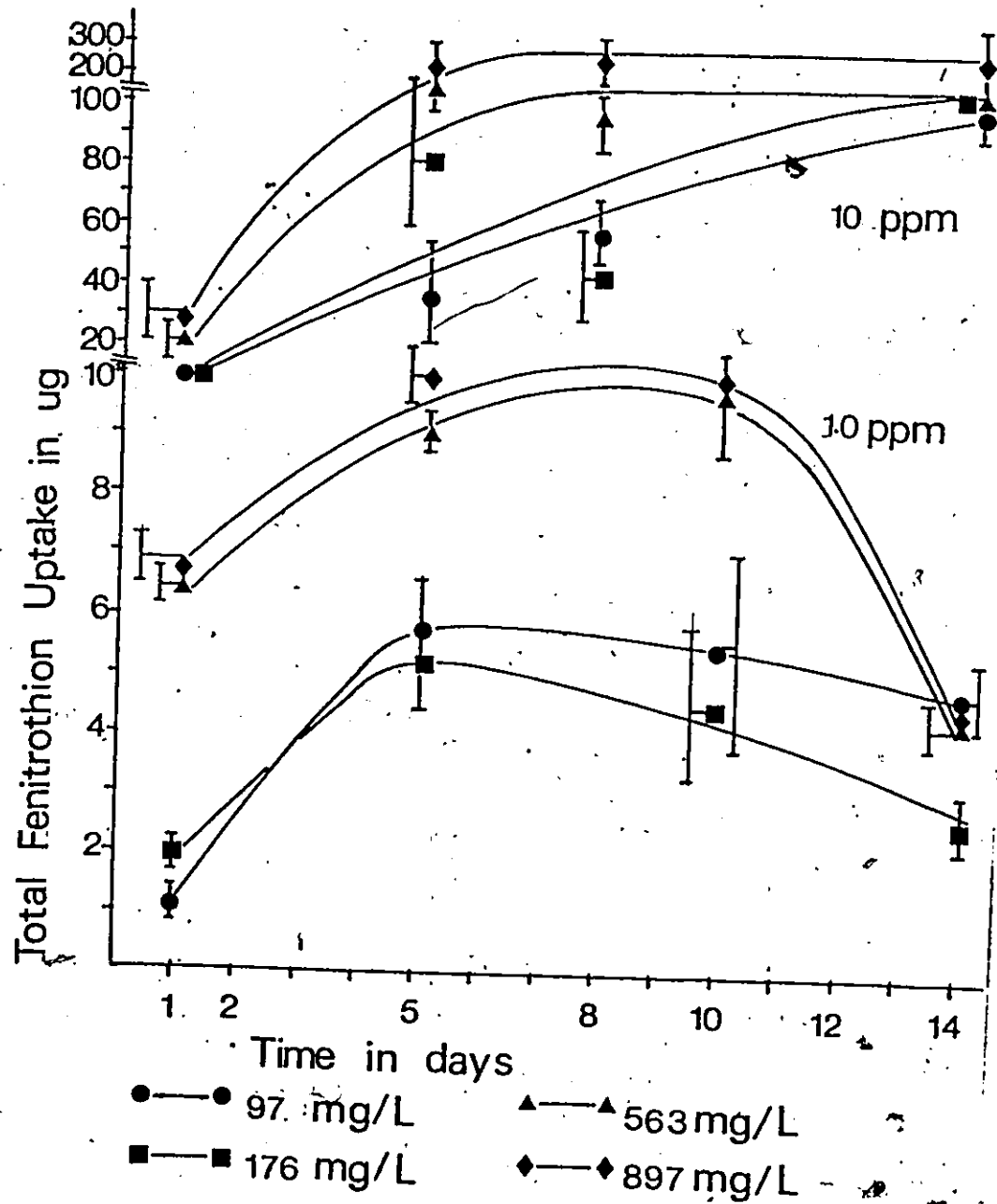


Figure 9: Fenitrothion concentration in *E. canadensis* in model three.

fenitrothion solution.

Whereas the concentration in Elodea increased constantly up to 14 days in the 10 µg/g solution, absorption by Elodea in the 1.0 µg/g solution peaked at 5 days followed by a decline in pesticide concentration. The estimated time of reduction of fenitrothion in Elodea to 1.0 µg/g levels was from 20 to 37 days depending on the plant segment activity in the 1.0 µg/g fenitrothion solution.

Further, the smaller Elodea segments showed greater pesticide absorption than the larger segments (Figure 9). In both the 1.0 µg/g and 10.0 µg/g experiments, the order of decreasing concentration in the tissue was in the 1.2, 2.5, 7.6 and 10.2 cm plant segments.

The water in this plant-water system was also analysed to recover all ¹⁴C-activity and to determine the extent of pesticide degradation.

Examination of the water extracts by TLC showed that some derivatives were present (Plate 10) and this was confirmed by autoradiography.

Quantitation of the toxic derivatives and the 3-methyl 4-nitrophenol are shown in Tables 9A and 9B.

Small amounts of fenitro-oxon, S-methyl fenitrothion, amino-fenitrothion, 3-methyl 4-nitrophenol and unknown (Rf 0.38) were detected and quantitated (Tables 9A and 9B). Fourteen days post-treatment, 94.15% of the ¹⁴C-activity in the chloroform extract was fenitrothion, with 3.5% as 3-methyl 4-nitrophenol, 1.6% as fenitro-oxon and the remainder being minor amounts of S-methyl fenitrothion and amino-fenitrothion.

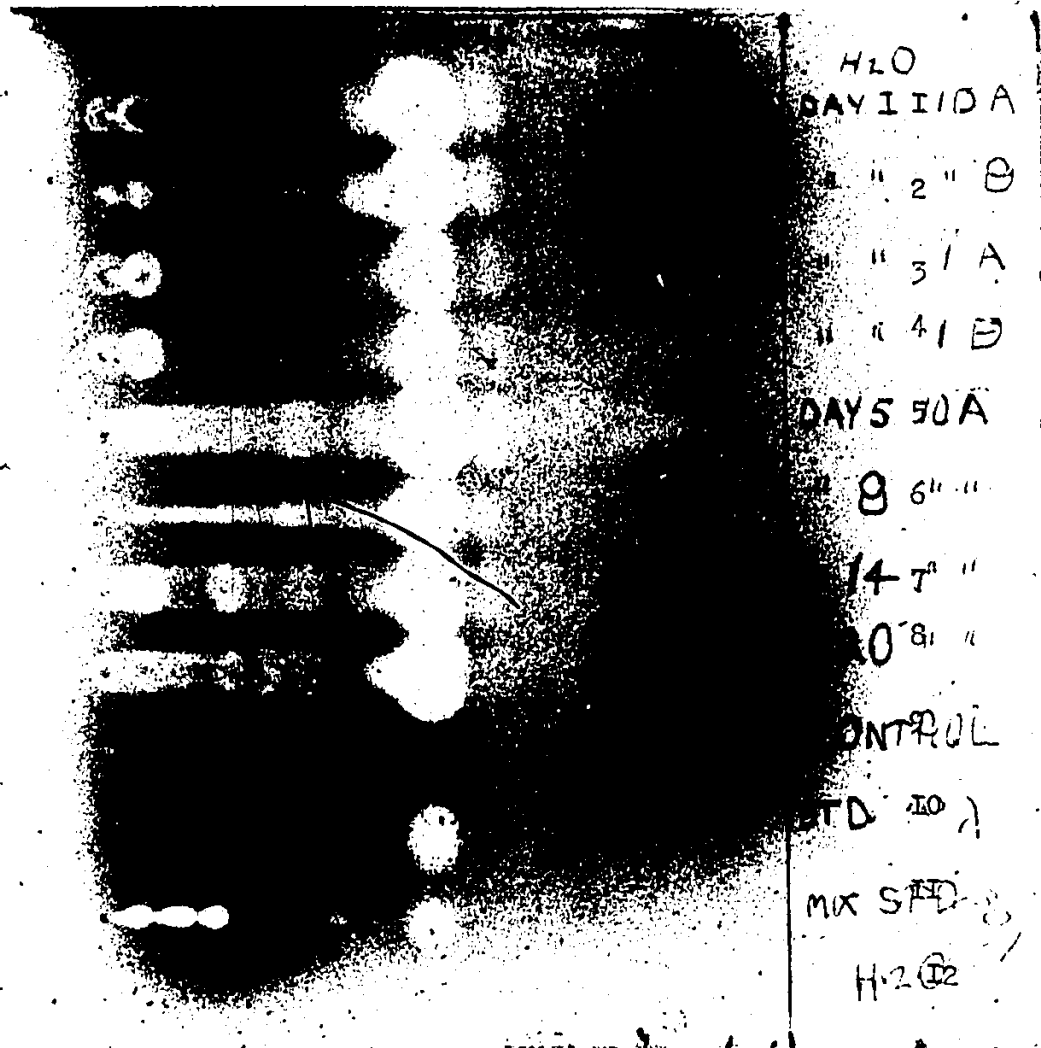


Plate 10

Plate 10. Model 3: T.L.C. of chloroform extracts of water from

E. canadensis model.

one day sample #1,2 from 10 μ g/g solution

one day samples #3,4 from 1 μ g/g solutions

Plant samples #5,6,7,8, -5,8,14 and 20 days

Control water extract #9

¹⁴C-labelled fenitrothion #10, 10 μ l stock

Mixed standard #11, 30 μ l of solution containing 8.8 NG/ μ l

fenitrothion (RF=0.48), 16.4 NG/ μ l fenitro-oxon (RF=0.06)

22.4 NG/ μ l Smethyl fenitrothion (RF=0.11), and 22.4 NG/ μ l

aminofenitrothion (RF=0.16)

Table 9A
Model 3- E. canadensis Study
Concentration of Fenitrothion and Derivatives in Water
ng/g

<u>Time</u> <u>(days)</u>	<u>Fenitro-</u> <u>-oxon</u>	<u>S-methyl-</u> <u>fenitro-</u> <u>-thion</u>	<u>Amino-</u> <u>fenitro-</u> <u>-thion</u>	<u>Unknown</u>	<u>3-methyl</u> <u>4-nitro-</u> <u>phenol</u>	<u>Formyl-</u> <u>fenitro-</u> <u>-thion</u>	<u>Fenitro-</u> <u>-thion</u>
<u>Rf</u>	<u>0.05</u>	<u>0.11</u>	<u>0.16</u>	<u>0.22</u>	<u>0.28</u>	<u>0.36</u>	<u>0.48</u>
1A ¹	17.52			(0.67) ³			806.4
1B ¹	34.58			(2.06) ³			762.8
1A ²	12.62	8.04		(0.64) ³		9.29	8851.5
1B ²	16.59	14.82		(0.46) ³		36.00	9142.4
5 ²	22.59	15.81			80.42	14.75	5633.0
8 ²	17.00	13.36	11.64		38.01	14.80	6543.9
14 ²	60.78	n/a	28.06		76.08	n/a	3705.9
20 ²	50.17	23.95	20.83	(0.68) ³	118.3	29.95	3712.9

- 1-The initial concentration of the 1.0ppm solution was 9832 ng/g.
2-The initial concentration of the 10.0ppm solution was 983.2ng/g.
3-The number in brackets refers to the percent of the total recovered ¹⁴C-activity in the chloroform extract of water found at Rf 0.28.

Table 9B

Model 3- E. canadensis Study

Fenitrothion and Derivatives as a Percent of Total
¹⁴C-Activity Recovered from the Water

<u>Time</u> <u>(days)</u>	<u>Fenitro-</u> <u>-oxon</u>	<u>S-methyl-</u> <u>Fenitro-</u> <u>-thion</u>	<u>Amino-</u> <u>fenitro-</u> <u>-thion</u>	<u>Unknown</u>	<u>3-methyl</u> <u>4-nitro-</u> <u>phenol</u>	<u>Formyl-</u> <u>fenitro</u> <u>-thion</u>	<u>Fenitro-</u> <u>-thion</u>
<u>Rf</u>	<u>0.05</u>	<u>0.11</u>	<u>0.16</u>	<u>0.22</u>	<u>0.28</u>	<u>0.36</u>	<u>0.48</u>
1A ¹	2.24			0.67			97.09
1B ¹	6.91			2.06			91.03
1A ²	0.15	0.29		0.64		0.10	99.08
1B ²	0.19	0.16		0.46		0.36	98.83
5 ²	0.41	0.27			2.50	0.25	96.57
8 ²	0.27	0.20	0.23		1.03	0.21	98.07
14 ²	1.64	n/a	0.82		3.50	n/a	94.05
20 ²	1.31	0.58	0.56	0.68	5.25	0.69	90.9

1-The initial concentration of the 10 ppm solution was 9832 ng/g.

2-The initial concentration of the 1.0ppm solution was 983.2ng/g.

Table 9C

Model 3- E. canadensis study

Bioaccumulation Ratio of Fenitrothion in E. canadensis

Day	Sample	1.0 p.p.m. solution	10.0 p.p.m. solution
1	a	396	165
	b	256	177
	c	223	90
	d	213	98
5	a	1669	990
	b	839	1113
	c	618	535
	d	557	536
10	a	1209	1899
	b	636	755
	c	667	814
	d	539	868
14	a	1607	8968
	b	334	n/a
	c	267	1607
	d	150	1360
20	c	263	1060
28	c	233	n/a

-The sample designations 'a,b,c,d,' represent plant segments of length 1.2, 2.5, 7.6 and 10.2 cm respectively.

After 20 days, fenitrothion was present as 90.9% of the total ^{14}C -activity in the chloroform extract of water, 5.3% as 3-methyl 4-nitrophenol and 1.3% as fenitro-oxon (Table 9B).

Model 4

In this model, species differences in the absorption and degradation of fenitrothion from a $1.02 \mu\text{g/g}$ solution by 3 submerged aquatic angiosperms was followed.

The distribution of ^{14}C -activity among the total plants, the chloroform extract of water, the lyophilized water and the unrecovered portion is shown in Figure 10.

The plants as a group sequestered $6.7\% \pm 0.5\%$ to $8.0\% \pm 0.9\%$ of the total applied ^{14}C -activity in this system at a plant density of 221 mg dry weight per litre of water and this compared favourably with the results of model 3.

The amount of total ^{14}C -activity in the chloroform extract of the water declined from $79.0 \pm 12\%$ of the total at one day post-treatment to $34.0 \pm 6.0\%$ at 14 days. During the course of the experiment from one to 14 days, the total ^{14}C -activity in the lyophilized fraction increased from $3.0 \pm 0.7\%$ to $21.9 \pm 2.0\%$ and this also compared favourably with previous studies. The unrecovered portion increased from $11.9 \pm 11.0\%$ to $36.5 \pm 10.1\%$ of the total ^{14}C -activity and this was unusually high compared with 3 previous studies in which the unrecovered portion did not exceed 25%.

The equivalent fenitrothion concentration in the 3 plant

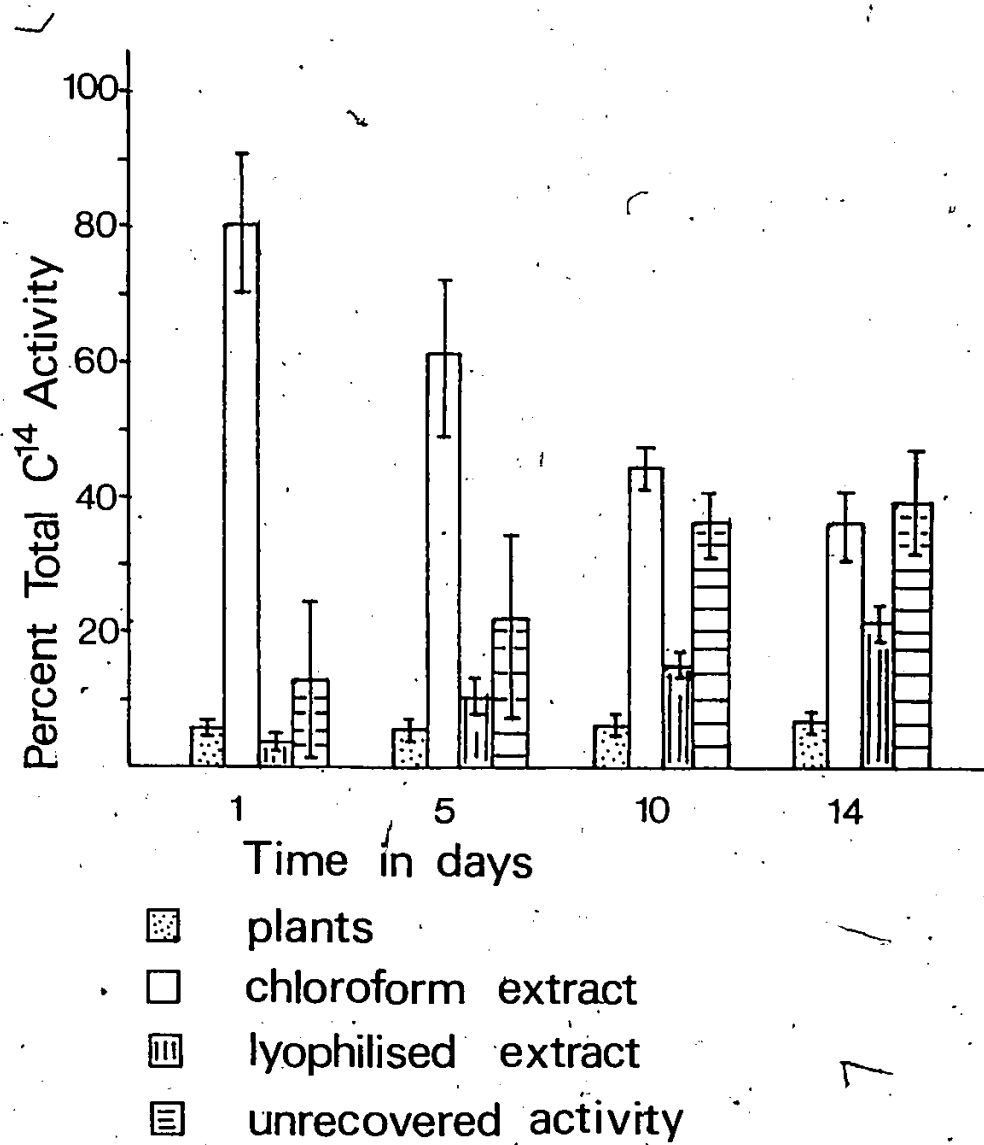


Figure 10: Distribution of C¹⁴ activity in model four.

species is shown in Figure 11, the "equivalent concentration" being used in the same sense as in model 2. Within the experimental period of one to 14 days, all 3 species, Elodea densa, Sagittaria graminea and Myriophyllum sp. showed fenitrothion equivalent concentrations within the range of 266 to 557 $\mu\text{g/g}$ dry weight. All 3 species demonstrated a fast absorption of fenitrothion in the first 24 hours at levels of from $268.6 \pm 62.9 \mu\text{g/g}$ to $384.9 \pm 144 \mu\text{g/g}$ dry weight. After 24 hours a slower rate of pesticide absorption occurred until a 10 day maximum showed a concentration in the range of $496 \pm 68.1 \mu\text{g/g}$ of fenitrothion equivalent in Myriophyllum sp. and $431.4 \pm 29 \mu\text{g/g}$ in E. densa. A slight decline in pesticide concentration occurred at 14 days with a concentration of $434.0 \pm 73 \mu\text{g/g}$ in Myriophyllum sp. and $283.5 \pm 125 \mu\text{g/g}$ in E. densa. A slower rate of pesticide absorption was observed in S. graminea after 24 hours as compared to the other 2 species but S. graminea showed continuous absorption after 10 days to a 14 day maximum concentration of $556.8 \pm 50 \mu\text{g/g}$ of fenitrothion equivalent. This 14 day maximum in Sagittaria surpassed the 10 day maximum concentration of the other two plant species. No visible damage to the plants was observed.

TLC, autoradiography and scintillation counting of the toxic derivatives and the 3-methyl 4-nitrophenol area of the plate revealed the derivatives fenitro-oxon, S-methyl-fenitrothion, 3-methyl 4-nitrophenol and unknowns at the origin (Plate 12). Quantitation of these derivatives are shown in Tables 10A and 10B.

Apart from Myriophyllum sp., most of the ^{14}C -activity in

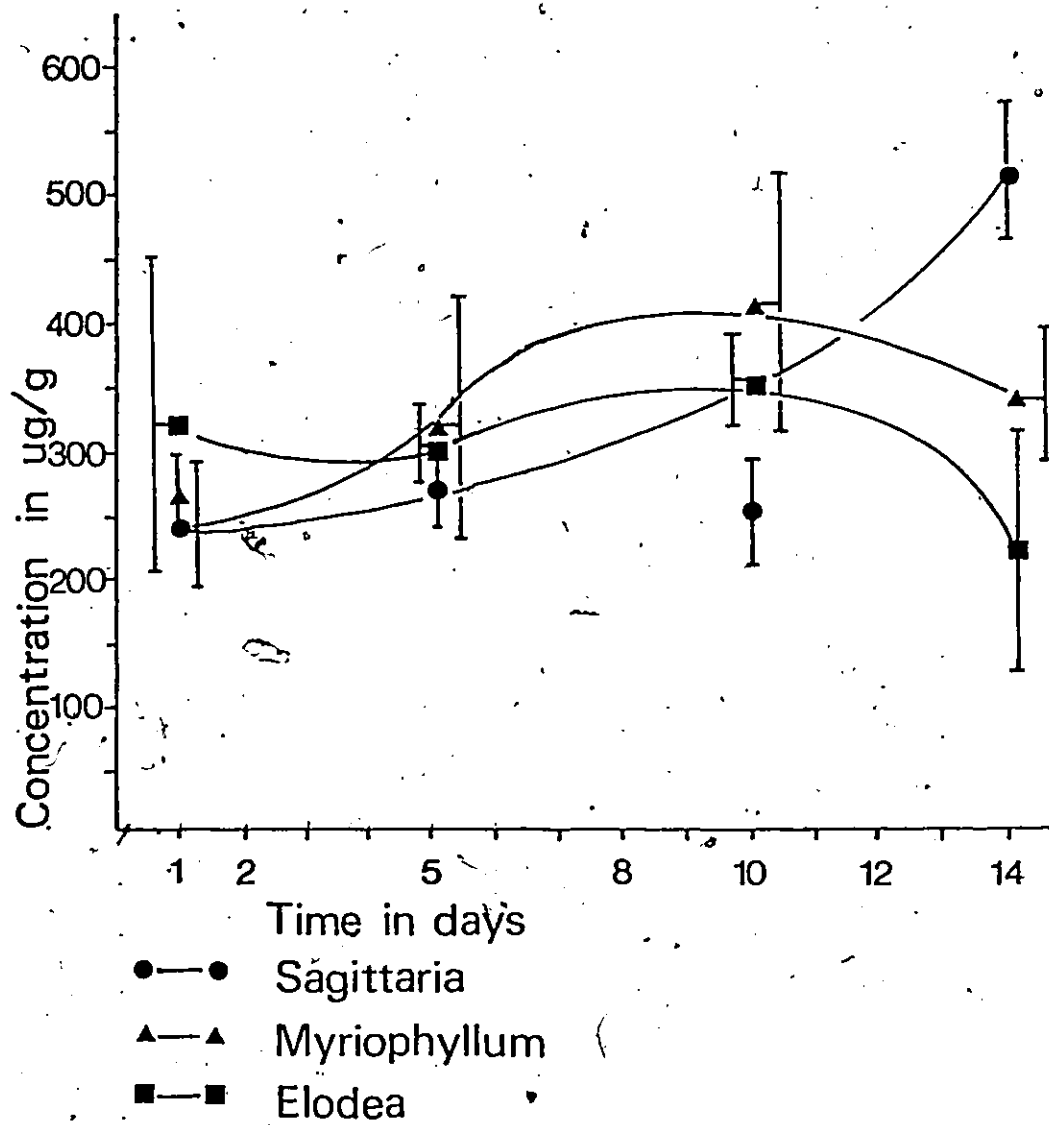


Figure 11: Total fenitrothion equivalent concentration in several plant species.

