# THE DISSIPATION OF CHLORPYRIFOS AND CYPERMETHRIN AND THEIR METABOLITES IN ELM BARK, LITTER AND SOIL AND THEIR PERSISTENCE IN THE CONTROL OF THE NATIVE ELM BARK BEETLE

BY

HENG JIN

A Thesis

Submitted to the Faculty of Graduate Studies
in Partial Fulfillment of the Requirements
for the Degree of

DOCTOR OF PHILOSOPHY

Department of Soil Science
University of Manitoba
Winnipeg, Manitoba

ISBN 0-612-16171-4

Name _____

*Dissertation Abstracts International* and *Masters Abstracts International* are arranged by broad, general subject categories. Please select the one subject which most nearly describes the content of your dissertation or thesis. Enter the corresponding four-digit code in the spaces provided.

SUBJECT TERM

Environmental Sciences

0768

SUBJECT CODE

UMI

## Subject Categories

# THE HUMANITIES AND SOCIAL SCIENCES

### COMMUNICATIONS AND THE ARTS
- Architecture ..... 0729
- Art History ..... 0377
- Cinema ..... 0900
- Dance ..... 0378
- Design and Decorative Arts ..... 0389
- Fine Arts ..... 0357
- Information Science ..... 0723
- Journalism ..... 0391
- Landscape Architecture ..... 0390
- Library Science ..... 0399
- Mass Communications ..... 0708
- Music ..... 0413
- Speech Communication ..... 0459
- Theater ..... 0465

### EDUCATION
- General ..... 0515
- Administration ..... 0514
- Adult and Continuing ..... 0516
- Agricultural ..... 0517
- Art ..... 0273
- Bilingual and Multicultural ..... 0282
- Business ..... 0688
- Community College ..... 0275
- Curriculum and Instruction ..... 0727
- Early Childhood ..... 0518
- Elementary ..... 0524
- Educational Psychology ..... 0525
- Finance ..... 0277
- Guidance and Counseling ..... 0519
- Health ..... 0680
- Higher ..... 0745
- History of ..... 0520
- Home Economics ..... 0278
- Industrial ..... 0521
- Language and Literature ..... 0279
- Mathematics ..... 0280
- Music ..... 0522
- Philosophy of ..... 0998
- Physical ..... 0523
- Reading ..... 0535
- Religious ..... 0527
- Sciences ..... 0714
- Secondary ..... 0533
- Social Sciences ..... 0534
- Sociology of ..... 0340
- Special ..... 0529
- Teacher Training ..... 0530
- Technology ..... 0710
- Tests and Measurements ..... 0288
- Vocational ..... 0747

### LANGUAGE, LITERATURE AND LINGUISTICS
- Language
  - General ..... 0679
  - Ancient ..... 0289
  - Linguistics ..... 0290
  - Modern ..... 0291
  - Rhetoric and Composition ..... 0681
- Literature
  - General ..... 0401
  - Classical ..... 0294
  - Comparative ..... 0295
  - Medieval ..... 0297
  - Modern ..... 0298
  - African ..... 0316
  - American ..... 0591
  - Asian ..... 0305
  - Canadian (English) ..... 0352
  - Canadian (French) ..... 0355
  - Caribbean ..... 0360
  - English ..... 0593
  - Germanic ..... 0311
  - Latin American ..... 0312
  - Middle Eastern ..... 0315
  - Romance ..... 0313
  - Slavic and East European ..... 0314

### PHILOSOPHY, RELIGION AND THEOLOGY
- Philosophy ..... 0422
- Religion
  - General ..... 0318
  - Biblical Studies ..... 0321
  - Clergy ..... 0319
  - History of ..... 0320
  - Philosophy of ..... 0322
- Theology ..... 0469

### SOCIAL SCIENCES
- American Studies ..... 0323
- Anthropology
  - Archaeology ..... 0324
  - Cultural ..... 0326
  - Physical ..... 0327
- Business Administration
  - General ..... 0310
  - Accounting ..... 0272
  - Banking ..... 0770
  - Management ..... 0454
  - Marketing ..... 0338
- Canadian Studies ..... 0385
- Economics
  - General ..... 0501
  - Agricultural ..... 0503
  - Commerce-Business ..... 0505
  - Finance ..... 0508
  - History ..... 0509
  - Labor ..... 0510
  - Theory ..... 0511
- Folklore ..... 0358
- Geography ..... 0366
- Gerontology ..... 0351
- History
  - General ..... 0578
  - Ancient ..... 0579
  - Medieval ..... 0581
  - Modern ..... 0582
  - Church ..... 0330
  - Black ..... 0328
  - African ..... 0331
  - Asia, Australia and Oceania ..... 0332
  - Canadian ..... 0334
  - European ..... 0335
  - Latin American ..... 0336
  - Middle Eastern ..... 0333
  - United States ..... 0337
- History of Science ..... 0585
- Law ..... 0398
- Political Science
  - General ..... 0615
  - International Law and Relations ..... 0616
  - Public Administration ..... 0617
- Recreation ..... 0814
- Social Work ..... 0452
- Sociology
  - General ..... 0626
  - Criminology and Penology ..... 0627
  - Demography ..... 0938
  - Ethnic and Racial Studies ..... 0631
  - Individual and Family Studies ..... 0628
  - Industrial and Labor Relations ..... 0629
  - Public and Social Welfare ..... 0630
  - Social Structure and Development ..... 0700
  - Theory and Methods ..... 0344
- Transportation ..... 0709
- Urban and Regional Planning ..... 0999
- Women's Studies ..... 0453

# THE SCIENCES AND ENGINEERING

### BIOLOGICAL SCIENCES
- Agriculture
  - General ..... 0473
  - Agronomy ..... 0285
  - Animal Culture and Nutrition ..... 0475
  - Animal Pathology ..... 0476
  - Fisheries and Aquaculture ..... 0792
  - Food Science and Technology ..... 0359
  - Forestry and Wildlife ..... 0478
  - Plant Culture ..... 0479
  - Plant Pathology ..... 0480
  - Range Management ..... 0777
  