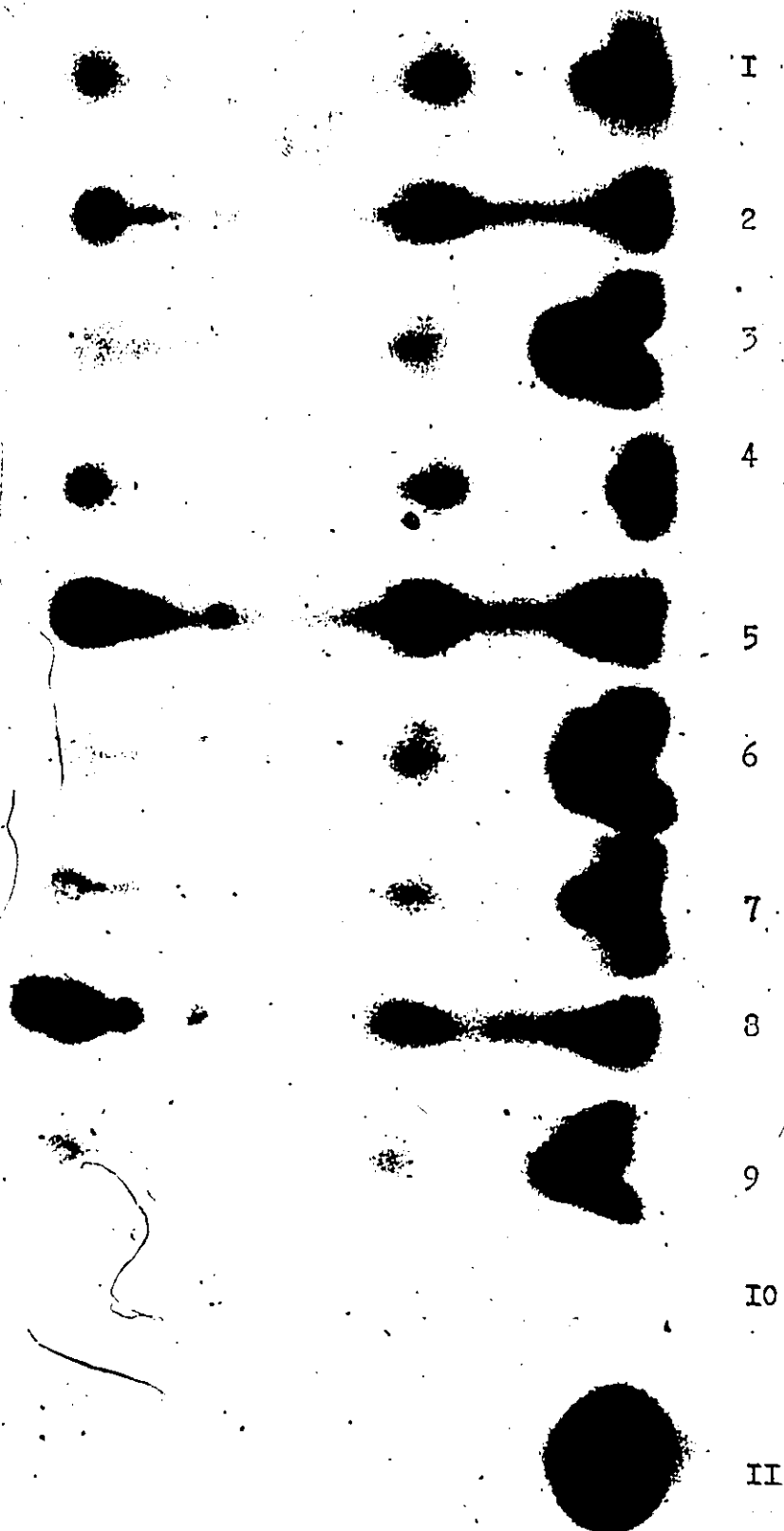


Plate I2

12

Plate 12. Model 4: Autoradiograph of T.L.C. of plant extracts from mixed plant study.

#1,2,3-5 day plant extracts from Sagittaria, Myriophyllum and Elodea respectively.

#4,5,6-10 day plant extracts from Sagittaria, Myriophyllum and Elodea respectively.

#7,8,9-14 day plant extracts from Sagittaria, Myriophyllum and Elodea respectively.

#10 30µl of solution containing unlabelled 8.8 NG/µl fenitrothion

#11 10.1 of stock ^{14}C fenitrothion

Table 10AModel 4- Mixed Plant Study

Concentration of Fenitrothion and Derivatives in Several Plant Species¹
ug/g (dry weight)

Time (days)	Species	Unknown ²	Fenitro-oxon	S-methyl- fenitrothion	3-methyl 4-nitro- phenol	Fenitro- thion
	<u>Rf</u>	<u>0</u>	<u>0.05</u>	<u>0.11</u>	<u>0.28</u>	<u>0.48</u>
5	Sag.	(3.4%)	0.08	2.63	14.17	244.2
	Myr	(10.5%)	15.41	7.99	38.17	239.1
	Elo	(1.7%)	2.51	0.99	5.15	350.8
10	Sag.	(6.9%)	4.87	3.90	20.4	206.2
	Myr	(17.0%)	64.52	15.33	38.88	257.7
	Elo	(1.6%)	2.36	4.06	7.72	404.0
14	Sag	(3.0%)	8.65	7.18	58.37	417.8
	Myr	(31.5%)	52.44	19.93	26.92	164.9
	Elo	(6.0%)	3.85	3.23	26.67	210.8

- Initial concentration in the water was 1.022 g/g fenitrothion
- The number in brackets refers to the percent of the total ¹⁴C-activity in the plant extract which remained at the origin of the TLC plate.
- The plant species included Sagittaria graminea, ('Sag'), Myriophyllum sp. ('Myr'), and Elodea densa ('Elo').

Table 10B

Model 4- Mixed Plant Study

Fenitrothion and Derivatives as a Percent of Total ¹⁴C-Activity in Several Plant Species¹

Time (days)	Species	Unknown	Fenitro-oxon	S-methyl- fenitrothion	3-methyl- 4-nitro phenol	Fenitro- thion
	<u>Rf</u>	<u>0</u>	<u>0.05</u>	<u>0.11</u>	<u>0.28</u>	<u>0.48</u>
5	Sag ³	3.4	0.03	0.93	9.1	86.6
	Myr	10.5	4.4	2.15	18.6	64.3
	Elo	1.70	0.72	0.27	2.52	94.8
10	Sag	6.92	1.91	1.44	13.61	76.12
	Myr	16.95	13.81	3.09	14.20	51.95
	Elo	1.59	0.58	0.94	3.24	93.65
14	Sag	3.03	1.65	1.29	18.99	75.04
	Myr	31.50	13.31	4.72	11.56	38.90
	Elo	6.04	1.44	1.14	17.04	74.34

¹-The initial concentration in the water was 1.022 µg/g fenitrothion

²-The unknowns were unidentified derivatives which remained at the origin of the TLC plate.

³-The plant species included Sagittaria graminea ('Sag'), Myriophyllum sp. ('Myr'), and Elodea densa ('Elo').

the plants was in the form of fenitrothion. In S. graminea the major derivative was 3-methyl 4-nitrophenol which represented 19.0% of the total activity in the plant at 14 days, with fenitrothion showing 75.0% of the total in the plants and from 3.0% to 6.9% as unknowns at the origin of the plate. Similarly in E. densa the 3-methyl 4-nitrophenol increased to 17.0% of the total in the plant extract at 14 days with fenitrothion as 74.3% of the ^{14}C -activity and 6.0% as unknowns at the origin of the plate.

Myriophyllum sp. showed exceptionally high levels of fenitrothion derivatives. The proportion of the ^{14}C -activity in the plant in the form of fenitrothion declined from 64.3% to 38.9% from five to 14 days with the major known derivatives being 3-methyl 4-nitrophenol and fenitro-oxon at 14.2% and 13.8%, respectively of the total activity by 10 days. However, the unknowns at the origin increased substantially from 10.5% to 31.5% of the total activity in the plants over 14 days.

TLC, autoradiography and scintillation counting of the water from model 4, in which the plants represented a mixed plant load of 221 mg/l showed several derivatives. Most of the ^{14}C -activity in the chloroform extract of water was fenitrothion, with lesser relative amounts of derivatives in the chloroform extract (ng/g level) of water, compared to the concentration of the same derivatives in the plants ($\mu\text{g/g}$ level). (Tables 11A, 11B and Plates 13, 14)

The proportion of ^{14}C -activity in the chloroform extract of water as fenitrothion, declined from 98.1% to 84.3% from one to

Table 11A

Model 4-Mixed Plant Study

Concentration of Fenitrothion and Derivatives in the Water¹ ng/g.

Time (days)		Fenitro- -oxon	S-methyl fenitrothion	Amino- fen.	3-methyl- 4-ethyl- phenyl- Unknown	Formyl- fen.	Fenitro- thion
	<u>Rf</u>						
	<u>0</u>	<u>0.05</u>	<u>0.11</u>	<u>0.16</u>	<u>0.26</u>	<u>0.34</u>	<u>0.57</u>
1 W ²	(0.06%)	2.04	1.42			5.57	794.7
LW ³							21.9
5 W	(0.45%)	6.18	0.74	2.26	1.43	20.30	584.9
LW	(3.5%)						71.0
10 W	(1.04%)	5.28	2.13	5.63	Rf0.22 15.28	26.01	380.9
LW	(3.80%)						81.1
14 W	(1.05%)	5.41	1.11	3.36	13.09	12.91	297.4
LW	(4.8%)						70.8

¹- The initial fenitrothion concentration was 1.022 g/g

²- 'W' represents the chloroform extract of water.

³- 'LW' represents the lyophilised water after chloroform extraction.

Table 11B

Model 4- Mixed Plant Study

Fenitrothion and Derivatives as a Percent of Total ¹⁴C-Activity Recovered

from Water¹ ng/g

Time (days)	Rf	Fenitro- -oxon	S-methyl- fenitrothion	Amino- fen.	3 BENZYL 4 NITROPHENYL Unknown	Formyl- fen.	Fenitro- thion
	<u>0</u>	<u>0.05</u>	<u>0.11</u>	<u>0.16</u>	<u>0.26</u>	<u>0.34</u>	<u>0.57</u>
1 W ²	0.06	0.26	0.11		0.33	0.65	97.0
LW ³	0.45						1.1
5 W	0.29	1.03	0.12	0.39	3.23	3.23	93.1
LW	3.5						0.9
10 W	1.04	1.18	0.45	13.6	Rf0.22 5.82	5.20	80.1
LW	3.8						2.3
14 W	1.05	1.62	0.30	1.09	6.68	3.00	83.8
LW	4.97				0.43		0.7

¹- The initial fenitrothion concentration was 1.022 g/g.

²- 'W' represents the chloroform extract of water.

³- 'LW' represents the lyophilised water after chloroform extraction.

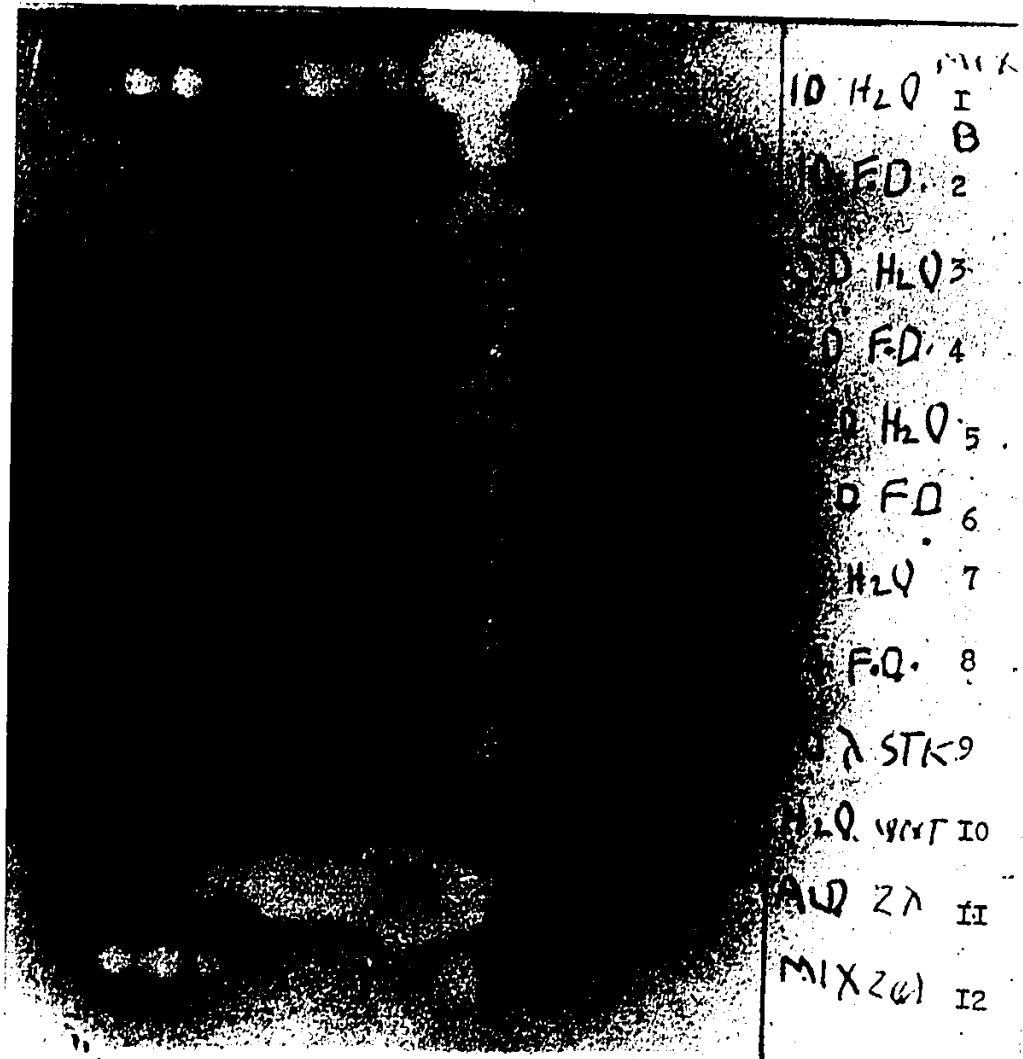


Plate 13

Plate 13. Model 4. T.L.C. of extracts of water from the mixed plant model.

Chloroform extracts of water: #1,3,5,7- 1,5,10 and 14 day samples.

Lyophilized extracts of water following chloroform extraction: #2,4,6,8 -1,5,10 and 14 day samples

¹⁴C fenitrothion: #9,10 μ l stock

Control #10, chloroform extract of water

Concentrated formylfenitrothion #11, 2 μ l unpurified

Mixed standard #12, 20 μ l of solution containing

8.8 NG/ μ l-fenitrothion (RF=0.48/16.4 NG/ μ l fenitro-oxon

(RF=0.06/y 22.4 NG/ μ l S methyl fenitrothion (RF=0.11)

and 22.4 NG/ μ l aminofenitrothion (RF=0.16)

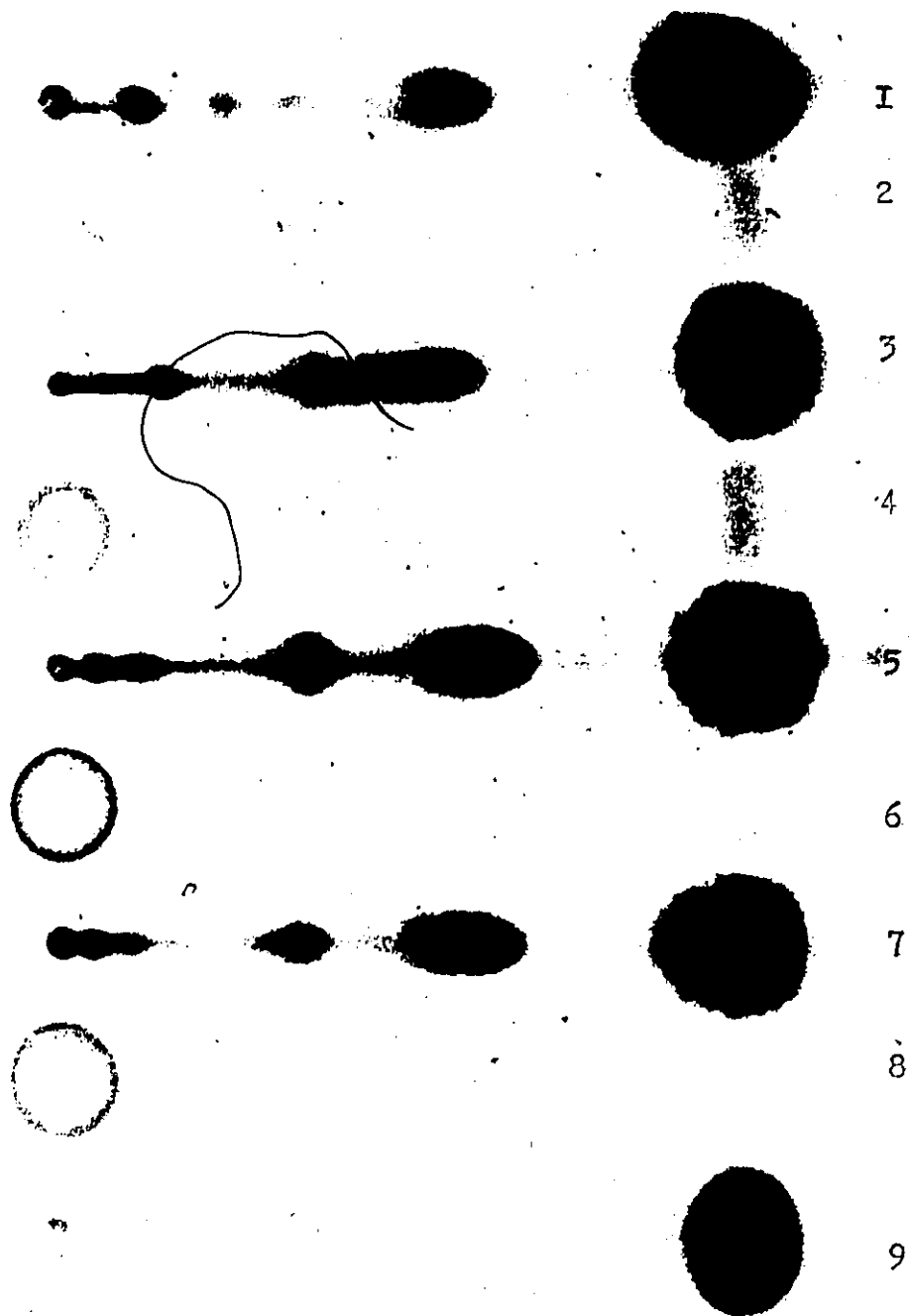


Plate I4

3

Plate 14. Model 4: Autoradiograph of T.L.C. of extracts of water
from the Mixed Plant Model.

Chloroform extracts of water: #1,3,5,7-1,5,10 and 14
day samples.

Lyophilized extracts of water following chloroform
extraction #2,4,6,8-1,5,10, and 14 day samples

Stock ¹⁴C-fenitrothion: #9, 10μl

14 days with amounts of 3-methyl-4-nitrophenol ranging from 0.7% to 5.2% and the unknown (Rf 0.22) at 5.8% to 6.7% of the total activity in the chloroform extract of water (Table 11B). As mentioned previously, the lyophilized water contained up to 21.5% of the total applied activity and this represents the water-soluble derivatives. The unrecovered portion contained up to 36.5% of the total applied ¹⁴C-activity; leaving 34% in the chloroform extract of water comprised of fenitrothion (28.6%) 1.7% as 3-methyl 4-nitrophenol and 2.3% as the unknown (Rf 0.22).

Bioaccumulation Ratios

The rate of pesticide metabolism was shown to be quite low in the aquatic plants in model 4, except in the case of Myriophyllum sp. where the rate and amount of pesticide degradation was much higher. Of all the plant-water models, model 4 showed the greatest levels of derivatives in the plant tissue. The presence of derivatives in the plant tissue might be the result of the bioaccumulation of the same derivatives from the surrounding water. To test this hypothesis, bioaccumulation ratios for fenitrothion and its derivatives were calculated and shown in Table 12 using the data from model 4 (Tables 10A and 10B).

Bioaccumulation ratios are here defined as the concentration of fenitrothion or derivative in the plant, divided by the concentration of the pesticide or derivative in the surrounding water. It may be suggested that all derivatives could be absorbed into the plant as easily as fenitrothion and that if the derivatives in the plants were the result of simple absorption from the surrounding water, the bio-

Table 12

Model 4- Mixed Plant Study

Bioaccumulation Ratios of Fenitrothion and Derivatives (concentration in plants/concentration in water).

Time (days)	Species	Fenitro-oxon	S-methyl-Fenitrothion	3-methyl-4-nitrophenol	Fenitrothion
5	Sag	12	3541	696	372
	Myr	2494	10,797	1876	364
	Elo	406	1338	253	535
10	Sag	922	183	780	446
	Myr	12,220	7197	1495	558
	Elo	447	1906	297	875
14	Sag	1599	6469	4521	1135
	Myr	9693	17,954	2085	448
	Elo	712	2909	2065	572

accumulation ratios of the derivatives should be the same as those calculated for fenitrothion. The calculated bioaccumulation ratios are shown in Table 12.

The plants in model 4 showed bioaccumulation ratios for fenitrothion of 364 to 1135 depending on species and time of exposure to the pesticide solution. These ratios were significantly higher for S-methyl-fenitrothion and 3-methyl 4-nitrophenol (Table 12).

Bioaccumulation ratios for S-methyl fenitrothion ranged from 1338 to 10,972 at 5 days, 183 to 7197 at 10 days, and 2909 to 17954 at 14 days depending on the plant species. Ratios for 3-methyl 4-nitrophenol ranged from 253 to 1876 at 5 days, 297 to 1,495 at 10 days and 2065 to 4,521 at 14 days. The ratios for fenitro-oxon were not considerably different from those of fenitrothion itself except in the case of Myriophyllum sp. which showed ratios of 12,220 and 9,693 at 10 and 14 days respectively. Thus it would appear that the presence of some derivatives in the plant is not the result of simple absorption of these derivatives from the surrounding solution.

Field Model

The field model consisted of 1 m³ containers filled with natural lake water, plants, algae and sediment and these were used to represent natural systems.

During the first 28 hours post-spray, there was a significant difference in pesticide concentrations between the upper and lower water samples but after this time the pesticide was quite

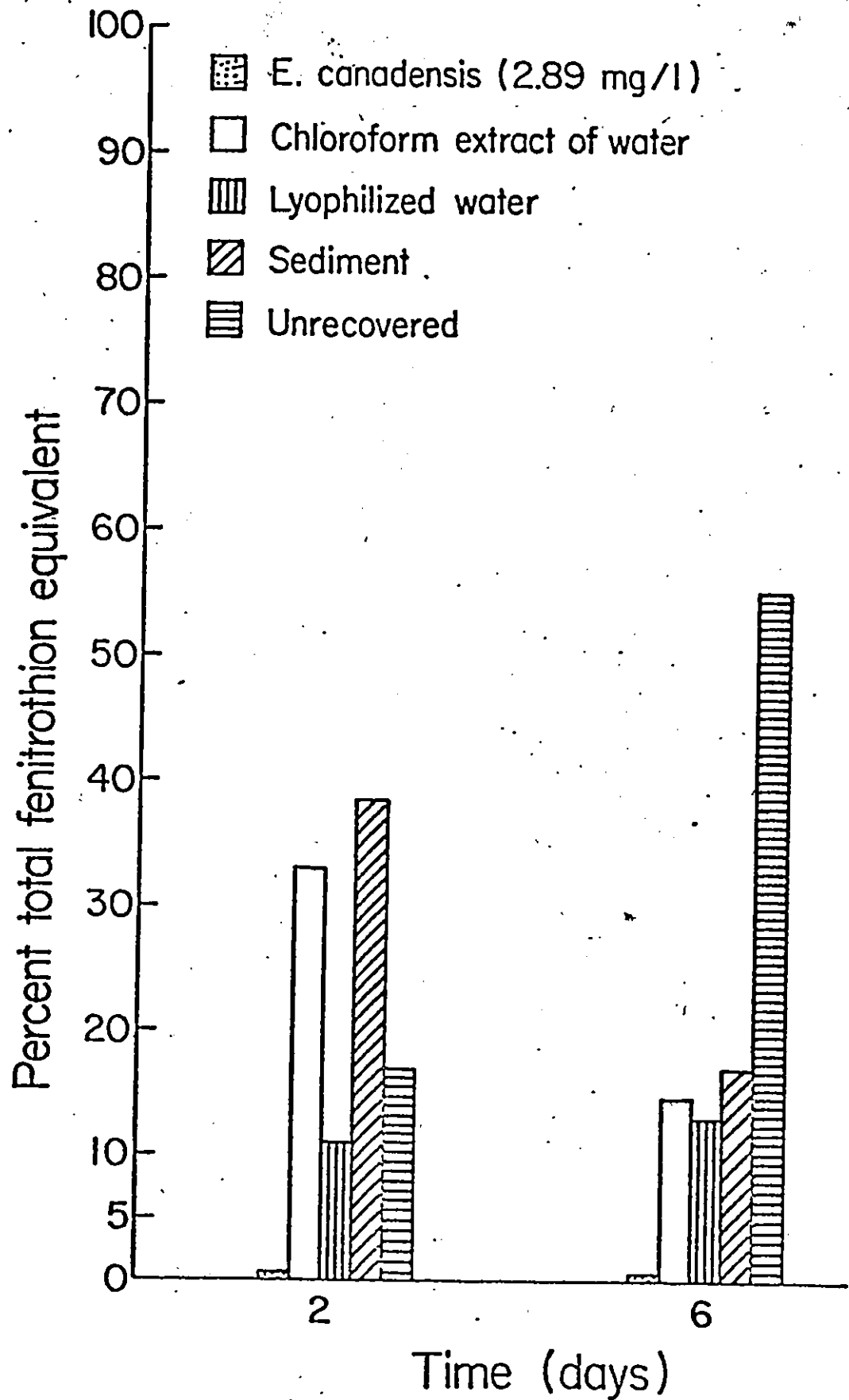


Figure 12 Fenitrothion Distribution in the Field Model.

well mixed. A gradual decline in pesticide concentration was observed, from about $6.0 \mu\text{g/g}$ in the water at 28 hours to $0.1 \mu\text{g/g}$ in the water at 28 days in the lighted model (Figure 13A). This 'disappearance' of fenitrothion indicated an estimated initial half-life of 46 hours in the lake water. Similar results were observed in the water of the dark model (Figure 13B).

Fenitrothion absorption by E. canadensis in the lighted model is shown in Figure 14. During the first 28 to 46 hours there was a significant difference in absorption by the plants obtained from the upper and lower levels. This probably reflects the observed differences in pesticide concentrations between the upper and lower waters, in this period, before complete mixing of the pesticide. A high initial fenitrothion concentration of $1,010 \pm 80 \mu\text{g/g}$ in the lower plant samples and $4,180 \pm 230 \mu\text{g/g}$ in the upper samples was (Figure 14) shown in the first one-half hour post-spray. The corresponding pesticide concentrations in the water at this time, were $2.96 \pm 0.8 \mu\text{g/g}$ in the lower water samples and $15.93 \pm 3.3 \mu\text{g/g}$ in the upper water samples. (Figure 13A) In this case and in the laboratory experiments, pesticide absorption was proportional to surrounding concentrations in the water. After 28 hours, upper and lower plant samples showed comparable pesticide concentrations.

This rapid initial uptake of pesticide was followed by a steady rate of uptake until a maximum of $71,235 \pm 3055 \mu\text{g/g}$ was reached at 74 hours. Following this there was a slight decline with concentrations in the range of $28,080 \mu\text{g/g}$ to $58,990 \mu\text{g/g}$ from 2 to 10 days. By 14 days the concentration declined to $11,639 \pm 1306 \mu\text{g/g}$ in E. canadensis. (Figure 14)

Fenitrothion absorption by E. canadensis in the dark model,

Figure 13A: Fenitrothion concentration in the water
of the lighted model: Upper (0-35 cm.)
and lower (36-60 cm.) samples.

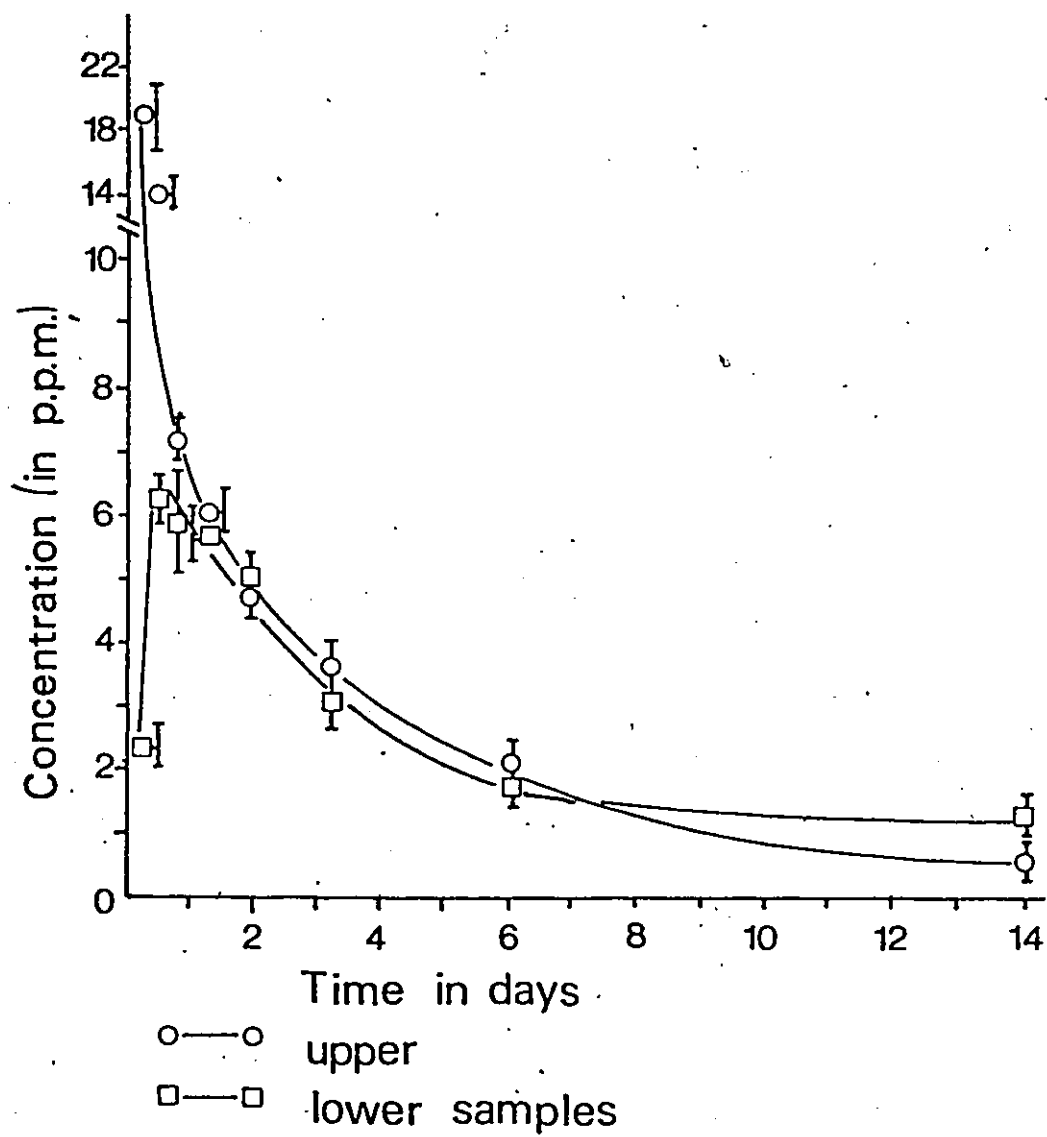


Figure 13B: Fenitrothion concentration in the water
of the dark model: upper(0-35 cm.) and
lower(36-60 cm.) samples.

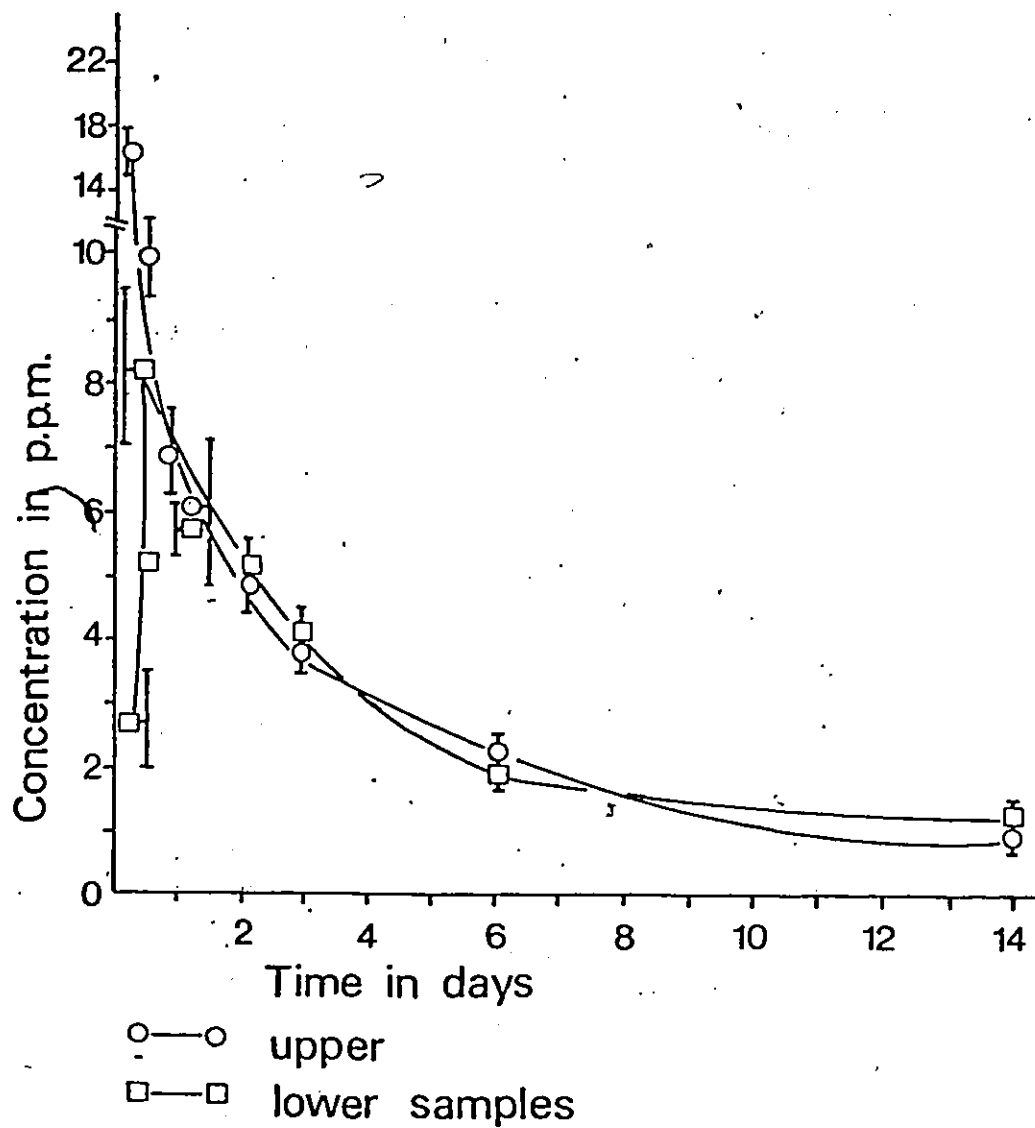
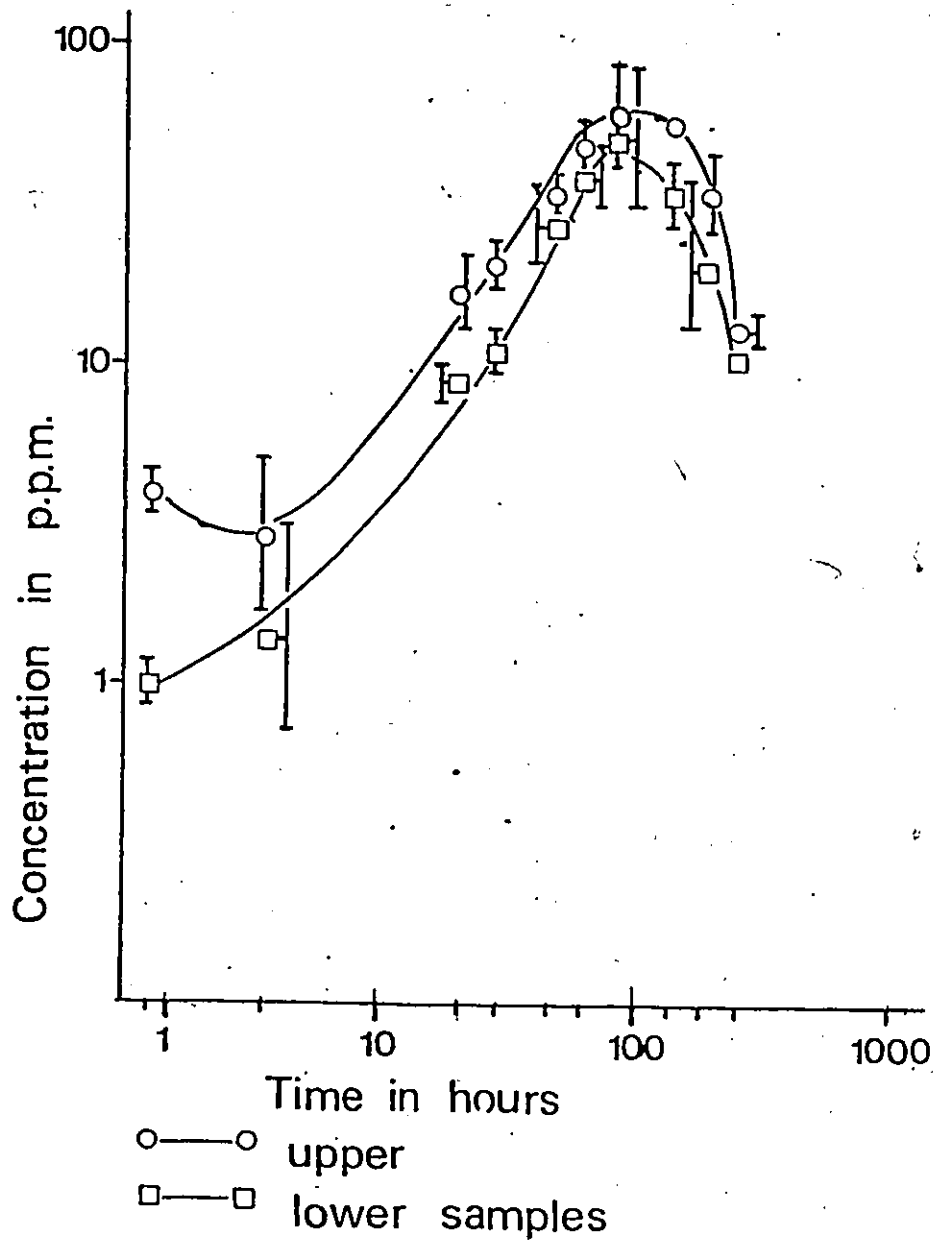


Figure 14: Fenitrothion concentration in E.canadensis
in the lighted field model:upper(0-12 cm.)
and lower(40-60 cm.) samples.



where light intensity was 0.1% of the lighted container at 20 cm depth, is shown in Figure 15. A rapid initial uptake giving a concentration of $844 \pm 213 \mu\text{g/g}$ of fenitrothion was observed in the first one-half hour post-spray followed by a rise to a maximum concentration of $4120 \pm 310 \mu\text{g/g}$ at 22 hours. Much the same concentration was maintained for 6 days followed by a rapid decline to $489 \pm 106 \mu\text{g/g}$ by 14 days.

No fenitrothion metabolites were detected in the plant extracts, either by gas chromatography or by TLC (Plate 15).

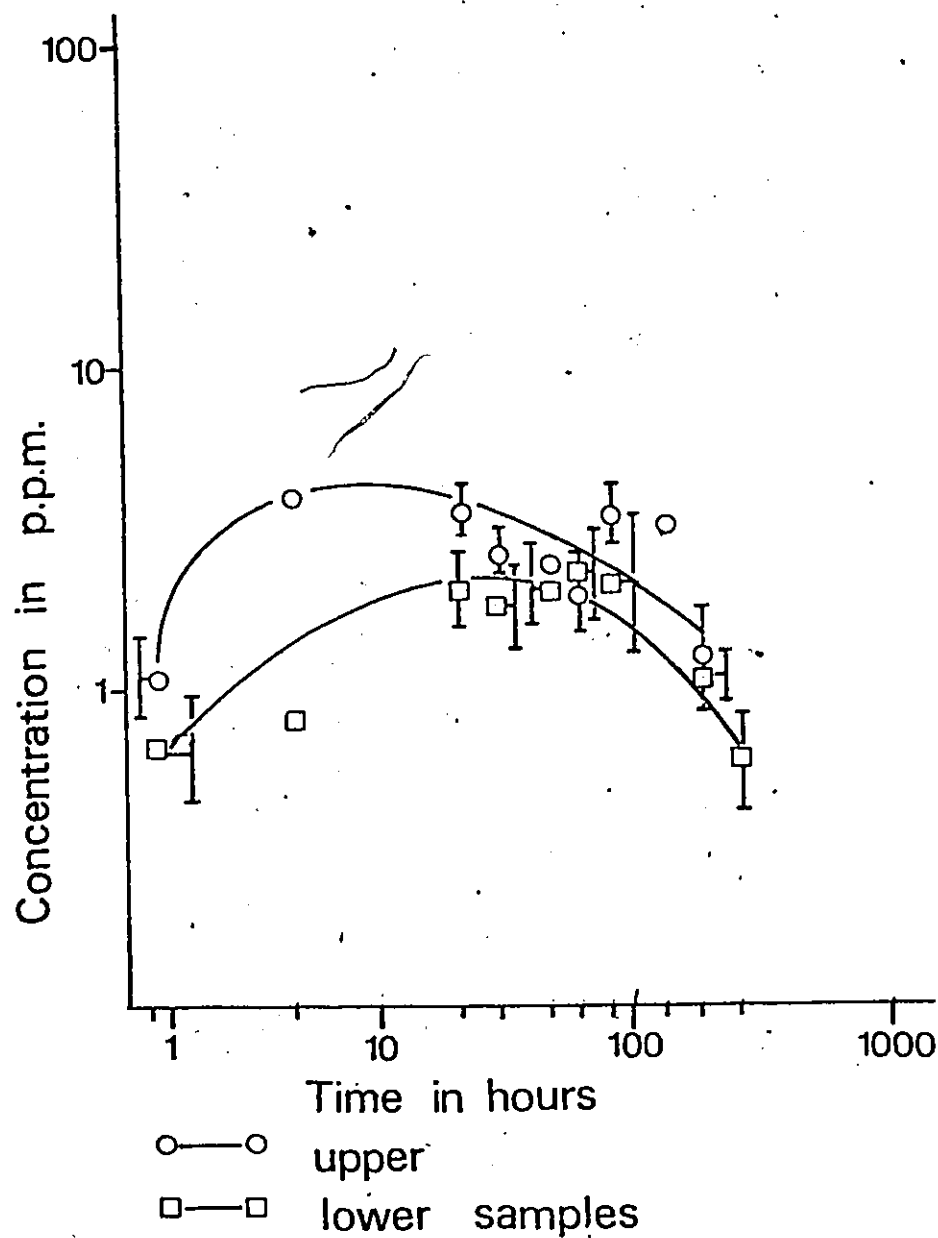
The sediment, which formed a 4 cm sediment-water slurry at the bottom of the light and dark containers was examined for traces of pesticide by using the extraction method of Takimoto et al (1976).

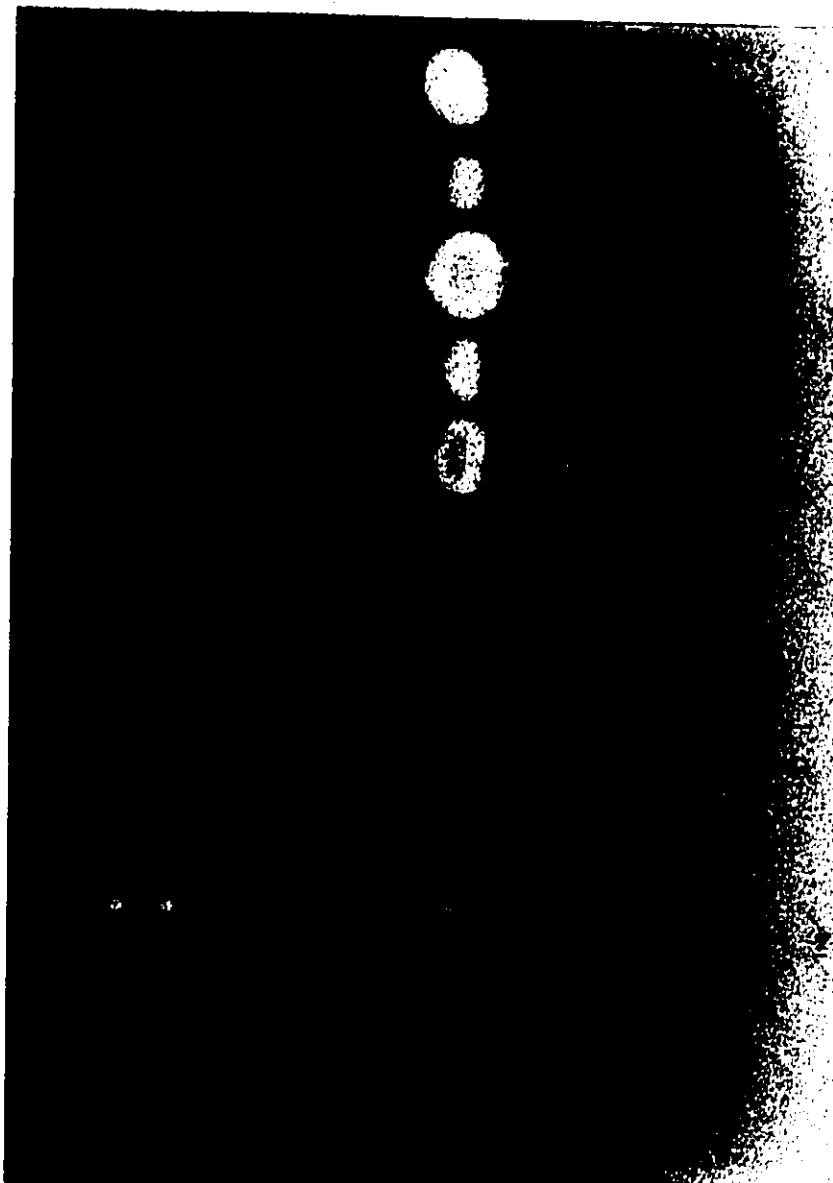
The total fenitrothion extracted from the sediment in the light and dark containers is shown in Figure 16. An initial concentration of $94.7 \pm 2.1 \mu\text{g/g}$ in the light model and $94.4 \pm 2.4 \mu\text{g/g}$ in the dark models at 2 days was followed by a gradual decline to $0.6 \pm 0.1 \mu\text{g/g}$ and $1.5 \pm 0.2 \mu\text{g/g}$ respectively at 28 days.

Together with fenitrothion, high levels of pesticide derivatives were also found in the sediment (Plate 16 and 17) and these were quantitated for both the light and dark models at 2 and 6 days (Table 13). The levels of derivatives were equal to or much greater than those of fenitrothion, indicating extensive pesticide degradation in the sediment.

In the light model at two days post-spray, the sediment showed

Figure 15: Fenitrothion concentration in *E. canadensis*
in the dark field model: upper(0-12 cm.)
and lower(40-60 cm.) samples.





5DU	I
5FL	2
7DU	3
9FU	4
10DU	5
10FU	6
3GU	7
10HU	8
1B MEOH	9
2.5A A	10
2500 NG	
DES	II

Plate I5

Plate 15. Model 5:-TLC of E. canadensis plant extracts from the field model.

Plant samples: #1, 3, 5 = 5, 7 and 10 day sample from the light container.

Plant samples: #2, 4, 6 = 5, 9 and 10 day samples from the dark container.

Control plant samples: #7, 8 = 3, and 10 day samples.

Desmethylfenithion: #11, 2500 ng.

Mixed Standard: #10; 2.5 μ l of solution containing 8.8 ng/ μ l fenitrothion ($R_f=0.48$) 16.4 ng/ μ l fenitrooxon ($R_f=0.06$), 22.4 ng/ μ l S-methylfenitrothion ($R_f=0.11$) and 22.4 ng/ μ l aminofenitrothion ($R_f=0.16$).

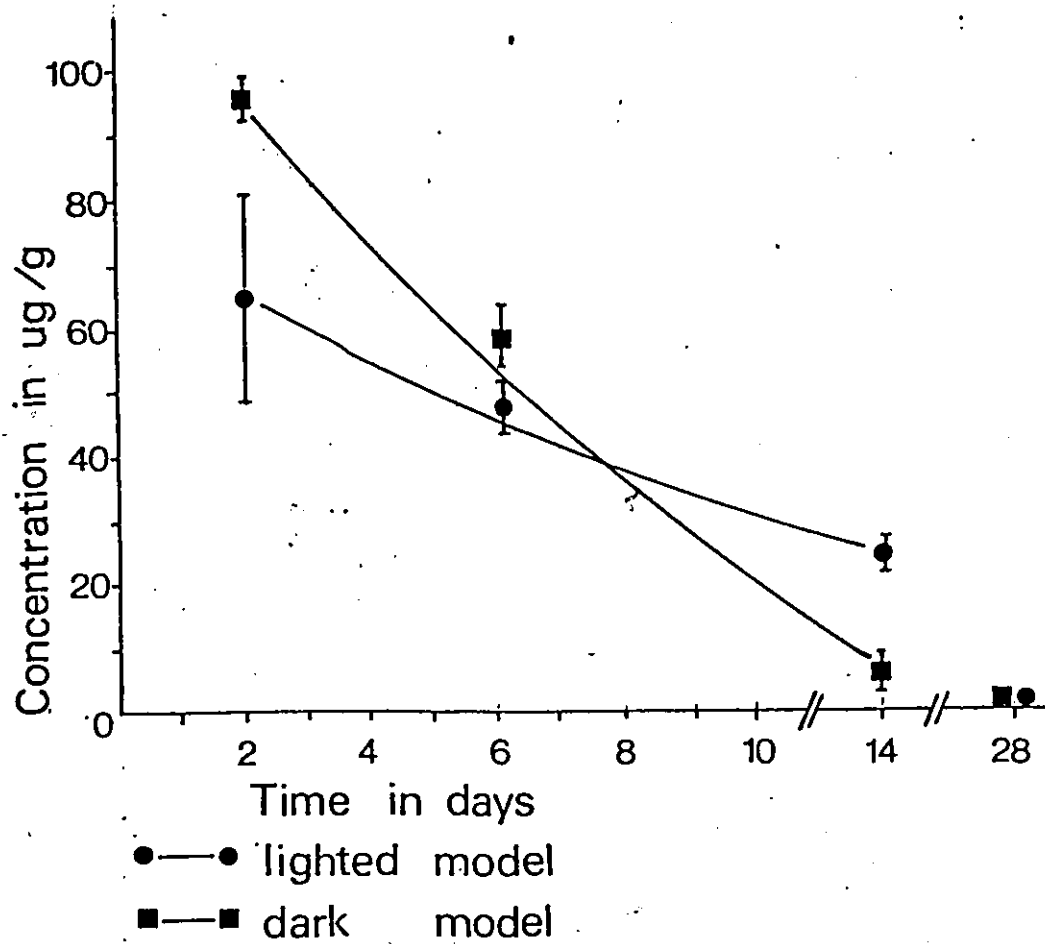


Figure 16: Fenitrothion concentration in the field sediment.

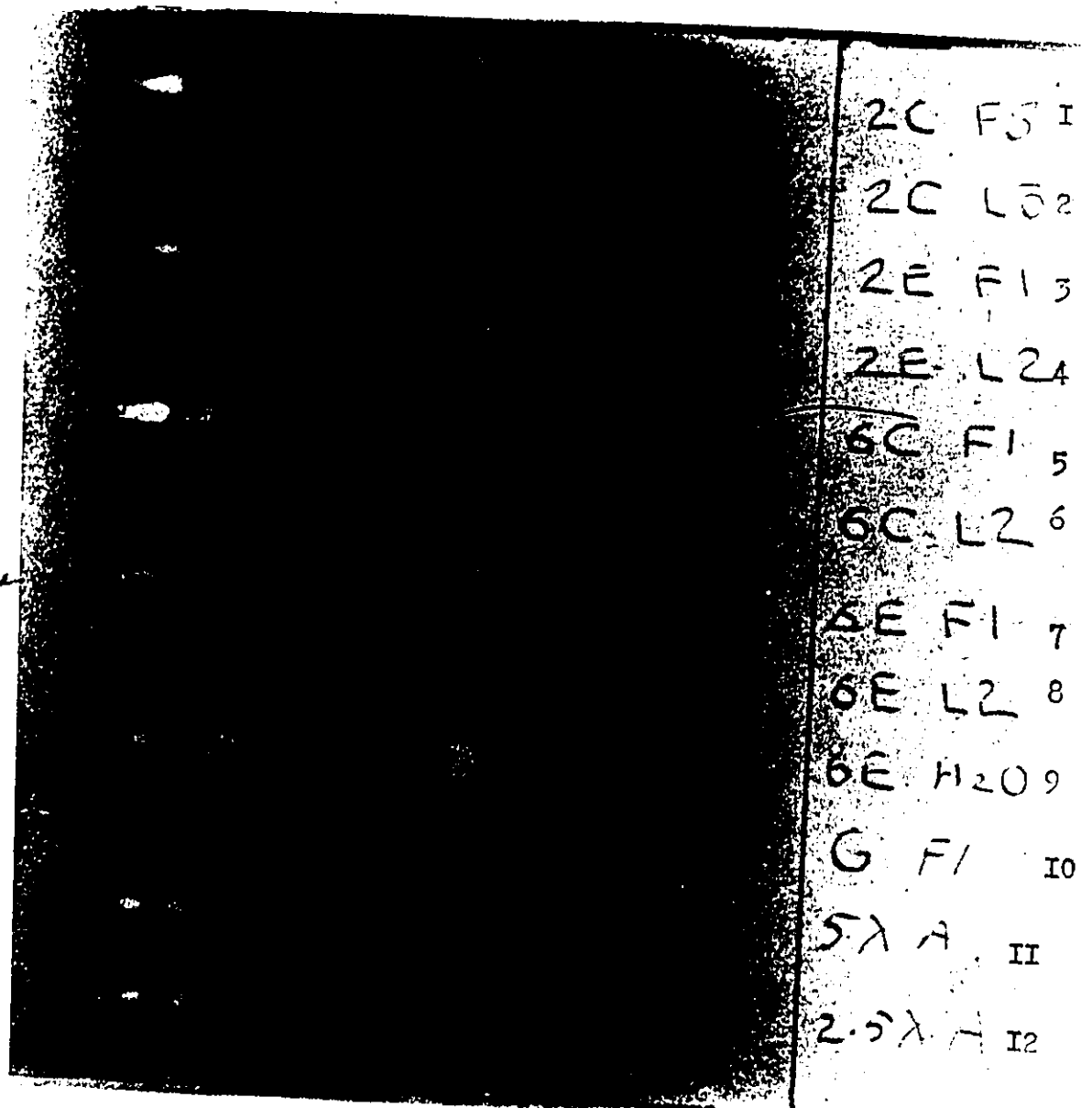


Plate I6

<Plate 16. Model 5: Sediment extracts and extracts of the water wash of the sediment from the field model.

Legend: "Free" sediment #1,5-2 and 6 day fractions from the light model respectively.

"Loose" sediment #2,6-2 and 6 day fractions from the light model respectively.

"Free" sediment #3,7-2 and 6 day fractions from the dark model respectively.

"Loose" sediment #4,8-2 and 6 day fractions from the dark model respectively.

Water wash of the sediment #9- 6 day sample from the dark model.

Control #10- free sediment fraction

Mixed Standard #11,12 -5 and 2.5 μ l of the stock solution respectively containing 8.8 NG/ μ l fenitrothion (RF=0.48)

16.4 NG/ μ l fenitrothion (RF=0.06), 22.4 NG/ μ l S methyl fenitrothion (RF=0.11) and 22.4 NG/ μ l aminofenitrothion (RF=0.16).

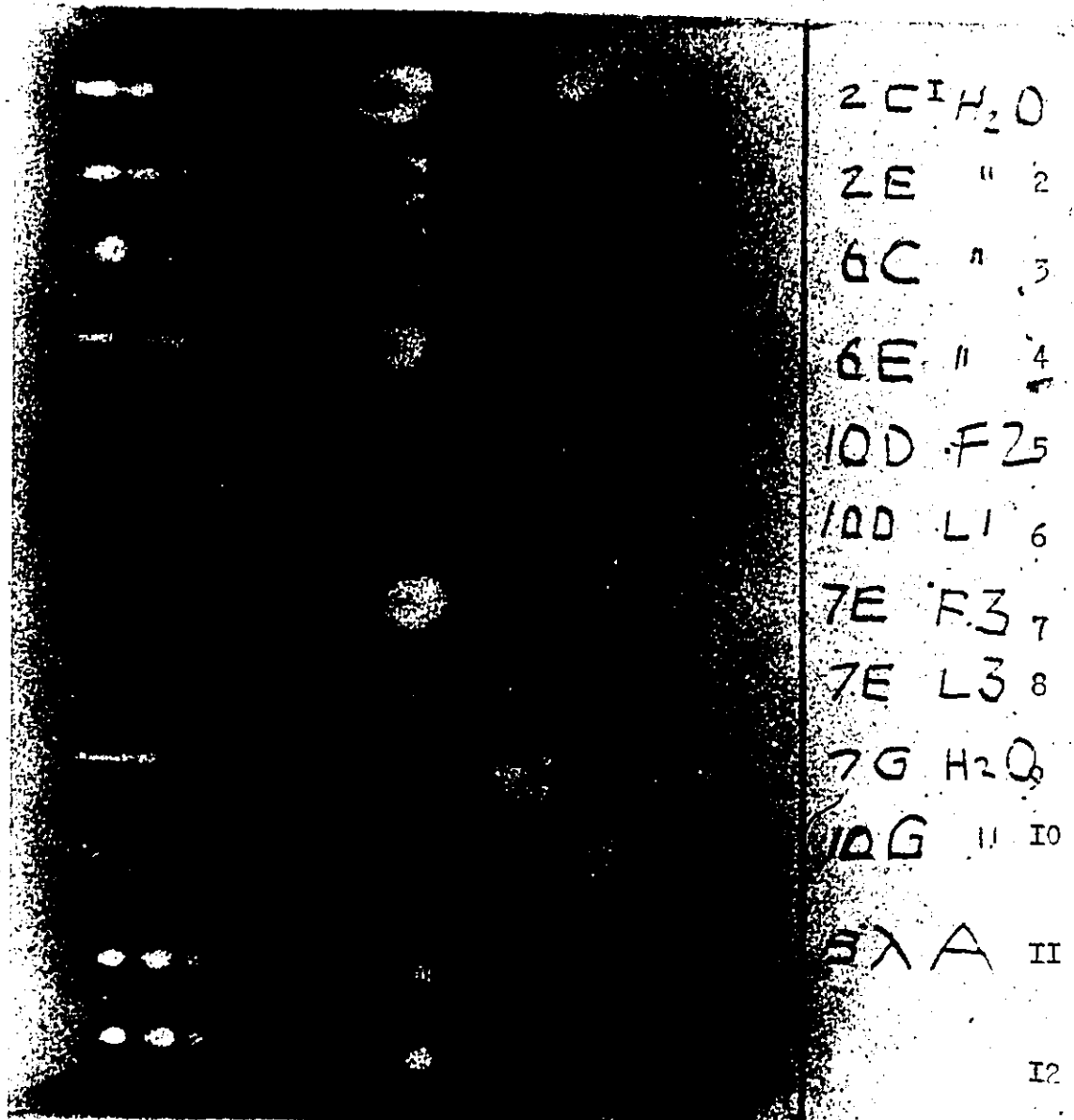


Plate I7

Plate 17. Model 5: Sediment extracts and extracts of the water wash of the sediment from the field model.

Water washes: #1, 3 = 2 and 6 day sample extracts of the sediment from the light model.

Water washes: #2, 4 = 2 and 6 day sample extracts from the dark model.

Free and loose sediment fractions: #5, 6 = sample extracts from 10 day samples from the light model.

Free and loose sediment fractions: #7, 8 = sample extracts from 7 day samples from the dark model.

Water washes: #9, 10 = 7 and 10 day of control sediment.

Mixed Standard: #11, = 5 μ l solution containing 8.8 ng/ μ l fenitrothion (R_f = 0.48), 16.4 ng/ μ l fenitro-oxon (R_f =0.06), 22.4 ng/ μ l s-methylfenitrothion (R_f =0.11) and 22.4 ng/ μ l aminofenitrothion (R_f =0.16).

Table 13-Model 5
Metabolites in the Sediment

	<u>Concentration (ug/g)</u>			
	<u>Amino-</u> <u>fenitrothion</u>	<u>Fenitro-</u> <u>-thion</u>	<u>Fenitro-</u> <u>-oxon</u>	<u>S-methyl</u> <u>fenitrothion</u>
1) 2 Day Light-Free-	179.0	63.7	210.2	69.2
Loose-	2.5	2.2	3.4	7.9
Total-	181.5	65.9	213.6	77.1
2) 2 Day Dark- Free-	97.9	88.7	111.4	33.4
Loose-	0.6	8.1	0.6	n.d.
Total-	98.5	96.8	112.0	33.4
3) 2 Day Dark- Free-	49.2	85.2	112.6	28.2
Loose-	1.6	6.8	2.4	27.3
Total-	50.8	92.0	115.0	55.5
4) 6 Day Light-Free-	25.3	38.9	79.2	38.6
Loose-	23.0	3.9	27.4	n.d.
Total-	48.3	42.8	106.6	38.6
5) 6 Day Light-Free-	12.7	34.4	68.4	46.5
Loose-	31.6	4.0	33.3	n.d.
Total-	44.3	38.4	101.7	46.5
6) 6 Day Dark-Free-	40.9	68.4	50.1	n.d.
Loose-	22.3	4.5	29.5	40.8
Total-	63.2	72.9	79.6	40.8
7) 6 Day Dark-Free-	13.8	62.1	n.d.	n.d.
Loose-	43.1	2.5	37.1	62.8
Total-	56.9	64.6	37.1	62.8

concentrations of 182 $\mu\text{g/g}$ amino-fenitrothion, 65.9 $\mu\text{g/g}$ fenitrothion, 214 $\mu\text{g/g}$ fenitro-oxon and 77.1 $\mu\text{g/g}$ of S-methyl-fenitrothion. In the dark model, the sediment showed a slightly higher level of fenitrothion ($94.4 \pm 2.4 \mu\text{g/g}$) but lower levels of amino-fenitrothion ($74.6 \pm 23.9 \mu\text{g/g}$), fenitro-oxon ($113.5 \pm 1.5 \mu\text{g/g}$) and S-methyl-fenitrothion ($44.5 \pm 11 \mu\text{g/g}$). After 2 days the amounts of fenitrothion and derivatives in the sediment declined (Table 13).

The 3-methyl 4-nitrophenol derivative was not detected by the alkali flame ionisation detector but the product may have been present in the samples. Another method may have detected this derivative.

Water Wash of the Sediment

Associated with the sediment was approximately 10 cm of the overlying water plus the water in the sediment-water slurry which was filtered out and extracted separately. In 70% of the samples, the amount of filtered water varied from 530 to 590 ml although when all water samples were considered, this wash ranged from 320 to 670 ml. The amount of water filtered through the sediment appeared to make little difference to the amount of derivatives found in the water wash.

Low levels (less than 15 $\mu\text{g/g}$ dry weight sediment) of each derivative were found in the water wash of the sediment, and these were the same type of derivatives as were present in the sediment itself (Figures 17A and 17B). Since these derivatives, except the S-methyl fenitrothion in the light model, were not detected in the water above the sediment, it could be assumed that these derivatives were desorbed or leached from the sediment.

Therefore the concen-

Figure 17A: Concentration of fenitrothion-derivatives
in the water wash of the sediment.

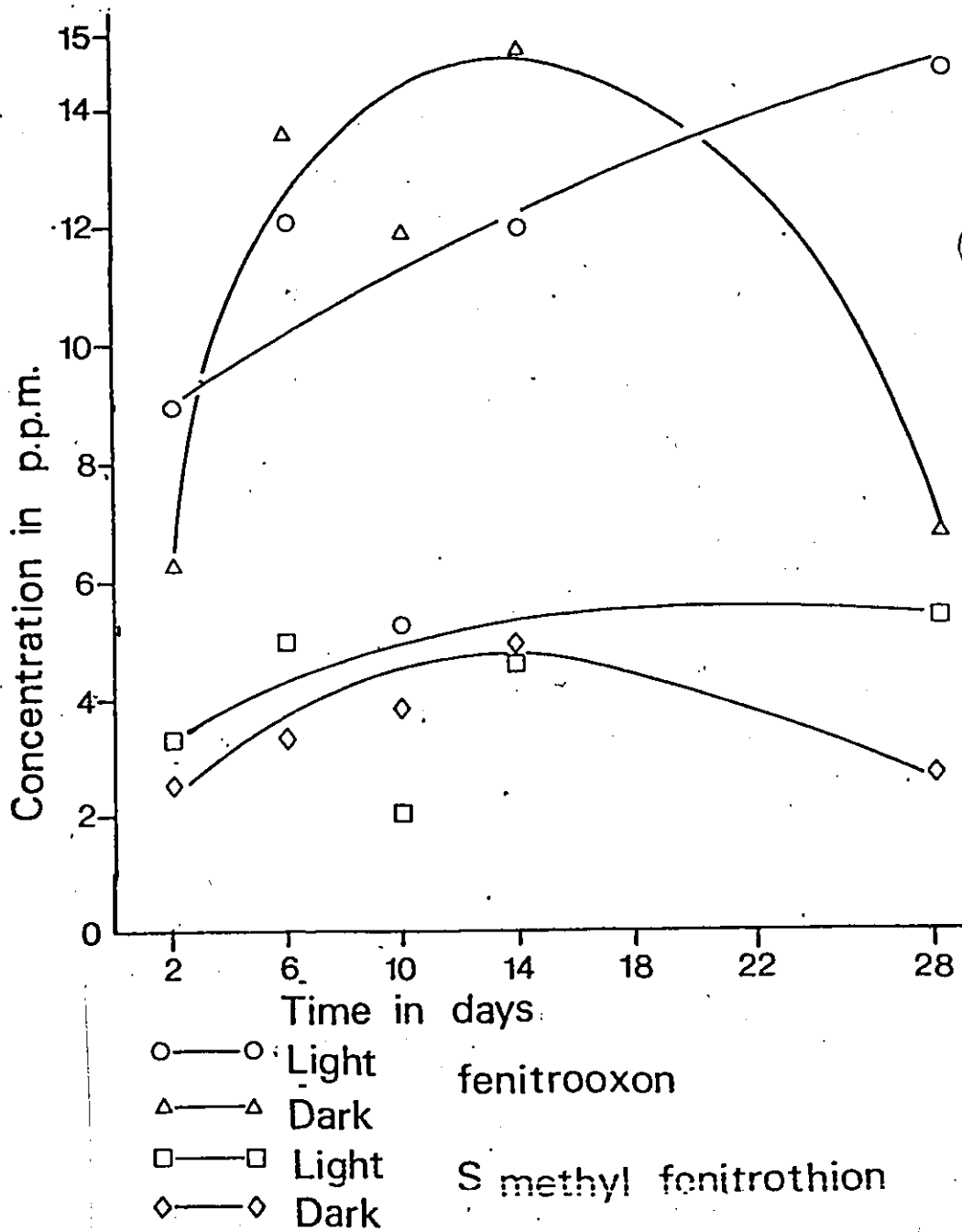
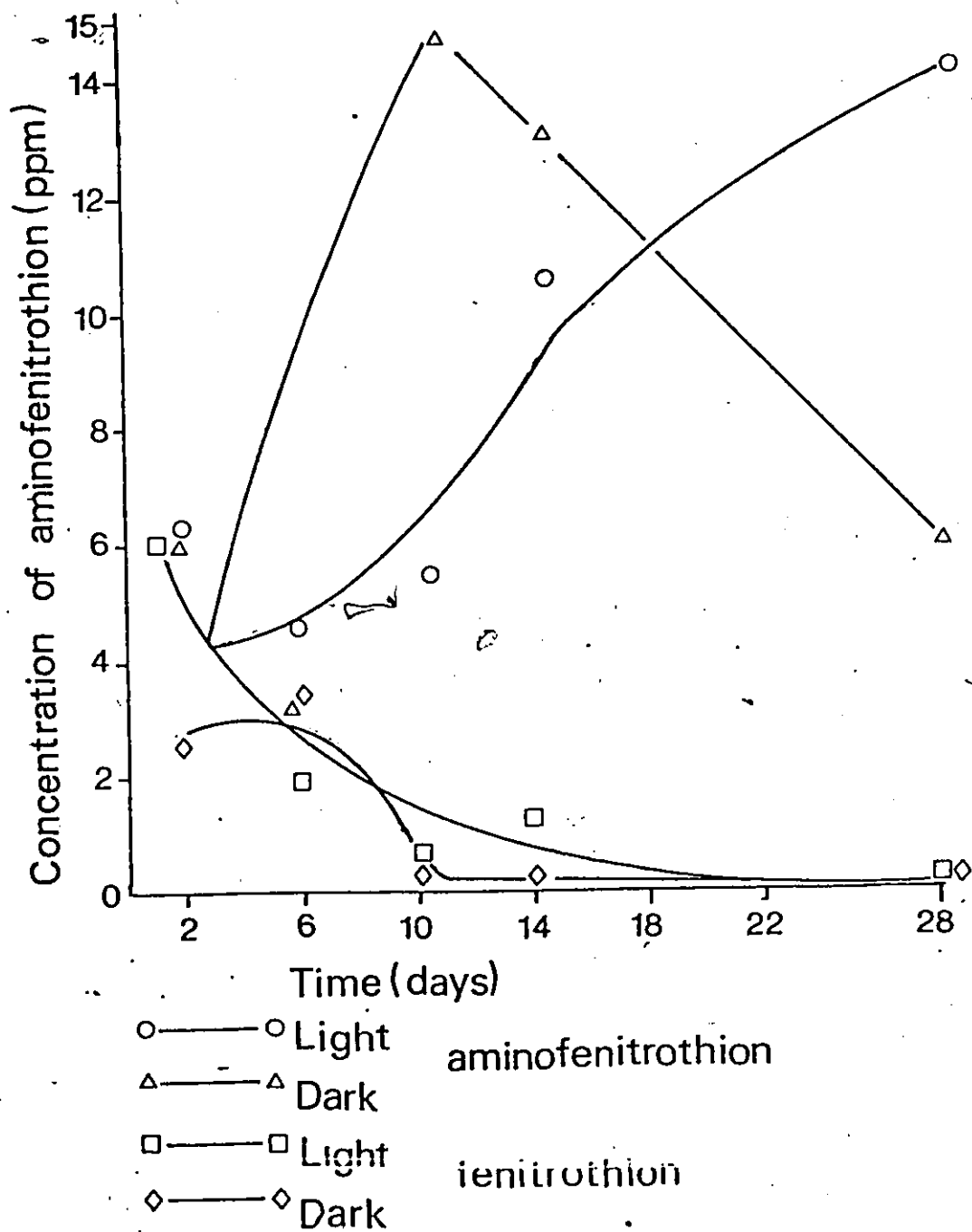


Figure 17B: Concentration of fenitrothion derivatives
in the water wash of the sediment.



trations referred to in the water wash are not the concentrations relative to water mass, but are concentrations relative to the total dry weight of the sediment that was filtered to obtain this water wash. The pesticide residue in the water wash is considered part of the total pesticide load in the sediment.

Amounts of fenitrothion in the water wash varied from 5.9 to 0.05 $\mu\text{g/g}$ in the period of 2 to 28 days in the lighted model and from 2.9 $\mu\text{g/g}$ to 0.10 $\mu\text{g/g}$ dry weight of sediment in the dark models over the same period (Table 14).

The S-methyl-fenitrothion in the water wash showed similar levels in the light and dark ranging from 2.33 $\mu\text{g/g}$ to 5.22 $\mu\text{g/g}$ dry weight sediment from 2 to 28 days.

For the first 14 days, fenitro-oxon was present in comparable amounts in the water wash of both lighted and dark models (6.5 $\mu\text{g/g}$ to 14.6 $\mu\text{g/g}$). By 28 days, fenitro-oxon was detected at levels of 14.3 $\mu\text{g/g}$ in the lighted model and 7.5 $\mu\text{g/g}$ in the dark model (Table 14).

Close similarities were seen in amino-fenitrothion levels in the wash of the sediment in light and dark at 2 days (5.9 $\mu\text{g/g}$ light; 5.7 $\mu\text{g/g}$ dark, 7 days (4.3 $\mu\text{g/g}$ light; 3.2 $\mu\text{g/g}$ dark and 14 days (10.9 $\mu\text{g/g}$ light; 12.5 $\mu\text{g/g}$ dark). There were less similarities in amino-fenitrothion levels between light and dark models in the sediment wash at 10 and 28 days. (Table 14)

Mass Balance

A materials balance for fenitrothion and its derivatives

Table 14
Model 5-Field Study--

Fenitrothion and Derivatives in the Water Wash of The
Sediment, with Concentration Relative to the Dry Weight
of the Sediment

<u>Time</u> <u>(days)</u>	<u>Amino-</u> <u>fenitrothion</u>	<u>Fenitrothion</u>	<u>Fenitrooxon</u>	<u>S-methyl</u> <u>fenitrothion</u>
2 L ¹	5.87	5.80	8.83	3.09
2 D ²	5.67	2.93	6.54	2.54
7 L	4.34	1.82	12.08	5.05
7D	3.17	2.58	13.67	3.44
10L	5.33	0.89	5.41	2.23
10D	13.95	0.22	12.12	3.95
14L	10.89	1.15	12.18	4.97
14D	12.46	0.06	14.57	5.12
28L	13.16	0.05	14.25	5.22
28D	6.31	0.10	7.47	2.68

1-'L' represents the lighted container.

2-'D' represents the dark container.

was determined from the information of the calculated mass of all components and the concentration of pesticide and derivatives in each component of this aquatic field model. The total amount of pesticide in each component was summed for all components to determine the extent of accountability for the pesticide in this model. The compartments in the field model were the water, sediment, water wash of the sediment, plants, algae and water-borne particulate matter. The water was analysed in 2 parts, the chloroform extract of the water and the lyophilized extraction. The materials balance for 2 and 6 days in the light and dark models are shown in Tables 15 to 18. and Figure 12 for the light model.

The mass balance in the light model at 46.5 hours post-spray is shown in Figure 15, where 83.2% of the originally applied fenitrothion was accounted for. The water held 33.2% of the total initial deposit as chloroform extractables and 10.7% as chloroform non-extractables. The chloroform non-extractables were derivatives such as desmethyl-fenitrothion aldehyde, carboxy-fenitrothion and dimethylphosphorothioate (Weinberger et al 1977).

The sediment contained 37% of the total fenitrothion equivalent in the form of fenitrothion (4.4%) or derivatives (32.6%).* The derivatives were amino-fenitrothion (12.2%), fenitro-oxon (15.2%), and S-methyl fenitrothion (5.2%). The amount of amino-fenitrothion could easily be twice the amount shown here because of poor recovery from the charcoal clean-up column. The water wash of sediment contained 1.7% of the total fenitrothion equivalent applied to the model and the particulate matter sequestered another 0.6% of the total.

Table 15-Partitioning of Fenitrothion in an Aquatic System

Light Container-Two Days Post-Spray (46.5 hours)

<u>Components</u>	<u>Mass of Components</u>		<u>Mass Percent of System</u>		<u>Concentration in component (dry weight) ug/g.</u>	<u>As Percent of total fenitrothion applied</u>		<u>Bioaccumulation Ratio (dry weight)</u>
	<u>Wet wt.</u>	<u>Dry wt.</u>	<u>Wet</u>	<u>Dry</u>				
<u>Water Metabolites</u>								
** #1					0.52	1.61		
#2					0.03	0.23		
#3					0.16	1.11		
#4					0.52	7.78		
1. Water	767 kg		97.1		4.33 (wet)	33.2		
2. Sediment:		22.8 kg.	6.73kg.	2.89	0.87			
a) fenitrothion					65.9	4.40		
b) amino-fenitrothion					181.5	12.20		
c) fenitro-oxon					213.6	15.20		
d) S-methyl-fenitrothion					77.1	5.20		
3. Water Wash of Soil:								
a) fenitrothion					5.80	0.39		
b) amino-fenitrothion					5.87	0.44		
c) fenitro-oxon					8.83	0.63		
d) S-methyl-fenitrothion					3.09	0.21		

Table 15-Partitioning of Fenitrothion in an Aquatic System (cont.)
Light Container-Two Days Post-Spray (46.5 hours)

<u>Components</u>	<u>Mass of</u>		<u>Mass Percent</u>	<u>Concentration</u>	<u>As Percent</u>	<u>Bionccu-</u>
	<u>Components</u>	<u>of System</u>				
	<u>Wet wt.</u>	<u>Dry wt.</u>	<u>Wet</u>	<u>Dry</u>	<u>of total</u>	<u>mulatio</u>
			<u>ug/g</u>	<u>(dry weight)</u>	<u>fenitrothion</u>	<u>Ratio</u>
					<u>applied</u>	<u>(dry weight)</u>
4. Sediment total:						
a) fenitrothion			71.7		4.60	
b) amino-fenitrothion			187.4		12.66	
c) fenitro-oxon			222.4		15.80	
d) s-methyl-fenitrothion			80.2		5.40	
5. Total in initial plants	15.2g	2.56g	1.9×10^{-3}	3.3×10^{-4}		
6. Remaining plants-2 days	10.50g	1.76g	1.4×10^{-3}	2.3×10^{-4}		
7. Total initial algae	$3.6 \times 10^{-1}g$	$6.0 \times 10^{-2}g$	4.5×10^{-5}	7.7×10^{-6}	0.52	6.66×10^3
8. Remaining total algae	$2.9 \times 10^{-1}g$	$4.37 \times 10^{-2}g$				
a) Euglena sp.	$2.54 \times 10^{-1}g$	$4.24 \times 10^{-2}g$	1.27×10^3		5.39×10^{-4}	2.93×10^2
b) Chlamydomonas sp.	$1.58 \times 10^{-2}g$	$2.64 \times 10^{-3}g$	8.43×10^3		2.23×10^{-4}	1.95×10^3
c) Chlorella sp.	$1.7 \times 10^{-2}g$	$2.77 \times 10^{-3}g$	1.84×10^4		5.08×10^{-4}	4.25×10^3
9. Water borne particulates			8.36×10^{-3}		0.06	
10. System total	789kg	773kg			83.17%	

* Average of two or more samples on a dry weight basis

** Water metabolites: 1) Carboxy-fenitrothion, 2) S-methyl-fenitrothion

3) carboxy-fenitrothion 4) DMPT(dimethyl thiophosphate)

Table 16- Partitioning of Fenitrothion in an Aquatic System
'Dark' Container-Two Days Post-Spray (46.5 hrs.)

Components	Mass of Components		Mass Percent of System		Concentration in component (dry weight) ug/g	As Percent of total fenitrothion applied	Bioacc-mulation Ratio (dry weight)
	Wet wt.	Dry wt.	Wet	Dry			
**							
1. Water metabolite#1					0.35	5.23	
2. Water	767kg		96.9		4.60	35.3	
3. Sediment:	24.6kg	7.0kg	3.11				
a) Fenitrothion					95.9	6.72	
b) Amino-fenitrothion					74.7	5.87	
c) Fenitro-oxon					113.2	8.42	
d) S-methyl-fenitrothion					44.5	3.12	
4. Water Wash of Soil							
a) Fenitrothion					2.93	0.20	
b) Amino-fenitrothion					5.67	0.44	
c) Fenitro-oxon					6.54	0.48	
d) S-methyl-fenitrothion					2.54	0.17	
5. Sediment Total							
a) Fenitrothion					98.8	6.93	
b) Amino-fenitrothion					80.4	6.32	
c) Fenitro-oxon					119.7	8.91	
d) S-methyl-fenitrothion					47.0	3.30	
6. Total initial plants	6.76g	1.14g	8.5×10^{-4}	1.47×10^{-4}			
7. Remaining plants, 2 days	4.0g	0.67g	5.04×10^{-4}	8.7×10^{-5}	3.01×10^3	2.02×10^{-2}	

Table 16-Partitioning of Fenitrothion in an Aquatic System(cont.)
'Dark' Container-Two Days Post-Spray(46.5 hrs.)

Components	Mass of Components		Mass Percent of System		Concentration in Component (dry weight)		As Percent of total fenitrothion applied		Bioaccumulation Ratio (dry weight)	
	Wet wt.	Dry wt.	Wet	Dry	ug/g					
8. Total Initial Algae	3.59×10^{-1}	5.98×10^{-2}	4.5×10^{-5}	7.7×10^{-6}						
9. Remaining Algae (2 days)	2.87×10^{-1}	4.78×10^{-2}	3.62×10^{-5}	6.18×10^{-6}						
a) Euglena	2.54×10^{-1}	4.24×10^{-2}			2.38×10^3				517	
b) Chlamydomonas	1.58×10^{-2}	2.64×10^{-3}			6.35×10^3				1380	
c) Chlorella	1.66×10^{-2}	2.77×10^{-3}			4.25×10^3				924	
10. System Total	791.6kg.	774kg					65.99%			

* Average of two or more samples on a dry weight basis.
** Water metabolite#1 is DMPT, dimethyl phosphorothioate.

Table 17-Partitioning of Fenitrothion in an Aquatic System
Light Container- 6 Days Post-Spray(144 hrs.)

Components	Mass of Components	Mass Percent of System	Concentration in components (dry weight)	As Percent of total fenitrothion applied	Bioacc-mulation Ratio (dry weight)
**					
1. Water metabolites#1					
#2			0.30	2.28	
#3			0.06	0.46	
#4			0.28	1.92	
			0.57	8.45	
2. Water	760kg	97.1	1.90	14.4	
3. Sediment:					
a) Fenitrothion	22.7kg	6.67kg	40.6	3.46	
b) Amino-fenitrothion			46.3	7.38	
c) Fenitro-oxon			104.2	7.68	
c) S-methyl-fenitrothion			42.6	2.84	
4. Water wash of Soil:					
a) Fenitrothion			1.82	0.12	
b) Amino-fenitrothion			4.34	0.29	
c) Fenitro-oxon			12.1	0.81	
c) S-methyl-fenitrothion			5.05	0.34	
5. Sediment Total:					
a) Fenitrothion			38.52	3.58	
b) Amino-fenitrothion			23.34	7.67	
c) Fenitro-oxon			91.28	8.19	
c) S-methyl-fenitrothion			47.35	3.18	

Table 17-Partitioning of Fenitrothion in an Aquatic System(cont.)

Light Container-6 Days Post- Spray(144 hours)

Components	Mass of		Mass Percent		Concentration		As Percent		Bioacc-	
	Components		of System		in components		of total		-mulation	
	Wet wt.	Dry wt.	Wet	Dry	(dry weight)	ug/g	fenitrothion applied	Ratio	(dry weight)	
6. Total initial plants	15.23g	2.56g	1.96×10^{-3}	3.3×10^{-4}						
7. Remaining plants	5.24g	0.88g	6.7×10^{-4}	1.14×10^{-4}	3.55×10^{-4}		0.31		1.87×10^{-4}	
8. Total initial algae	3.6×10^{-1}	6.0×10^{-2}								
9. Total algae(6 days)	2.1×10^{-1}	3.6×10^{-2}				6.6×10^{-2}	2.1×10^{-4}		3.47×10^{-2}	
a) Euglena sp.	1.9×10^{-1}	3.18×10^{-2}				2.97×10^{-3}	5.88×10^{-3}		1.56×10^{-3}	
b) Chlamydomonas sp.	1.18×10^{-3}	1.98×10^{-3}				4.45×10^{-3}	9.21×10^{-5}		2.34×10^{-3}	
c) Chlorella sp.	1.23×10^{-2}	2.07×10^{-3}				6.83×10^{-3}	0.05			
10. Water borne particulates										
11. System Total	782kg	767kg								

* Average of two or more samples on a dry weight basis.

** Water metabolites: 1) Carboxy-amino-fenitrothion, 2) S-methyl-fenitrothion

3) Carboxy-fenitrothion, 4) dimethyl-phosphorothioate

Table 18-Partitioning of Fenitrothion in an Aquatic System
Dark Container-6 Days Post-spray (144 hours)

<u>Components</u>	<u>Mass of</u>		<u>Concentration</u>	<u>As Percent</u>	<u>Bioacc-</u>
	<u>Wet wt.</u>	<u>Dry wt.</u>		<u>of Total</u>	<u>-mulation</u>
	<u>Wet wt.</u>	<u>Dry wt.</u>	<u>in component</u>	<u>applied</u>	<u>Ratio</u>
	<u>Wet</u>	<u>Wet</u>	<u>(dry weight)</u>	<u>(dry weight)</u>	<u>(dry weight)</u>
1. Water	760kg	96.9	2.06	15.65	
2. Sediment	24.7kg	7.02	0.92		
a) Fenitrothion			68.8	4.83	
b) Amino-fenitrothion			60.1	4.73	
c) Fenitro-oxon			58.6	4.46	
d) S-methyl fenitrothion			51.8	3.64	
3. Water Wash of soil					
a) Fenitrothion			2.58	0.18	
b) Amino-fenitrothion			3.17	0.25	
c) Fenitro-oxon			13.87	1.02	
d) S-methyl-fenitrothion			3.44	0.24	
4. Sediment Total:					
a) Fenitrothion			71.38	5.01	
b) Amino-fenitrothion			63.27	4.98	
c) Fenitro-oxon			72.27	5.38	
d) S-methyl-fenitrothion			55.24	3.88	

Table 18-Partitioning of Fenitrothion in an Aquatic System(cont.)
 Park Container-6 Days Post-Spray(144 hours)

Components	Mass of		Mass Percent		Concentration		As Percent		Bioacc-
	Components	of System	of System	in Component	(dry weight)	ug/g	of Total	fenitrothion	
	Wet wt.	Dry wt.	Wet	Dry			applied	Ratio	(dry weight)
5.Total Initial Plants	6.76g	1.11g	8.6×10^{-6}	1.4×10^{-6}					
6.Remaining plants (6 days)	1.28g	0.22g	1.63×10^{-6}	2.9×10^{-7}	3.15×10^3		6.93×10^{-3}		1529
7.Total Initial Algae	3.6×10^1	6.0×10^2	4.54×10^{-7}	7.80×10^{-8}					
8.Total Algae, 6 days	2.1×10^2	3.2×10^2	2.72×10^{-2}	4.68×10^{-8}					
a)Euglena sp.	1.89×10^1	3.18×10^2			1.72×10^3		5.47×10^{-4}		835
b)Chlamydomonas sp.	1.18×10^1	1.98×10^3			4.41×10^3		8.73×10^{-5}		2141
c)Chlorella sp.	1.23×10^2	2.07×10^3			2.10×10^3		4.35×10^{-5}		1020
9.System total	784kg	767kg					34.9%		

*Average of two samples on a dry weight basis.

The plants, at a load of 2.9 mg/l dry weight, contained 0.5% of the total pesticide. The natural plant load in the lake was approximately 100 times higher (224 mg/l field). Mass balance calculations at later times indicated that large amounts of fenitrothion equivalent were missing from the model. At 6 days in the light and dark, the total recovery was reduced to 47.0% and 34.9% in the (Tables 17,18 light and dark models respectively. Later experiment (Moody unpub.) showed that the plastic containers likely absorbed much of this pesticide. For this reason the partition values of the pesticide are not accurate beyond 2 days because the plastic reduced the amount of pesticide available for uptake by all the other components of the system.

Discussion

Model One

Following spray operation, the water is the initial macro-reservoir from which the pesticide partitions into other components. The array of compartments into which partitioning can occur depends on the model's complexity, which in a natural system could be defined as being formed of sediment, particulate matter, plants algae and water as the major components. In order to isolate the contribution of plants and sediment to pesticide degradation in an aquatic system it was necessary to study the degradation in natural water alone. Unfiltered lake water is not a simple system in which to study pesticide degradation because 3 modes of degradation, hydrolytic, photolytic and metabolic degradation are occurring simultaneously but at different rates.

The fenitrothion in the water of model one degraded to 66% of the initial deposit by 20 days and a half-life was roughly estimated to be 29 days in this model (pH 6.8; 22°C). This observed half-life in model one was shorter than that for fenitrothion in distilled water in the dark (70 days at pH 7.0, 22°C) (Greenhalgh et al 1979 in press) but shows a longer half-life than the 0.5 to 3.5 days for fenitrothion in natural waters with sediment, particulate matter, plants and natural sunlight (Sundaram 1974). Thus light and biotic factors influence the degradation of pesticides in water. The influence of light can be especially important in pesticide degradation as seen in Table B where a comparison of several works are shown in the following:

Table B

Half-life of Fenitrothion in Water

1) distilled water	dark	pH 7.0	23°C	70 days (Greenhalgh <u>et al</u> 1979)
2) lake water	laboratory light	pH 6.8	23°C	*29 days (Boulton <u>et al</u> unpub.)
3) distilled water	sunlight	pH 7.0	23°C	20 hours (Mikami <u>et al</u> 1976)
4) distilled water	sunlight	pH ?	23°C	*17.5 hrs. (Ohkawa <u>et al</u> 1974)

*estimated from data

It would appear from Table B that natural sunlight increases the rate of fenitrothion degradation in the water.

The chloroform extractable derivatives in the water of model one, including fenitro-oxon, S-methyl-fenitrothion, 3-methyl-4-nitro-phenol and the unknown at Rf 0.38 were all at low levels, from 1.0 to 20.0 ng/g, and these derivatives are identical to fenitrothion photo-decomposition products found in distilled water or on chromatoplates exposed to sunlight (Ohkawa et al 1974).

The unknown water-soluble degradation products, which rose to 23.5% of the total applied ¹⁴C-activity in the water of model one were not identified. However, these derivatives may be similar to the derivatives found in the lyophilized extract of the water from the field model. These derivatives in the field model were identified as carboxy-fenitrothion, desmethyl-fenitrothion and perhaps carboxy-amino-fenitrothion (Weinberger et al 1977 (NRC)). If this were true it would compare favourably with the work of Ohkawa et al (1974) who identified

carboxy-fenitrothion (38.4%), fenitro-oxon (1.6%), formyl-amino-carboxy-fenitrothion (1.5%), 3-carboxy-4-nitrophenol, humic acids (51.3%) and 6 other unidentified products in distilled water under natural sunlight at 80 hours. In buffered distilled water at pH 3.0 and 7.0 the same products were formed in only slightly different relative amounts. Thus the degradation of fenitrothion in water appears to undergo hydrolytic and photolytic degradation and the model one appears to follow quite closely the types of degradation products found by other workers in the field. However, the slower rate of this process in model one may be due to the lower light levels available in the laboratory measured from the water surface; intensities which were 40 to 66 times less than in the field.

Model Two

The E. densa in model 2 (463 mg/l dry weight) sequestered a maximum of 8.1% of the total ^{14}C -activity from the 1.0 $\mu\text{g/g}$ fenitrothion solution, reaching a peak concentration of 137 $\mu\text{g/g}$ of fenitrothion equivalent and then a gradual decline was noted to 27 $\mu\text{g/g}$ at 25 days. This apparent reduction in ^{14}C -activity in the plant may be caused by release of the pesticide or derivatives into the surrounding water, or the result of the production of 'bound' or conjugated pesticide residues in the plant. If this apparent reduction in pesticide residues were caused by release to the water, this change would be noted by an increase in the ^{14}C -activity of the total water. This was not seen, possibly because this change would be masked by the large increase in the unrecovered portion of the ^{14}C -activity. Little new Elodea growth was noted, so the effect of growth in the dilution of the pesticide is not

a significant factor in the disappearance of pesticide from the plant.

Volatilisation of the pesticide or $^{14}\text{CO}_2$ from the model was not monitored, and no attempt was made to recover the possible bound residues from the plant material.

'Bound residues' is a term used here to describe a pesticide residues which cannot be removed by a solvent extraction method and in no way indicates a mechanism of pesticide binding to a substrate.

Bound fenitrothion residues have been found in rice (Miyamoto et al 1965), 'conjugated' parathion residues in sorghum (Ching-Chieh Yu 1975), 'unextractable' dieldrin residues in E. canadensis (Sanborn and Ching-Chieh Yu 1973), 'unextractable' Phorate residues in E. nuttali (Walter-Echols 1978), and unextractable triforine residues in barley (Rauchaud 1978). It was suggested that the parathion residues in sorghum may be water-soluble derivatives that conjugated with cellular material so that it was unextractable by organic solvent. Much of this conjugated residue was removed by heating the extracted residue with 0.025M HCL at 70°C for twenty hours (Ching-Chieh Yu 1975).

The nature of bound residues was further investigated by Rauchaud (1978). Approximately 33% of ^3H -triforine fungicide could not be removed by a methanol extraction. Further residue was removed by acidified methanol (18%) and hot dimethyl sulfoxide (13%). The residue from the dimethyl sulfoxide extract could be freed by amyloglucosidase and β glucosidase (13%) indicating some pesticide binding to starch. It was hypothesised that the remaining bound residue (4%) was bound to lignin (Rauchaud 1978). Thus, the conju-

gation of the pesticide with cell constituents could explain the 'apparent' decline of fenitrothion in E. densa in model two.

Volatilisation of fenitrothion from the model may explain some of the unrecovered pesticide in this model. The calculated theoretical value of volatilisation of fenitrothion is slow, such that the half-life in a water column one metre deep would be 93 days in the absence of any degradative pathway (Marshall and Roberts 1977). Volatilisation of aldrin, deildrin, DDT and lindane from tap water ranged from 0.9% to 25% in 24 hours but for the organophosphorus pesticides, parathion, diazinon and azinphosmethyl, the volatilisation ranged from 0.96% to 1.93% in tap water over 24 hours. The addition of soil, algae or detergents substantially reduced this volatilisation (Lichtenstein and Schulz 1970). Addition of parathion to soil resulted in no volatilisation (Lichtenstein and Schulz 1964) and fenitrothion solution added to several submerged soils resulted in the evaporation of only small amounts of ^{14}C -activity ($\leq 5.2\%$) in 30 days in the form of carbon dioxide (Takimoto et al 1976). However, the experiment of Takimoto was performed in the dark, where the influence of light was negligible in the process of volatilisation. Thus it would be expected that volatilisation would be a slow process compared to the competition from other degradative processes, but it remains a possibility. The plant could concentrate the pesticide from solution, and translocate it to the exposed apex where it might be released to the air.

The differences in the types and amounts of fenitrothion

degradation products in the water of model 1 (no plant) and model 2 (with plant) are easily seen in the comparison of the autoradiographs (Plate 4 and Plate 8 respectively). The major difference is the much greater concentration of water-soluble products at the origin in the plate of model 2 (up to 69% to 83% of the total ^{14}C -activity by 20 to 25 days) than of model one (23.5% by 20 days). Therefore, the presence of the E. densa segment increased the levels of water-soluble derivatives in the water by up to 300% to 400%. Whether this increase was caused by direct metabolism of the pesticide by the plant followed by its release to the water, or whether this was caused by some indirect effect of the plant on the system is not known. Such indirect effects may be due to increased oxygen levels in the water, the stimulation of bacterial activity in the water, the release of photosensitizing compounds or extracellular enzymes like phosphatases or the possibility that the plant may act as a surface for some photolytic reaction.

Elodea nuttallii has been shown to cause the production of phorate-sulfone in the water containing phorate and this may be caused by the oxygen released from the plant to the water (Walter-Echolls and Lichtenstein 1978).

The plant extraction method would not remove these water-soluble derivatives from the plant tissue, although traces of ^{14}C -activity were noted at the origin of the TLC plate (3 or 4). This may indicate traces of these derivatives in the plant. Thus the plants could have metabolised the fenitrothion to water-soluble derivatives and excreted them to the water.

It is well known that aquatic plants release nutrient elements such as phosphorous (De Marte and Hartman 1974) and organic compounds (Wetzel 1974) to surrounding waters and this could stimulate microbiotic activity and aid in the formation of well known water-soluble, bacterial metabolites of fenitrothion such as desmethyl-fenitrothion and desmethyl-aminofenitrothion (Miyamoto 1977, Miyamoto et al 1966). Increased bacterial activity could explain the larger amount of water-soluble products in the model 2 (plant-water model) as compared to model 1 (water model).

An aqueous extract of dead algal cells was shown to increase the photodecomposition of DDT and mexacarbate by a sunlamp (Boush and Matsamura, 1975) and similar photosensitisation of fenitrothion by Xanthone, Baytex, and dyfonate has also been shown (Ivie and Casida 1971). Organic ligands of less than molecular weight 1400 have been implicated in the binding of mercury in river water (Ramamoorthy et al 1977) and similarly sized organic compounds released by the plant could interact with the pesticide in the natural pond water.

Thus some or all of these factors; oxygen content, bacterial activity or photosensitizing activity of some released compounds from the plant may have contributed to the large quantity of water-soluble products in model 2.

Minor differences in the type and amount of chloroform extractable products in the water of models 1 and 2 were noted. Two to four times more 3-methyl 4-nitrophenol was found in the water of the plant-water model 2 than in the water of model 1, but there were

no significant differences in fenitro-oxon and S-methyl-fenitrothion. The nitrocresol is a known bacterial and photolytic product so its increased formation in the plant-water model may be for the same reasons as previously mentioned for the water-soluble derivatives. Also, plants, algae, bacteria and zooplankton are also known to release extra-cellular phosphatases that cleave phosphate esters and these could possibly cleave the fenitrothion into the nitrophenol and the DMPT moieties (Jansson 1976). These reasons may account for the higher concentrations of the nitrophenol in the water of the plant-water model (2).

The fenitro-oxon and S-methyl-fenitrothion are considered mostly photodecomposition products (Ohkawa et al 1974, Miyamoto et al 1977). Their equal formation in the water model 1 and the plant-water model 2 is not surprising and the productions of the 2 products in the water is independent of the activities of the plant. Photodecomposition of a related pesticide, parathion, in distilled water gave para-oxon and S-ethyl parathion as major photoproducts as well as 10 minor products. This formation of oxidation and isomerisation products in the light is a typical reaction of organophosphorus pesticides (Joiner and Baetcke 1974).

Model Three

Model 3 was designed to test the potential absorption of fenitrothion by a typical aquatic macrophyte, Elodea canadensis, using a biomass-to-water ratio similar to that found in natural conditions.

E. canadensis was chosen as a typical submerged macrophyte because of its widespread growth in North America (Sculthorpe 1967),

its distribution over a wide range of lake types as measured by conductivity (75 to 350 μg per mHos) and pH (6.3 to 8.5) (Crowder et al 1977) its presence in many lakes of Gatineau Park (Aiken and Gillet 1974), its ease of growth and its ability to grow when floating in the water. It is a widely used study plant for research in plant physiology (Sculthorpe 1967) and for the target and non-target actions of herbicides and insecticides (Seaman and Thomas 1965, Maki and Johnson 1977, Sanborn and Ching-Chieh Yu 1975).

Plant density in continuous beds of Chara sp. in Lac Bourgeois (pH 7.5) showed a biomass of 210 g/m^2 (263 mg/L at 1 m depth) dry weight and this compared to a mean plant biomass of 248 g/m^2 (range of 1.0 to 1,154 g/m^2) in Lake Opinicon (mean depth 2 m) in eastern Ontario. Using these figures as a guideline, model 3 was designed with plant densities ranging from 97 to 897 mg/L dry weight to represent a wide range of possible plant densities found in a shallow pond system (< 2-3 m depth).

Using the top plant load (897 mg/L) E. canadensis removed 45% of the total fenitrothion from a 10.0 $\mu\text{g/g}$ and 29% of the pesticide from the 1.0 $\mu\text{g/g}$ solution. Field studies showed that under natural light conditions, the pesticide concentrations in the plant were even higher.

Pesticide absorption could be divided into 2 groups. For both the 10 $\mu\text{g/g}$ and 1.0 $\mu\text{g/g}$ pesticide solutions, the total uptake was identical for the 1.2 cm and 2.5 cm segments and total uptake was very similar for the 7.6 cm and the 10.2 cm segments (Figure 8, 9).

This could be explained by the fact that most of the observed radioactivity was in the Elodea leaves and not as much in the stem as shown in the

whole plant autoradiographs of Elodea densa, a plant similar to E. canadensis. When it is noted that internodal distance in E. canadensis progressively increased from one-half to 1.0 cm as the plant length increased, then it is easy to see that leaf area and number could be similar between the 2 smallest and the 2 largest plant segments used in this model. By the same explanation, one could explain the decreasing pesticide concentrations in a decreasing series of sizes from the 1.2, 2.5, 7.6, and 10.2 cm segment sizes. A greater number of leaves and hence surface area per unit weight is shown by the smallest apical segments, and this surface area to mass ratio declined as the plant segment size increased. Thus the smallest apical segments should show the greatest pesticide concentrations as they do (Figure 9). Another factor causing higher pesticide concentrations in the smaller apical segments may be the higher physiological activity of the apical segments, as they represent younger and growing tissue.

Pesticide absorption by Elodea was dependent on the surrounding concentrations of pesticide in solution. Increasing concentrations of fenitrothion in the water increased fenitrothion concentration in the plants. During the first 5 days of continual pesticide uptake, the plants from the 10 µg/g solution took up an average of 7 times more pesticide than the plants from the 1.0 µg/g solution. After 5 days, the concentrations in the plants from the 1.0 µg/g solution declined steadily, while the concentration in the plants from the 10 µg/g solution climbed continually until the end of the experiment at 14 days. This decline in pesticide concentrations after 5 days from the 1.0 µg/g

solutions in Model 3 was identical to a similar decline in E. densa in model 2 from the 1.0 $\mu\text{g/g}$ fenitrothion solution. The continual absorption by E. canadensis up to 14 days in model 3 from the 10 $\mu\text{g/g}$ solution was not similar to the absorption of the same plant in model 5 (field) at the same concentration. This difference may be caused by lower light levels in Model 3 and a saturation capacity in E. canadensis. Absorption in the field model peaked at 71,235 $\mu\text{g/g}$ (74 hrs) whereas absorption in Model 3 was still increasing at 14 days (47,207 $\mu\text{g/g}$) because saturation levels had not been reached. The reason for the decline in pesticide levels in the plants from the 1.0 $\mu\text{g/g}$ solution in Model 3 cannot be easily explained. There was no visible toxic effect of the one or ten $\mu\text{g/g}$ fenitrothion solution on the plant. Some sub-lethal effect of the pesticide may have influenced the plants ability to metabolise, bind or excrete the pesticide from the tissue, such that plants could eliminate pesticide when exposed to a 1.0 $\mu\text{g/g}$ solution but not a 10 $\mu\text{g/g}$ solution.

Bioaccumulations ratios of 2,000 for Lemna sp. for Carbaryl (Kanazawa et al 1975), ratios of 1,280 for dieldrin in Elodea sp. (Sanborn and Ching-Chieh Yu 1975) and a high value for diphenamid in Myriophyllum sp. (Bingham and Shaver 1977) show that pesticide absorption by aquatic plants is significant. This high bioaccumulation of pesticides is not unusual when compared to the accumulations of phosphorus, magnesium, iron and other elements from the sediment by Scirpus validus to levels of 1,000 to 10,000 parts per million in the tissue (Small and Gaynor 1975) and the large accumulation of non-essential elements such as cadmium and lead by E. canadensis (Mayes and McIntosh 1977). Aquatic plants tend to bioaccumulate nutrients to levels up to thousands of times greater than

the water or substrate concentrations and at levels far greater than that needed for growth in a phenomenon termed as a "luxury consumption of nutrients" (Wetzel 1975). Thus the high bioaccumulation of pesticides is not unusual and it may occur by the same mechanism as ion transport across the membrane, via active transport. Uncouplers of oxidative phosphorylation, such as 2,4-dinitrophenol and sodium azide were shown to reduce potassium absorption by 'dark' corn leaf segments to 20% of the controls in the dark; however, the presence of light increased potassium absorption to 60% of the 'lighted' controls in the presence of these uncouplers. This indicates that ATP produced in photophosphorylation compensated partially for the lack of ATP formed by oxidative phosphorylation (Rains 1968). Therefore, if pesticide absorption is due to ATP-dependent active transport, then there should be significant differences between pesticide absorption between light and dark conditions and possibly differences when the light intensity and quality changes. This was observed in the comparison of fenitrothion absorption in the light and dark containers of model 5 and the light model in the laboratory (model 3). In a comparison of all three models, in which the fenitrothion concentration was 10 µg/g, two to 16 times greater fenitrothion absorption was noted in the lighted models containing Elodea (Figure 9, 14) as compared to the Elodea in the dark field model (Figure 15). Light intensity readings just above the water surface in the dark container of less than 60 Lux were much less than the 1,500 Lux in the lighted laboratory model and the 60,000-100,000 Lux measured above the lighted field model. Thus the evidence would indicate that the fenitrothion uptake is likely active uptake and that varying the light levels may influence active uptake of the fenitrothion.

Analysis of the water from model 3 showed derivatives similar to the water of model 2. Although the water of model 3 showed 7 to 25 times more fenitro-oxon, 13 to 15 times more S-methyl-fenitrothion and one to 4 times more 3-methyl 4-nitrophenol than the water of model 2 it must be noted that the fenitrothion concentration in model 3 (10 µg/g) was 10 times higher than in model 2 (1.0 µg/g). The water of model 3 showed traces of amino-fenitrothion from 8 to 20 days and an unknown (Rf 0.36) that the water of model 2 did not show. An explanation for this may be as follows: The concentration of Aerotex in model 3 was 1.0 µg/g whereas that in model one was 0.1 µg/g. One µg/g Aerotex solutions were shown to cause 36% mortality of Scenedesmus sp., 27% mortality of Chlamydomonas sp. and 84.5% mortality of Chlorella sp. Also, 10.0 µg/g fenitrothion solutions caused a lag phase in algal growth (Moody unpublished). Together, both these factors may have caused significant algal death and cell lysis and this may have increased bacterial activity. The increased bacterial activity may have formed the amino-fenitrothion in model 3, this reduced product being a well-known bacterial degradation product of fenitrothion (Miyamoto et al 1966).

Thus far, the potential absorption and degradation of fenitrothion by Elodea, and the contribution of this aquatic plant to the degradation of the pesticide in the water, has been shown.

Model Four

A mixed plant model containing one plant each of E. densa, Sagittaria graminea, and Myriophyllum sp. at a plant load of 221 mg/l

dry weight, sequestered 6% to 8% of the total ^{14}C -activity over the 14 day period from the 1.0 $\mu\text{g/g}$ solution and this compared favourably with similar results in model 3.

All three plants showed similar absorptions in the range of 266 to 557 $\mu\text{g/g}$ of fenitrothion equivalent and the slight differences in the rate and total pesticide absorption may be due to differences in surface area and metabolic activity. The delayed absorption by Sagittaria graminea (Figure 11) may be caused by its lower surface area to mass ratio because of the thickness of the leaf blade compared to the finely reticulate Myriophyllum sp. and the thin Elodea densa leaf. These 3 species showed much more pesticide degradation than E. canadensis and this was best shown by the example of Myriophyllum sp. which showed levels of 27 to 39 $\mu\text{g/g}$ of 3-methyl 4-nitrophenol, 8.0 to 20 $\mu\text{g/g}$ of S-methyl-fenitrothion, and 15 to 65 $\mu\text{g/g}$ of fenitro-oxon. The water soluble products which were less than 7.0% in Elodea and Sagittaria formed up to 32% of the total ^{14}C -activity in Myriophyllum sp. and their identities were unknown.

E. densa in model 4 showed 4 to 30 times more fenitro-oxon 5 to 10 times more 3-methyl 4-nitrophenol and 10 to 50 times more S-methyl-fenitrothion than the E. densa in model 2. Conditions such as light, temperature, water, water pH, plant load (463 mg/l , model 2 and 221 mg/l , model 4) and initial fenitrothion concentration were similar in both experiments so that one should expect similar degradation in the pesticide in the plant.

An explanation may lie in the fact that the 2 systems were

significantly different in several ways. Model 4 had a surface area to volume ratio 70% greater than that of model 2 and the plants of model 4 had more extensive portions of the apex floating on the surface than that of model 2. These two factors may expose some plant tissue and hence some of the absorbed pesticide to the atmosphere and light where photodecomposition to the fenitro-oxon, S-methyl-fenitrothion, and the 3-methyl 4-nitrophenol could occur. All 3 of these derivatives are known photodecomposition products on bean leaf surfaces (Ohkawa et al 1974). These same reasons could be used to explain the greater unrecovered ^{14}C -activity in model 4 which reached 37% of the initially applied dose (Figure 10). A greater amount of the pesticide or derivative may have volatilised from the water or the surface of the plant. However, another reason which could explain the unrecovered pesticide may be due to a possible loss as conjugated residue in the plant.

The derivatives found in the water of the mixed plant model 4 were compared to the levels found in the E. densa model 2 and the levels of fenitro-oxon, S-methyl-fenitrothion, and the 3-methyl 4-nitrophenol were very similar (Table 8A, 11a). In model 4, however, amino-fenitrothion (2.3 to 5.6 $\mu\text{g/g}$) and unknowns at RF 0.33 and 0.22 (1.4 to 15.3 $\mu\text{g/g}$) were found that were not in model 2. Perhaps slight differences in the microflora of the water caused the production of these bacterial metabolites in the water. Aquatic macrophytes are known to release much of their net daily photosynthate to the surrounding waters, to feed a host of micro-biota (Wetzel and Manny 1972, Wetzel 1969) and it may be that the Myriophyllum sp. or the S. graminea of model 4 released products more conducive to bacterial growth than the E. densa of model 2.

Bioaccumulation Ratios

The presence of high levels of pesticide derivatives in the plants might be a simple absorption of these derivatives from the surrounding water and not caused by fenitrothion absorption followed by pesticide degradation. To test this, hypothetical bioaccumulation ratios for fenitrothion and its derivatives were compared with each other. The bioaccumulation ratio, as mentioned earlier, is defined as the concentration in the plants divided by the concentration of pesticide or derivative in the water. The basic assumption was that if the derivatives found in the plant were due to simple uptake from the surrounding waters, then the bioaccumulation ratios for the derivatives should be the same as for fenitrothion. This assumes that all derivatives are absorbed at the same rate as fenitrothion, and this may seem reasonable considering the similarity in structure of at least two of the three derivatives found in this work, fenitro-oxon and S-methyl-fenitrothion. Phorate, Phorate sulfone, and Phorate sulfoxide, were absorbed at the same rate and amount by Elodea nuttallii so this assumption may be valid (Walter-Echols 1978).

Bioaccumulation ratios for fenitrothion were 372 to 534 at 5 days, 446 to 875 at 10 days and 448 to 1,135 at 14 days depending on the species of plant in model 4 (Table 12).

Bioaccumulation was significantly higher for S-methyl-fenitrothion and 3-methyl 4-nitrophenol in all species for most of the time and bioaccumulation ratios for fenitro-oxon were higher for Myriophyllum sp. and S. graminea for most of the time (Table 12). As

an example, the bioaccumulation for S-methyl-fenitrothion was 1,338 to 10,797 at 5 days, 183 to 7,197 at 10 days and 909 to 17,954 at 14 days depending on the plant species used.

The higher bioaccumulation ratios for at least S-methyl-fenitrothion and 3-methyl 4 nitrophenol and possibly fenitro-oxon, in some species, suggest that these compounds were largely absorbed as fenitrothion and then degraded to these derivatives within the plant.

Whether the pesticide degradation is caused by the activity of plant enzymes, by a photochemical reaction, or by a combination of these processes has not been determined.

The chloroform extractable derivatives in the plants of model 4 comprise 5.3% to 66.2% of the total ^{14}C -activity in the plant depending on the species and time considered, however, only the S-methyl fenitrothion and fenitro-oxon are significantly toxic (several times the toxicity of fenitrothion in vivo) and these derivatives make up 1.0% to 17% of the total ^{14}C -activity in the plant, again depending on the time and species considered. As mentioned previously, the occurrence of these toxic derivatives in the plant at levels at least 1,000 times higher than in the surrounding water (Table 10A, 11A) (initial H_2O conc. 1.022 $\mu\text{g/g}$) may provide a danger to the approximately 20 taxa of macroinvertebrates that have been shown to colonize E. canadensis (Berry Jr. et al 1975). Given that aquatic plants are important foci for microfaunal activity that often equals or exceeds that of the top active layers of the sediment (Mortimer et al unpub.), that the plants can show bioaccumulation values of up to 17,000 for toxic derivatives

that are themselves 2 or 3 times more toxic than the parent compound, and that the median level of toxicity ($LC_{50}^{24 \text{ hrs}}$) for fenitrothion to aquatic insects in general (Symons 1977) is 0.06 ppm, then it may be valid to assume that a great danger may exist to the aquatic invertebrates even at environmental concentrations of fenitrothion (0.025 ppm Eidt and Sundaram 1975). The danger to the microbiota in contact with the plant surface, or those grazing on the plant, cannot be estimated because the relative amounts of pesticide on the surface, and in the tissue, is not known, nor is data available on any possible desorption of the pesticide or derivatives from the plants which could harm the invertebrates. Thus the 4 laboratory models demonstrated the potential absorption and degradation of the pesticide in a plant water system and the contribution of the plants to pesticide degradation in the water.

Model Five

Model 5 was designed to show the importance of the aquatic plants in a field model also containing sediment, algae and the natural sunlight, and in one set of containers, to show the effect of lack of light on all the processes.

Fenitrothion disappeared quite quickly from the water with an estimated half-life of about 46 hours in the light and dark models (Figure 12 and 13). Although water-borne degradation products were found to form about 10% of the total applied dose at 2 days, and that about 34% remained as fenitrothion in the water at 2 days in the light, much (56%) was missing (Table 15).

Further analyses revealed much of the missing amount in the sediment, plants, algae and particulate matter (Table 15)

The rapid pesticide absorption by Elodea up to a peak of $71,235 \pm 3035$ $\mu\text{g/g}$ in 74 hours in the light was followed by a decline to $11,639 \pm 1306$ $\mu\text{g/g}$ at 14 days (Figure 14) and the corresponding peak of $4,120 \pm 310$ $\mu\text{g/g}$ at 22 hours in the dark followed by a decline to 489 ± 108 $\mu\text{g/g}$ and the influence of light in this absorption has been discussed (Figure 15). The cessation of net pesticide absorption by Elodea in the dark model was likely caused by the lack of high energy compounds such as ATP to continue what was likely the active uptake of fenitrothion by the plant. The maintenance of relatively constant levels of fenitrothion from 22 hours to 6 days with no pesticide derivatives (chloroform extractable) being formed in the dark model may have indicated that all active uptake had ceased in the dark model (Figure 15).

The lack of pesticide degradation by the E. canadensis in the laboratory models and the field models, unlike the other three species may be due to species differences in the ability to metabolize fenitrothion. A subtle toxic effect of the 1.0 $\mu\text{g/g}$ Aerotex solution that was present in the field, a concentration that was shown to be significantly toxic to some algae (Moody), may have impeded the plant's ability to detoxify the pesticide, but the E. canadensis in the 1.0 $\mu\text{g/g}$ fenitrothion solutions (0.1 $\mu\text{g/g}$ Aerotex), also did not show pesticide degradation in the laboratory. E. densa, a related species, showed some pesticide degradation in the 1.0 $\mu\text{g/g}$ solutions of pesticide but

it was still low compared to the S. graminea and the Myriophyllum sp. The use of some other plant species in the field model (5) may have shown more pesticide degradation in the plants.

Although no visible damage occurred in E. canadensis in the field, the generally high concentration range of 28,080 to about 58,990 µg/g fenitrothion in the plant indicated that 2.8% to 5.0% of the total dry weight of the plant was pesticide and this could easily have a sub-lethal effect on the plant.

TFM, 3-trifluoromethyl-4-nitrophenol, is a larval lampricide and a herbicide which is similar in structure to 3-methyl-4-nitrophenol. This 3-methyl-4-nitrophenol was a significant derivative found in 3 of the 4 species examined in several of the models. TFM was found to inhibit the growth of E. canadensis by 5% to 10% at concentrations of 5.0 µg/g and inhibit the growth of Myriophyllum spicatum 60% to 85% at concentrations of 10 to 25 µg/g of TFM in the water (Maki and Johnson 1977). Environmental concentrations of TFM after a typical spray, 6 to 7 µg/g in water, were expected to reduce the biomass of E. canadensis by 5 to 10% and Myriophyllum spicatum by 20%. Concentrations of TFM in the tissue were not specified (Maki and Johnson 1972). Thus the presence of the nitroresol in the plant may be sub-lethally effective in reducing net plant production. A possible toxic effect of 3-methyl-4-nitrophenol, or any other derivative may impair the ability of that plant to detoxify the pesticide and thus the pesticide or derivative would remain in the system for that much longer, posing a potential hazard through delayed release into the aquatic system.

The decline in the apparent fenitrothion concentration in E. canadensis in the field model may be caused by the reasons mentioned previously, such as release to the water, or conjugation to the plant material.

Thus, the field model showed that plants can be an important reservoir of the pesticide. The use of other species in other models (2,4) showed that much significant pesticide degradation occurred in the aquatic plants leading to the hypothesis that aquatic plants are an important site of fenitrothion decomposition.

Sediment

The relatively high levels of amino-fenitrothion (44 to 182 $\mu\text{g/g}$) fenitro-oxon (37 to 14 $\mu\text{g/g}$), and S-methyl-fenitrothion (39 to 77 $\mu\text{g/g}$), compared to the levels of fenitrothion (38 to 94 $\mu\text{g/g}$) show that these derivatives are the important initial degradation products in the sediment in the light and dark for the first 6 days (Figure 13).

The actual amount of amino-fenitrothion in the sediment is likely twice the amount recovered because of the inadequate recovery from the charcoal cleanup method (Table 3). Thus amino-fenitrothion may actually be the major derivative in the sediment of the field system. Amino-fenitrothion has been shown to be the major derivative found in flooded, anaerobic or near-anaerobic soils, forming up to 65% of the total recovered ^{14}C -activity (Takimoto et al 1976). Similar results were shown for parathion in lake sediments (Graetz et al 1970).

The 2 to 3 times greater levels of S-methyl-fenitrothion and fenitro-oxon in the sediment of the light models as compared to the dark models are similar to the findings of Ohkawa, if these derivatives are considered photodecomposition products. About ten times more fenitro-oxon (3.3% light; 0.4% dark) and S-methyl-fenitrothion (3.9% light; 0.4% dark) were found in lighted water as compared to the water held in the dark (Ohkawa et al 1974).

The appearance of fenitro-oxon and S-methyl-fenitrothion together with amino-fenitrothion in the sediment is unusual as the two former derivatives are associated with a photodecomposition reaction in the presence of oxygen, while the latter is more associated with bacterial activity in an anaerobic system (Ohkawa et al 1974, Miyamoto et al 1966, 1977). This paradox may be explained by a suggestion that the sediment was both aerobic and anaerobic. Previous experiments with fenitrothion in anaerobic sediments did not show the production of fenitro-oxon nor S-methyl-fenitrothion for a possible reason that these experiments were performed in the dark and that the sediments were truly anaerobic (Takimoto et al 1976). Fenitro-oxon and S-methyl-fenitrothion are well known photoproducts of fenitrothion on bean leaf surface (Ohkawa et al 1974) and these derivatives may have formed on the sediment surface in the fully oxygenated and sunlit waters of model 5. Even the top few millimeters of mostly anaerobic sediments can be fully oxygenated and this region would likely be sufficient to explain the production of these 2 derivatives by a purely photochemical means.

The oxidation and reduction of the same pesticide molecule

in an aquatic system must also be considered. ^{14}C -Phorate added to the sediment was found to desorb (50%) from flooded loam sediments to be oxidised to ^{14}C -Phorate sulfoxide, and vice versa, that ^{14}C -phorate sulfoxide added to the flooded loam-water system was found to be reduced in the sediment to ^{14}C -Phorate (Walter-Echols and Lichtenstein 1978). A similar reaction may have occurred when carboxy-amino-fenitrothion was tentatively identified as one of the water-soluble derivatives found in the water of model 5 (Weinberger *et al* unpub.). In this case, amino-fenitrothion formed in the sediment was likely desorbed from the sediment, and then oxidised to the carboxy-amino-fenitrothion or the opposite sequence was followed.

Other derivatives should have been found in the sediment, such as 3-methyl-4-aminophenol, formyl-amino-fenitrothion, S-methyl 4-nitrophenol, desmethyl fenitrothion, desmethyl-amino-fenitrothion (Takimoto *et al* 1976) and perhaps other products were present. However, incomplete or inadequate methodology were employed so that some products such as the 3-methyl 4-nitrophenol, the water soluble derivatives, and other possible products in the sediment could not be extracted or analysed. The water-soluble derivatives such as desmethyl-fenitrothion, carboxy-fenitrothion and others would not be removed from the sediment and these products were estimated to be 6% to 17% of the total applied pesticide in some submerged soil systems (Takimoto *et al* 1976).

Tightly bound residues, substances not removed by solvent extraction, could also be expected in the field sediment study, as up to 23.7% of the applied pesticide has been shown to be tightly bound to some soils by 60 days (Takimoto *et al* 1976). However, no attempt was

made to recover some of the bound residues, by more rigorous extraction methods. The binding of pesticides to soils, such that they are not extractable by the usual solvent methods, is a common occurrence and has been shown for innumerable pesticides such as parathion, dieldrin, propanil, methyl-parathion and others (Ching-Chieh Yu and Sanborn 1975, Sanborn and Ching-Chieh Yu 1975; Hsu and Bartha 1976; Fuhremann and Lichtenstein 1978).

Attempts to determine the type of residue binding to the sediment and the nature of bound residues has best been shown by an approach by Hsu and Bartha (1976), in their work with dichloroaniline-humus complexes. A complex extraction involving solvent extraction, wet combustion, alkaline hydrolysis and steam distillation removed undegraded herbicide that was physically adsorbed, and removed covalently bound but hydrolysable DCA that could be analysed by GC, TLC and scintillation counting methods (Hsu and Bartha 1976). They suggested that there is a covalent binding of the nitrogen atom of the DCA to the carbon of the carbonyl group or an aromatic ring of humic compounds.

The preferential binding of amino-parathion to submerged sediments as observed by Katan et al 1976 could occur via the amino group in a mechanism similar to that proposed by Hsu and Bartha (1976). This mechanism could explain the most rapid disappearance of aminofenitrothion from the sediment from very high initial levels in the field model(5) compared to the more moderate disappearance of the other derivatives.

There is a need to know the nature of this tight binding to the sediment and the identity of this bound residue because these bound residues have been shown to disassociate from the sediment and recirculate

in the aquatic system as the parent compound or as a derivative.

Penicillium frequentans was shown to degrade the tightly bound DCA-humic acid complex to free DCA plus other unknown compounds believed to be "humic oligomers with attached DCA" (Hsu and Bartha 1976). This biotic reaction could release bound residues into the environment where it could harm some organisms.

In another study, methylparathion treated soil was extracted with solvents and 32.5% of the initial application was left in the soil as bound residue. Earthworms (Lumbricus terrestris) and oat plants (Avena sativa) were allowed to grow in that soil for 2 to 6 weeks and sizable amounts of ^{14}C -residues were found in the tissues of both oat and earthworm. The majority of the ^{14}C residue taken up by the earthworm (58% to 68%) again became bound in the tissue and it was not determined if this bound residue was again in the form of methylparathion (Fuhremann and Lichtenstein 1978). If this bound residue in the earthworm were the intact methylparathion it would mean that there could be a continuous recycling of this organophosphorous pesticide between the soil and biota and this process would extend the persistence of a pesticide in the environment. Of further interest was that 46% to 62% of the ^{14}C -residues in the oat were translocated to the green part of the plant and most of these were benzene soluble residues which indicates that they were the parent compound or a residue very similar to the methylparathion. Since Elodea nuttalli has been shown to take up Phorate from the sediment, translocate it to the leaves, and release it to the surrounding waters (Walter Echols 1978), and translocation of "soil-bound" methyl parathion by oat plants has been shown, it might be hypothesized

that rooted aquatic plants have the ability to recirculate "bound" fenitrothion residues in the sediment to the surrounding waters. For these reasons an attempt should be made to recover these "bound" residues, determine their identity, and assess the dangers of a delayed release of this residue on the biota of an aquatic system. Much faster pesticide degradation was seen in the sediment of the lighted field model (5) than in the sediment of the dark model of Takimoto et al (1976) not only because of the presence of photoproducts, fenitro-oxon and S-methyl-fenitrothion in the field, but for the more rapid formation of amino-fenitrothion. Several reasons may account for this difference. Takimoto used air dried upland loam soil which was submerged for one week prior to use, while the field model used natural untreated lake sediment. The soil used by Takimoto may not have had a full complement of active bacteria compared to the field sediment and thus its activity may have been less than the field sediment. Secondly, the unconsolidated field water-sediment slurry may have dispersed the bacteria and sediment particles in a fine suspension thus exposing a greater surface area to which the pesticide could interact and be subjected to bacterial activity. Other subtle factors such as relative bacterial concentrations and differences in soil characteristics could also influence the pesticide degradation and explain the difference between these field sediment results and the work of Takimoto et al (1976).

In submerged soils the half-life of fenitrothion ranged from 4 to 20 days in different soil types in the dark (Takimoto et al 1976), whereas the half-life was estimated to be 7 and 9 days in the light and dark respectively in the field model (5) (measured after 2 days

post-spray), this compares favourably to the work of Takimoto et al 1976.

The eventual fate of the pesticide and degradation products is the likely hydrolysis to a phenol such as 3-methyl-4-nitrophenol and the DMPT. The nitrocresol and similar derivatives could polymerize to the humic acid components to become part of the soil, or the cresols could be used as a carbon source by the bacteria resulting in the eventual production of carbon dioxide (Munnecke and Hsieh 1974).

Pesticide degradation in the sediment really refers to degradation performed by the sediment bacteria and fungi. At the sediment-water interface, bacterial activity increases 10^3 to 10^7 times compared to the overlying water (Wetzel 1975) and this activity has been found essential for most pesticide degradation. The irradiation of soils with gamma radiation resulted in no fenitrothion degradation in several soils that normally showed extensive pesticide decomposition (Takimoto et al 1976). Some pesticide hydrolysis has been shown in irradiated and sterile soils, but this was a limited and slow hydrolysis, such as for parathion, compared to the faster biotic degradation (Yaron 1975).

Isolated bacterial and fungal cultures have been shown to degrade several organophosphorus pesticides such as fenitrothion parathion and methyl-parathion so their role in the process is quite well proven (Miyamoto et al 1966, 1977; Sethunathan and Yoshida 1972, 1973; Siddaramappa 1973).

Cell free extracts of Flavobacterium sp. and Pseudomonas sp.

hydrolysed parathion and a purified inducible enzyme parathion hydrolase extracted from a mixed cell culture, could hydrolyse 8 of 10 organophosphorus pesticides at rates up to 416 n mol/min/mg. protein (Munnecke and Hsieh 1974). Further, mixed cell cultures succeeded in using parathion or p-nitrophenol as a sole carbon source. When p-nitrophenol was used as a carbon source, the nitro group was removed as nitrate and the hydroquinone used as the carbon source.

Thus, bacteria and fungi have been shown to have the potential to completely degrade some O-P pesticides, but whether they do so in natural sediments remains to be proven. Rhizopus arrhizus, which could degrade Dyfonate (o-ethyl-S-phenyl ethyl phosphonodithioate), in liquid culture, failed to do so when the fungal spores were added to the soil. Scanning electron microscopy showed that the soil was a poor substrate for microbial growth (Flashinski and Lichtenstein 1974).

Competition between microorganisms can also inhibit pesticide degradation. Penicillium paraherquei, a fungus, could degrade bromacil in pure culture but not when added to a non-sterile bromacil treated soil. However, when the fungus was added to sterilised bromacil treated soil, it proceeded to metabolise bromacil (Flashinski and Lichtenstein 1974).

It may be concluded that microbial activity, either bacterial or fungal activity, was responsible for the fenitrothion reduction to amino-fenitrothion, and that fenitro-oxon and S-methyl fenitrothion were the result of photochemical reactions on the

sediment surface.

Although there are bacteria in natural waters, few reports have shown the presence of amino-fenitrothion in the water, perhaps because of their relatively lower concentration in the water as compared to the sediment (Greenhalgh, et al 1979, Yasuno et al 1965, Zitko and Cunningham 1974). However, small amounts of 3-methyl-4-nitrophenol were found in the water of the field model (5), (Table 15), and this hydrolysis product is both a bacterial and photodegradative product of fenitrothion (Miyamoto 1977).

Bacteria, phytoplankton and zooplankton may release extracellular enzymes, phosphomonoesterases, which may be able to hydrolyse fenitrothion at the P-O bond (Jansson 1971). Free and seston-associated enzymes from zooplankton (Bosmina obtusirostris), and green algal blooms (Schroderia setigera) hydrolysed the artificial substrate 3-O-methyl-flouescein phosphate. Equal amounts of these phosphatases were excreted by both phytoplankton and zooplankton so that both these types of organisms were equally important in this activity. Jansson found great increases in these free phosphatases in the water during algal blooms, presumably when the lack of free phosphate in the water is the limiting factor of growth during bloom periods. It has been shown that phosphorous and sulphur from pesticides becomes incorporated into cell membranes of algae (Daughton et al 1976). Thus, the introduction of fenitrothion into the aquatic system could offer a relatively bountiful and needed source of phosphorus to the algae and the production of these enzymes may be induced by the presence of

fenitrothion. These enzymes may then assist in the hydrolysis of fenitrothion in natural waters. The fact that similar phosphatases isolated from potato were able to hydrolyse parathion may be further evidence that phosphatases may be significant in degrading O-P pesticides in natural waters (Heuer *et al* 1976). Also, more 3-methyl-4-nitrophenol was found in the water of the plant-water model 2 (24 to 58 ng/g) than in the water only model (1) (3 to 25 ng/g), which may indicate the activity of a phosphatase excreted by the aquatic plants. Further work could be done to assay the phosphatase activity in the water and its relation to O-P pesticide hydrolysis.

Mass Balance

Mass balance calculations attempted to account for all the pesticide added to the field model and to determine the importance of each component in the partitioning of the pesticide in the aquatic system (Tables 15 to 19).

At 2 days in the light, the total sediment sequestered 38.7% of the total added fenitrothion as fenitrothion or derivatives, the water held 47.7% of the initial dose as fenitrothion or derivatives, the plants held 0.5% of the applied fenitrothion and negligible amounts were held by the total algae and particulate matter. A total recovery of 83.2% of the pesticide or derivatives was obtained. These calculations emphasised the importance of the sediment as the main reservoir of the pesticide but also showed the sediment (bacteria) to be 3 times more active in pesticide degradation than the photochemical degradation observed in the water. In the water, 10.7% of the total

fenitrothion equivalent in the system (model 5) was in the form of fenitrothion derivatives, mostly photodecomposition products, whereas in the sediment 34.8% of the total pesticide equivalent in model 5 was in the form of fenitrothion degradation products.

Later mass balance calculations are inaccurate because of the likely extensive pesticide uptake by the poly-vinyl chloride plastic sheeting used in the field models as containers, so they cannot be considered to represent the partitioning of the pesticide in a natural aquatic system.

Algae and particulate matter sequestered a minor amount of the total pesticide in the field models, but these agents may pick up a greater percentage of the total pesticide at lower environmental concentrations (75 ng/g) and thus be a more significant reservoir of the pesticide. However, although algae showed bioaccumulation of 1,000-4,000 fold for fenitrothion, they did not show any pesticide degradation. The fate of this fraction of fenitrothion is release to the water or the sediment where degradation can occur (Weinberger et al 1977).

The plants at a density of 2.89 mg/l (1.98 gm/m) sequestered 0.5% of the total pesticide, but this plant density was low by a factor of 76 compared to Lac Bourgeois at a load of 221 mg/g (210.4 g/m), and compared to the mean density of a typical shallow (≈ 2 M) south eastern Ontario lake of 248g/m (Crowder et al 1977). Thus, in Lac Bourgeois (2 m mean depth), the natural plant load could possibly sequester up to 53% of the total pesticide. This extrapolation

tion cannot be exact because increasing absorption is not exactly proportional to increasing plant mass as shown in model 3; and absorption may differ because of the surrounding pesticide concentration. However, a comparison of bioaccumulation ratios for fenitrothion in E. canadensis in 1.0 and 10.0 µg/g solutions show similar ratios for up to the first 10 days so that it may be possible for the plants to show the same bioaccumulation ratios at the lower pesticide concentrations found in the water after an operational spray (Table 9C) at the 25 to 75 ng/g level (Eidt and Sundaram 1975). Therefore, it may not be unreasonable to speculate that aquatic plants could sequester up to one-half of the pesticide in an aquatic system. The mass balance study is incomplete because of possible losses to the plastic, to the air through volatilisation, and to the sediment, as water-soluble and bound residues were not monitored and analysis at later times was not followed. However, these results comprising the laboratory and field studies, together with the work of others, give a fairly clear picture as to the fate of fenitrothion in the aquatic system. Several intriguing problems remain and these include determining whether any secondary effects to the flora and fauna may occur in view of some of the information uncovered.

Secondary Effects in the Aquatic System

The aquatic system is a non-target area for the pesticide, so that incident spray must be viewed as an unwanted pollutant.

The primary observed effect of the spraying of an organophosphorus pesticide in an aquatic system is an immediate and/or

delayed kill of aquatic invertebrates and zooplankton (Flannagan 1973; Hurlbert 1975; Symons 1977). The spray effect is highly variable and may range from no effect to 80% to 90% kill with a median reduction of 2% to 5% at 140 to 210 g/ha and 10% kill at 280 g/ha dose. Recovery rates for populations of benthic insects ranged from 20 to 35 days at sprays of 140 to 210 g/ha (Flannagan 1973, Symons 1977). More importantly, there is a delayed kill effect of 2 to 3 days (Flannagan 1973) or 10 days post-spray in which a second decline in insect numbers is noted. (Symons 1977). This may be caused by the maturation of eggs to the susceptible larval stages while the concentration of fenitrothion or toxic degradation products is still high in the water. Given that the mean level of toxicity (LC_{50} 24 hrs) of fenitrothion to aquatic insects is 60 ng/g (ppb) (Wildish and Phillips 1972) and that environmental fenitrothion concentrations in water can briefly exceed this (75 ng/g, Eidt and Sundaram 1975), then a significant kill may be noted in the water. However, this effect may be brief because the pesticide disappears from the water quickly. If the sediment absorbs 30% to 50% of the pesticide in less than 2 days as shown in model 5 and this pesticide is likely to be in the top few millimeters of the sediment, then the concentration of the pesticide in this part of the sediment could be much higher than in any other part of the aquatic system except the plants. Therefore, this may explain the long-term and delayed insect kill which likely occurs in this top part of the sediment which contains a high concentration of stream invertebrates in many stages of growth. Thus the invertebrate kill in the sediment could last beyond the 1 to 3 day delayed kill in

the water because of increased persistence and concentration of fenitrothion in the sediment. However, this depends on the bioavailability of the pesticide.

Although benthic insect populations show resilience in returning to previous population levels quite quickly, the long-term effects of yearly spraying on benthic insect populations and pesticide-sediment-insect interactions should be investigated. The concentration, accumulation, persistence and toxicity of the fenitrothion degradation products may pose a hazard to invertebrates in the sediment.

Organophosphorus sprays, such as parathion and Dursban have caused significant zooplankton kill in several lakes resulting in a 'bloom' growth of green or blue-green algae, presumably caused by the removal of the predatory grazing on the phytoplankton by the zooplankton (Hurlbert 1975, 1970). The addition of Dursban at 56.0 g/ha to freshwater ponds, reduced crustacean zooplankton populations and caused a bloom of Anabaena sp., a blue-green algae, (Hurlbert et al 1970) and similar results were shown for parathion (Cook and Connors 1963). In the field model (5), a bloom of blue-green algae was observed in the sprayed containers but not in the control containers, indicating that a similar effect occurred with the use of fenitrothion in an aquatic system (Weinberger et al 1977). Given that the 24 hour LC_{50} for crustacean such as Daphnia pulex sp. is 0.045 µg/g fenitrothion in water (Symons, 1977) and that concentrations of fenitrothion in the field model ranged from 12.2 µg/g from one-half hour to 14 days respectively, then it is probable

that many zooplankton were killed and this allowed an algal bloom to occur. However, zooplankton populations quickly recover and natural phytoplankton and zooplankton populations return to natural levels.

Organophosphorus pesticides can have a sub-lethal effect on algal photosynthesis. Five out of 13 organophosphorus pesticides tested caused reductions in photosynthesis of greater than 10% in some algal species subjected to 1.0 µg/g pesticide concentrations in water (Butler 1963). This would be a minor, transitory effect on natural algal populations after a fenitrothion spray because of the low environmental concentrations used in the field and its low persistence in field waters.

However, as mentioned previously, the Aerotex used in the fenitrothion spray formulation, may be more toxic than the fenitrothion itself, causing mortality and/or inhibition of Scenedesmus sp., Chlamydomonas sp., and Chlorella sp. of 36%, 27% and 85% respectively at a concentration of 1.0 µg/g. At expected environmental concentrations of Aerotex, about 2.8 ng/g in the top 1.0 cm of water immediately after spray, Aerotex would not be expected to have a definite effect.

Another secondary effect of spraying may be the possibility of sub-lethal effects of the pesticide and derivatives on plant productivity even at environmental concentrations. At high enough levels of bioaccumulation in plants (up to 74,000 µg/g), even the most harmless chemical can be toxic.

As mentioned previously, the 3-methyl-4-nitrophenol deri-

vative found in the plants (up to 58.4 µg/g) may reduce photosynthesis in a way similar to 3-trifluoromethyl-4-nitrophenol (TFM) as shown by Maki and Johnson (1977). TFM at concentrations of 6.0 to 7.0 µg/g reduced the growth of E. canadensis by 10% (Maki and Johnson 1977).

Another relationship to be considered is pesticide transfer between plant and sediment. Myriophyllum exalbescens roots took up ^{32}P , translocated it through the tissues, and released it to the surrounding waters (De Marte and Hartman 1974). The reverse transfer of ^{32}P and labelled herbicide from the water through the plant and to the sediment was also shown for Myriophyllum brasiliense (Sutton and Bingham 1969). If this process occurred with fenitrothion, there could possibly be a delayed transfer of pesticide or derivatives to the soil or back to the water which could cause a delayed toxic effect on the invertebrates associated with sediment or plant. Perhaps this relationship is part of the reason for the delayed mortality of 3 to 10 days observed in benthic insects following a spray operation (Flannagan 1973, Symons 1977).

The non-target effects of fenitrothion spray on the aquatic system may be direct, and indirect. They may be manifested as transitory changes in phytoplankton, zooplankton, and benthic insects, a delayed mortality in benthic insects (likely caused by pesticide persistence in aquatic plants and sediment), and the possibility of reduced productivity of algae and plants by components of the pesticide formulation or certain pesticide derivatives.

Summary and Conclusions

The uptake of formulated fenitrothion and the presence of several degradation products was studied in several species of submerged macrophytes and a sediment in several model systems. Fenitrothion appears to partition mainly into the sediment and lesser amounts into the aquatic plants.

The uptake of 30% to 50% of the fenitrothion in the system into the sediment in the field, and the high degree of pesticide degradation to aminofenitrothion, S-methyl fenitrothion, and fenitro-oxon shows that the sediment is a macro-reservoir for the pesticide in an aquatic system and is a major focus of pesticide degradation.

The uptake of up to 29% to 45% of the pesticide by plants in an appropriate plant-water system, the high bioaccumulation of pesticide, and the occasional presence of significant pesticide degradation, show that the plants are also a significant macro-reservoir of pesticide in aquatic systems. Plants may also contribute directly or indirectly to the production of water-soluble pesticide derivatives in the water.

The chemical nature of bound residues in the sediment and plants must be determined as they may be re-released into the aquatic system by biological action to exert a delayed toxic effect in an unknown time frame. This study shows that fenitrothion and degradation products are more persistent in an aquatic system than previous field studies have indicated.

Suggestions for further research

The fate of fenitrothion in an aquatic system is not fully known but a broad outline has been developed. Several intriguing areas of ignorance remain in the pesticide interactions with sediment, particulate matter, plants, invertebrates, algae, combinations of these components and the sub-lethal effects of the pesticide on some of these components.

The sediment is the likely end-point for most of the pesticide residue but the extent and nature of water-soluble and bound residues is largely unknown. There remains a need to know the nature of the residue and its mode of binding in order to estimate the hazard of re-release of toxic residues into the aquatic environment.

Related to this is the problem of pesticide/sediment/plant interrelations where some evidence shows that aquatic plants could release bound residues from the sediment and re-release them to the water by translocation through the plant tissues, thereby posing another potential hazard to the system.

At the lower environmental concentrations of pesticide residue, the particulate matter and algae could be more significant as a reservoir for these compounds. It has been suggested that this component is responsible for the long-term and low-level persistence of pesticides in natural waters. The absorption and desorption characteristics of the pesticide on particulate matter should be investigated as this process may

increase pesticide persistence in the aquatic environment.

Reports that certain phytoplankton and zooplankton release extra-cellular enzymes that hydrolyse phosphate esters suggest that these enzymes could also hydrolyse pesticide residues. If this process did occur, what proportion of pesticide hydrolysis in water would be caused by photochemical reactions or biotic reactions? Pesticides could be designed such that they would be very susceptible to biotic hydrolysis in the water so that pesticide persistence would be minimal in the aquatic system.

Aquatic plants are a foci of not only pesticide absorption and degradation but also of microinvertebrate activity. What potential hazards are there to aquatic invertebrates living and grazing on aquatic plants possessing high concentrations of pesticide residue? What is the extent of pesticide desorption from aquatic plants to the surrounding water and to the plant surface where invertebrates may be in contact? These questions remain to be answered.

The sub-lethal effects of pesticide and adjuvants used in the pesticide formulation on algal and plant productivity may be significant and warrants further study. These items for further study do affect the persistence of the pesticide, but pesticide levels are low, possible release from the different compartments is slow, so that the possible hazards they present are likely minimal. In conclusion it may be said that fenitrothion at present optional spray levels, offers little long-term persistence in the aquatic system.

References

- Ahmed, M.K. and J.E. Casida, 1958. "Metabolism of Some Organophosphorus insecticides by micro-organisms". J. Econ. Entomol. 51 (1): 59-63.
- Aiken, S. and J.M. Gillett. 1974. "The Distribution of Aquatic Plants in Selected Lakes of Gatineau Park, Quebec". Can. Fld. Naturalist 88: 437-488.
- Anderson, B., J.P.E., E.P. Lichtenstein and W.F. Whittingham, 1970. "The Effect of Mucor alternans on the persistence of DDT and dieldrin in culture and in soil". J. Econ. Entomol. 63(5): 1595-1599.
- Bailey G.W. and J.L. White, 1970. "Factors influencing the adsorption, desorption and movement of pesticides in soil". Res. Rev. 32: 29-92.
- Bartha, R. 1971. "Fate of Herbicide Derived Chloroanilines in Soil". J. Agr. Food. Chem. 19(2): 385-387.
- Bartsch, E. 1974. "Diazinon: Residues in plants, soil and water". Res. Reviews 51: 37-68.
- Berry, C.R. Jr., C.B. Schreck, S.L. Van Horn 1975. "Aquatic macro-invertebrate Response to Field Applications of the Combined Herbicides Diquat and Endothal". Bull. Env. Contam. and Toxicol. 14(3): 374-379.
- Bingham, S.W. and R.L. Shaver, 1977. "Diphenimid Removal from Water and Metabolism by Aquatic Plants. "Pest. Biochem. and Physiol. 7: 8-15.
- Boush, G.M. and F. Matsumura. 1975. "Pesticide degradation by marine algae" Nat'l. Tech. Infor. Serv. AD-A008, 275, 23 p. 1975.

- Bourne, S. 1978. "Preservation of Organophosphorous Pesticides in Water Samples". J. Env. Sci. Health B13(2): 75-86.
- Bowman, J.S. and J.E. Casida. 1957. "Metabolism of the Systemic Insecticide O,O, Diethyl-S-Ethylthiomethyl Phosphoridithioate (Thimet) in Plants". J. Agr. Food Chem. 5(3): 192-197.
- Brewer, D.G., G. Wood and J. Unger 1974. "The Photodecomposition of Fenitrothion". Chemosphere 3: 91-95.
- Butcher, J.E., M.G. Boyer, C.D. Fowle. 1977. "Some Changes in Pond Chemistry and Photosynthetic Activity following treatment with Increasing Concentrations of Chlorpyrifos". Bull. Env. Contam. and Toxicol. 17(6): 752-758.
- Carter, N.E. and N.R. Brown. 1975. "Seasonal Abundance of Certain Soil Arthropods in a Fenitrothion - Treated Red Spruce Stand". Can. Entomol. 105: 1065-1073.
- Ching-Chieh-Yu and J.R. Sanborn. 1975. "The Fate of Parathion in a Model Ecosystem". Bull. Env. Contam. and Toxicol. 13(5): 543-550.
- Cook, S.F. Jr. and J.D. Connors, 1963. "Short-term side effects of the insecticidal treatment of Clear Lake, Lake County, California". J. Econ. Entomol. 56: 819.
- Crowder, A.A., J.M. Bristow and M.R. King 1977. "Distribution, Seasonality and Biomass of Aquatic Macrophytes in Lake Opinicon (Eastern Ontario)". Le Naturaliste Canadien 104: 441-452.
- Crowder, A.A., J.M. Bristow, M.R. King and S. Vanderkloet. 1977. "Aquatic Macrophytes of Some Lakes in Southeastern Ontario". Le naturaliste Canadien 104: 457-464.
- Daughton, C.G. and D.B.H. Hsieh. 1977. "Accelerated parathion

- degradation in soil by inoculation with parathion-utilizing bacteria". Bull. Env. Contam. Toxicol. 18(1): 48-56.
- De Martre, J.A. and R.T. Hartman. 1974. "Studies on Absorption of ^{32}P , ^{59}Fe and ^{45}Ca by Water-milfoil (Myriophyllum exalbescens. Fernald) Ecology 55: 188-194.
- Edwards, C.A. and A.R. Thompson 1973. "Pesticides and the soil fauna". Res. Rev. 45: 1-79.
- Eichelberger, J.W. and J.J. Lichtenberg 1971. "Persistence of Pesticides in River Water" Env. Sci. Technol. 5: 541-544.
- Eidt, D.C. and K.M.S. Sundaram, 1975. "The insecticide fenitrothion in headwaters streams from large-scale forest spraying". Can. Entomol. 107: 735-742.
- Eto, M. 1974. "Organophosphorous pesticides: organic and biological chemistry" CRC Press; Cleveland, Ohio.
- Fassett, N.C. 1967. "A manual of Aquatic Plants". University of Wisconsin Press, Madison Wisconsin.
- Flannagan, J.F. 1973. "Field and Laboratory Studies of the Effect of Exposure to Fenitrothion on Freshwater Aquatic Invertebrates. "Man. Entomol. 7: 15-25.
- Flashinski, S.J. and E.P. Lichtenstein, 1974. "Degradation of Dyfonate in soil inoculated with Rhizopus arrhizus". Can. J. Microbiol. 20: 871-875.
- Forbes, M.A., B.P. Wilson, R. Greenhalgh and W.P. Cochrane, 1975. "Confirmation of Organophosphorous Insecticides by Chemical Reduction". Bull Env. Contam. and Toxicol. 13(2): 141-148.
- Fuhremann, T.W. and E.P. Lichtenstein. 1978. "Release of Soil-Bound methyl (^{14}C) parathion residues and their uptake by earthworms

- and oat plants". J. Agr. Food. Chem. 26(3): 605-610.
- Gerstl, Z. and B. Yaron 1978. "Adsorption and desorption of parathion by attapulgite as affected by the mineral structure". J. Agr. Food Chem. 26(3): 569-573 (1978).
- Graetz, D.A., G. Chesters, T.L. Daniel, E.W. Newland and G.B. Lee. 1970. "Parathion Degradation in Lake Sediments". Jour. Water Poll. Con. Fed. 1176-1194.
- Greenhalgh, R. and W.P. Marshall 1976. "Ultraviolet Irradiation of Fenitrothion and the Synthesis of the Photolytic Oxidation Products". Jour. Agr. Food Chem. 24(4): 708-713.
- Greenhalgh, R., W.D. Marshall and J. Kovacicova 1975. "Determination of S-Methyl Isomer in Technical Grade Fenitrothion by Gas Chromatography and High School Speed Liquid Chromatography". Bull Env. Contam. and Toxicol. 13(3): 291-296.
- Gunner, H.B., B.M. Zukerman, 1968. "Degradation of Diazinon by synergistic microbial action". Nature 217: 1183-1184.
- Hallett, P., R. Greenhalgh, P. Weinberger and R. Prasad. 1975a. "The Absorption of Fenitrothion during germination of stratified and non-stratified white pine seeds and identification of metabolites formed". Can. Jour. For. Res. 5: 84-89.
- _____ 1975b. "Comparative Uptake and Metabolism of Fenitrothion by 3 species of Tree Seedlings and One Species of a Rodent". Chem. Cont. Res. Inst. CC-X-107, Ottawa.
- Harris, C.R., and E.P. Lichtenstein, 1961. "Factors Affecting the Volatilization of Insecticidal Residues from Soils". J. Econ. Entomol. 54(5): 1038-1043.
- Heuer, B., Y. Birk and B. Yaron 1976. "The Effect of Phosphatases on

- the Persistence of Organophosphorous Insecticides in Soil and Water". J. Agr. Food Chem. 24(3): 611-614.
- Hosokawa, J. and J. Miyamoto. 1974. "Metabolism of ^{14}C -labelled Sumithion, O,O-dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate in apples" Botyu-Kagaku 39: 49-54.
- Hsu, T.S. and R. Bartha 1976. Hydrolyzable and Non-Hydrolyzable 3,4 Dichloroaniline-Humus Complexes and their Respective Rates of Biodegradation". J. Agr. Food Chem. 24: 118-122.
- Hurlbert, S.H. 1975. "Secondary Effects of Pesticides in an Aquatic Ecosystem". Res. Reviews. 57: 81-148.
- Hurlbert, S.H. 1970. "Biological Effects and Pesticides of Dursban in Freshwater Ponds". Jour. Econ. Entomol. 63 (1): 43-
- Ivie, G.W. and J.E. Casida 1971. "Sensitized Photodecomposition and Photosensitizer Activity of Pesticide Chemicals Exposed to Sunlight on Silica Gel Chromatoplates". J. Agr. Food Chem. 19(3): 405-409.
- Iwata, Y., W.E. Westlake, and F.A. Gunther 1973. "Persistence of parathion in 6 California soils under laboratory conditions". Arch. Env. Contam. and Toxicol. 1: 84-96.
- Joiner, R.L. and K.P. Baetcke 1974. "Identification of the Photoalteration Products Formed from Parathion by Ultraviolet Light". Jour. of Assoc. of Anal. Chem. 57(2): 408-415.
- Jansson, M. 1976. "Phosphatases in lake water. Characterization of enzymes from phytoplankton and zooplankton by gel filtration". Science 194 (4262): 320-321.
- Kanazawa, J., A.R. Isensee and P.C. Kearney, 1975. "Distribution of carbaryl and 3-5-xylyl-methylcarbamate in an aquatic model

- ecosystem". J. Agr. Food. Chem. 23(4): 760-763.
- Katan, J., T.W. Fuhremann and E.P. Lichtenstein 1976. "Binding of ¹⁴C-Parathion in Soil: A reassessment of Pesticide Persistence". Science 193: 891-894.
- Khan, M.A.Q. 1977. "Pesticides in aquatic environments". Plenum Press, New York, from Proceedings of a Symposium for the International Congress of Entomology". Washington, D.C. Aug. 22, 1976.
- Kingsbury, P.D. 1977. Fenitrothion in a lake ecosystem". Chem. Cont. Res. Inst. Ottawa Inf. Rep. CC-X-146.
- Kliger, L. and B. Yaron 1975. "Parathion Recovery from Soils After a Short Contact Period". Bull. Env. Contam. Toxicol. 13(6): 714-719.
- Kovacicova, J., V. Batora and S. Truchlik 1973. "Hydrolysis rate and in vitro anticholinesterase activity of fenitrothion and S-methyl-fenitrothion". Pest. Sci. 4: 759-763.
- Leuck, D.B. and M.C. Bowman 1969. "Persistence of O,O-Dimethyl O,4-nitro-M-tolyl phosphorothioate, Its Oxygen Analogue and Its Cresol in Corn and Grass Forage". J. Econ. Entomol. 62(6): 1282-1285.
- Lewis, D.L., D.F. Paris, and G.L. Baughman 1975. "Transformation of Malathion by a Fungus, Aspergillus oryzae, isolated from a Freshwater Pond". Bull. Env. Contam. and Toxicol. 13(5): 596-600.
- Lichtenstein, E.P. and K.R. Schulz 1970. "Volatilisation of Insecticides from Various Substrates". J. Agr. Food Chem. 18(5): 814-818.
- Lichtenstein, E.P. and K.R. Schulz 1964. "The Effects of Moisture and Micro-organisms on the Persistence and Metabolism of Some Organophosphorous Insecticides in soils, with special emphasis

on Parathion". J. Econ. Entomol. 57: 618-627.

Maki, A.W. and H.E. Johnson 1977. "The influence of Larval Lampricide (TFM; 3 Trifluoromethyl-4-nitrophenol) on Growth and Production of Two Species of Aquatic Macrophytes; Elodea canadensis (Michx.) Planchon and Myriophyllum spicatum L. Bull. Env. Contam. and Toxicol. 17: 57-63.

Marshall, W.K. and J.R. Roberts 1977. "Simulation Modelling of the Distribution of Pesticides in Ponds". in "Proceedings of a Symposium on Fenitrothion: The Long-Term Effects of Its Use in Forest Ecosystems". Ottawa, April 20-22, 1977. Ed. J.R. Roberts R. Greenhalgh and W.K. Marshall. NRCC No. 16073.

Mayes, R.A. and A.W. McIntosh 1977. "Uptake of Cadmium and Lead by a rooted Aquatic Macrophyte (Elodea canadensis)". Ecology 58: 1176-1180.

McNeil, J.N. and J.M. McLeod. 1977. "Apparent Impact of Fenitrothion on the Swaine Jack-Pine Sawfly, Neodiprion Swainei, Midd." in "Proceedings of a Symposium on Fenitrothion: The Long-Term Effects of Its Use in Forest Ecosystems". Ed. J.R. Roberts, R. Greenhalgh and W.K. Marshall. Ottawa April 20-22, 1977. NRCC No. 16073.

McRoy, C.P. and R.J. Barsdate, 1970. "Phosphate Absorption in Eelgrass". Limnol and Oceanog. 15: 6-13 (1970).

McTaggart-Cowan, P.D., F.W.H. Beamish, D.J. Clegg, G.S. Cooper, D.J. Obichon, R. Greenhalgh, J.M. McLeod, D.B. Peakall and J.R. Roberts. 1977. "Panel Report". In "Proceedings of a Symposium on Fenitrothion: The Long-Term Effects of Its Use in Forest Ecosystems". Ottawa, Apr. 20-22, 1977, NRCC No. 16073. Ed.

- J.R. Roberts, R. Greenhalgh and W.K. Marshall. NRCC No. 16073.
- Mendoza, C.E., P.J. Wales, H.A. McLeod, and W.P. McKinley 1968b.
- "Thin-layer Chromatographic - Enzyme Inhibition Procedure to Screen for Organophosphorous Pesticides in Plant Extracts without Elaborate Clean-up". Analyst 93: 173-177.
- Mendoza, C.E., P.J. Wales, H.A. McLeod, and W.P. McKinley 1968a.
- "Enzymatic Detection of Ten Organophosphorous Pesticides and Carbaryl on Thin-Layer Chromatograms: An Evaluation of Indoxyl, Substituted Indoxyl and 1-Naphthyl Acetates as Substrates of Esterases, Analyst 90: 34-38.
- Mikami, N., H. Ohkawa, and J. Miyamoto 1976. "Photodecomposition of Sumithion". Res. Dept. Pesticides Div., Sumitomo Chem. Co. Ltd.
- Miyamoto, J. 1977 "Degradation of Fenitrothion in Terrestrial and Aquatic Environments Including Photolytic and Microbial Reactions". in: Proceedings of a Symposium on Fenitrothion: The Long-Term Effects of Its Use in Forest Ecosystems". Ed. J.R. Roberts, R. Greenhalgh and W.K. Marshall. Ottawa April 20-22, 1977 NRCC No. 16073.
- Miyamoto, J., K. Kitagawa, and Y. Sato 1966. "Metabolism of Organophosphorous Insecticides by Bacillus subtilis, with Special Emphasis on Sumithion". Japan J. Exp. Med. 36(2): 211-225.
- Miyamoto, J. and Y. Sato. 1965. "Determination of Insecticide Residue in Animal and Plant Tissues. II. Metabolic Fate of Sumithion in Rice Plant Applied at the Preheading Stage and its Residue in Harvested Grains". Botyu-Kagaku: 30: 45-49.
- Miyamoto, J. and Y. Kawaguchi and Y. Sato. 1965. "Determination of Insecticidal Residue in Animal and Plant Tissues. I. Deter-

mination of Sumithion Residue in Bananas grown in Formosa.

"Botyu-Kagaku 30: 9-12.

Moody, R.P., R. Greenhalgh, L. Lockhart and P. Weinberger 1978.

"The Fate of Fenitrothion in an Aquatic Ecosystem". Bull.

Env. Contam. & Toxicol. 19(8): 8-14.

Moody, R.P., R. Prasad, R. Greenhalgh and P. Weinberger 1972a.

"Fate of Fenitrothion in Forest Trees. VI. Some Factors

Affecting Rate of Dissipation from Balsam Fir and White

Spruce". in "Pesticide Management" Ed. Academic Press,

1977.

Mortimer, D.C. and A. Kudo. 1975. "Interaction between aquatic plants

and bed sediments in mercury uptake from flowing water". J.

Env. Qual. 4: 491-495.

Munnecke, D.M. 1978. "Detoxification of pesticides using soluble or

immobilized enzymes". Process. Biochem. 13(2): 14-16.

Munnecke, D.M. 1976a. "Enzymatic hydrolysis of organophosphoate insecticides, a possible pesticide disposal method". Appl. Env.

Microbiol. 32: 7-13.

Munnecke, D.M. and D.P.H. Hsieh, 1974. "Microbial Decontamination of

Parathion and p-Nitrophenol in Aqueous Media". Appl. Microbiol.

28(2): 212-217 (1974).

Ohkawa, H., No. Mikami and J. Miyamoto 1974. "Photodecomposition of

Sumithion (O, O-dimethyl-O-(-3-methyl-4-nitrophenyl)-Phosphorothioate). Agr. Biol. Chem. 38 (11): 2247-2255 (1974).

Paris, D.F., D.L. Lewis and N.L. Wolfe, 1975. "Rates of Degradation

of Malathion by Bacteria Isolated from Aquatic Systems". Env.

Sci. and Technol. 9(2): 135-138.

- Poinke, H.B. and G. Chesters 1973. "Pesticide, Sediment-Water Interactions". J. Env. Qual. 2 (1) 29-45.
- Ponnamperuma, F.N. 1972. "The Chemistry of Submerged Soils". Adv. in Agron. 24: 29-96.
- Rains, D.W. 1968. "Kinetics and energetics of light enhanced potassium Absorption by corn leaf tissue". Plant Physiol. 43: 394-400.
- Ramamoorthy, S., S. Springthorpe and D.J. Kshner 1977. "Competition for Mercury Between River Sediment and Bacteria". Bull. Env. Contam. & Toxicol. 17 (5): 505-511.
- Rauchaud, J., J.R. Decallone, and J.A. Meyer 1978. "The nature of bound residues derived from triforine in barley plants". Pest. Sci. 9(1): 74-78.
- Roberts, J.R., R. Greenhalgh, W.K. Marshall 1977. "Proceedings of a Symposium on Fenitrothion: The Long-Term Effects of Its Use in Forest Ecosystems". Ottawa, April 20-22, 1977 NRCC No. 16073.
- Rorke, M.A., D.R. Gardner and R. Greenhalgh 1974. "Lethality and behavioural symptoms produced by some organophosphorous compounds in the snail (Helix aspera). Bull. Env. Contam. and Toxicol. 11: 417-424.
- Rosival L., M. Vargova, J. Szokoloyova and K. Cerey 1976. "Contribution to the toxic action of S-methyl Fenitrothion". Pest. Biochem. and Physiol. 6: 280-286.
- Ruzuicka, J.H.; J. Thompson and B.B. Wheald 1967. "The gas chromatographic determination of organophosphorous pesticides. Part II. A comparative study of hydrolysis rates". J. Chromatog. 31: 37.
- Saltzman, S., V. Mingelgrin, and B. Yaron. 1976. "Role of water in the hydrolysis of parathion and methylparathion on kaolinite". J. Agr.

Food Chem. 24(4): 739-743.

Sanborn, J.R. and Ching-Chieh Yu 1973. "The Fate of Dieldrin in a Model Ecosystem". Bull. Env. Contam. and Toxicol. 13(5): 543-550.

Sculthorpe, C.D. 1967. "The Biology of Aquatic Vascular Plants". Edward Arnold Pub. Ltd. London, England.

Seaman, D.E. and T.M. Thomas 1966. "Absorption of Herbicides by Submersed Aquatic Plants". In Proc. 18th Ann. Calif. Weed. Conf. San Jose, Calif. Jan. 18-20, 1966.

Sethunathan, N., R. Siddaramappa, K.P. Rajaram, S. Barik and P.A. Wakid 1977. "Parathion: Residues in soil and water". Res. Rev. 68: 91-122.

Sethunathan, N. and T. Yoshida 1973a. "Parathion degradation in submerged rice soils in the Philippines". J. Agr. Food Chem. 21: 504-506.

Sethunathan, N. and T. Yoshida 1973b. "A Flavobacterium sp. that degrades diazinon and parathion". Can. J. Microbiol.: 19: 873-875.

Siddaramappa, R., K.P. Rajaram and N. Sethunathan 1973. "Degradation of parathion by bacteria isolated from flooded soil". Appl. Microbiol. 26: 846-849.

Simpson, J.L. and E.C. Evans 1953. "The metabolism of nitrophenols by certain bacteria. Biochem. J. 52: XXIV.

Small, E. and J.D. Gaynor 1975. "Comparative Concentrations of 12 Elements in Substrates and Leaves of Scirpus validus and other Aquatic Plant Species in a Sewage Lagoon and in Unpolluted Habitats. Can. Field Nat. 89: 41-45.

Stewart, D.K.R., A.A. MacLean and E.R. Kimball 1976. "Insecticide

Residues in Bottom Sediments of Two New Brunswick Streams Draining Agricultural Land". Can. J. Soil Sci. 57: 15-20.

Stewart, D.K. and D. Chisolm 1971. "Long-term persistence of BHC, DDT and Chlordane in sandy-loam soil". Can. Jour. Soil. Sci. 51: 379-383.

Stewart, D.K.R., D. Chisolm and M.T.H. Ragab 1971. "Long-Term persistence of parathion in soil. Nature 229: 47.

St. John, H., 1965. "Monograph of the genus Elodea IV. The species of eastern and central North America and Summary!" Rhodora 67: 1-35.

Sundaram, K.M.S. 1974. "Distribution and Persistence of Fenitrothion Residues in Foliage, Soil and Water in Larose Forest". Chem. Con. Res. Inst. Ottawa Info Rep.. CC-X-64 April 1974. Env. Canada Forestry Service.

Sutton, D.L. and S.W. Bingham 1970. "Absorption and translocation of Simazine in parrotfeather". Weed Sci. 17: 431-435.

Sutton, D.L., D.A. Durham, S.W. Bingham and C.L. Foy. 1969. "Influence of Simazine on apparent photosynthesis of aquatic plants and herbicide residue removal from water". Weed Sci. 17: 56-59.

Suzuki, T. and M. Uchiyama 1975a "Pathways of nitroreduction of parathion by spinach homogenate". J. Agr. Food Chem. 23: 281-286.

Suzuki, T. and M. Uchiyama 1975b. "Inhibitory Effect of Parathion on the Photosynthetic Electron Transport System in Isolated Spinach Chloroplasts. Bull. Env. Contam. and Toxicol. 14(5): 552-557.

Symons, P.E.K. 1977 "Dispersal and toxicology of the insecticide fenitrothion: Predicting hazards of forest spraying". Bull. Env. Contam. and Toxicol. 68: 1-36.

Takimoto, Y., M. Hirota, H. Inui and J. Miyamoto 1976. "Decomposition and Leaching of Radioactive Sumithion in 4 Different Soils under

- Laboratory Conditions". J. Pest. Sci. 1: 131-143.
- Walter-Echols, G. 1978. "Fate of the insecticide phorate in an aquatic environment". Diss. Abst. Int. B38 (11): 5178. Univ. of Wisconsin, Madison, Wisconsin.
- Walter-Echols, G. and E.P. Lichtenstein 1978. "Movement and metabolism of ^{14}C -phorate in a flooded soil system". J. Agr. Food Chem. 26(3): 599-604.
- Watts, R.R., R.W. Storherr, J.R. Pardue and T. Osgood 1969. "Charcoal Column Clean-Up Method for Many Organophosphorous Pesticide Residues in Crop Extracts". Jour. Assoc. of Anal. Chem. 52(3): 522-536.
- Weinberger, P., R. Greenhalgh, R. Moody and B. Boulton, 1977. "Partitioning of fenitrothion in aquatic ecosystems". Report to N.R.C.
- Weinberger, P., L. Pomber, and R. Prasad 1978a. "The responses of White Spruce and Yellow Birch to Some Selected Organophosphorous Insecticides and the Adjuvants Atlox and Aeropex". Jour. Exp. Bot. 29(109): 497-487.
- _____ 1978b. "Phytotoxicity of fenitrothion to white pine seeds and seedlings". Can. Jour. For. Res. 8(2): 155-161.
- _____ 1978c. "Some toxic effects of fenitrothion on seed germination and early seedling growth of jack pine, spruce and birches". Can. Jour. For. Res. 8(2): 243-246.
- Wetzel, R.G. 1975. "Limnology" W.B. Saunders Co. Philadelphia, P.A.
- Wetzel, R.G. and B.A. Manny 1972b. "Secretion of dissolved organic carbon and nitrogen by aquatic macrophytes". Verh. Int. Ver. Limnol. 18: 162-170.

- Wetzel, R.G. and D.G. McGregor 1968. "Axenic culture and nutritional studies of aquatic macrophytes". Amer. Midland Natur. 80: 52-64.
- Wildish, D.J. and R.L. Phillips 1972. "Acute lethality of Fenitrothion to Freshwater Aquatic Insects". Fish. Res. Board Can. MS Rep. 1210 (10 pages).
- Yamaguchi, S. and A.S. Crafts 1958. "Autoradiographic Method for Studying absorption and translocation of Herbicides using ¹⁴C-labelled Compounds". Hilgardia 6(28): 161-191.
- Yaron, B. 1975. "Chemical Conversion of Parathion on Soil Surfaces". Soil Sci. Soc. of Amer. Proc. 39(4): 639-643.
- Yaron, B. and S. Saltzman 1978. "Soil-parathion interactions". Res. Rev. 69: 1-34.
- Yasuno, M., S. Hirakoso, M. Sasa and M. Vehida 1965. "Inactivation of Some organophosphorous Insecticides by Bacteria in Polluted Water". Japan J. Exp. Med. 35: 545-563.
- Yule, W.N. and J.R. Duffy, 1972. "The Persistence and Fate of Fenitrothion Insecticide in a Forest Environment". Bull. Env. Contam. and Toxicology 8(1): 10-17.
- Zitko, V., D.W. McLeese, W.C. Carson and H.E. Welch 1976. "Toxicity of alkyldinitrophenols to some aquatic organisms". Bull. Env. Contam. and Toxicol. 16(5): 508-515.
- Zitko, V. and T.D. Cunningham 1975. "Fish Toxicity of S-Methyl Fenitrothion". Bull. Env. Contam. and Toxicol. 14(1): 19-24.
- Zitko, V. and T.D. Cunningham 1974. "Fenitrothion, Derivatives and Isomers: Hydrolysis, Adsorption and Biodegradation". Fisheries and Marine Service Tech. Rep. 458, Env. Can.

Addendum

For brevity, pesticide concentrations are stated as 'ug/g (name of derivative)' and not as 'ug(derivative) per gram of substrate'. The identity of the substrate is mentioned in the same statement in which the concentration is given.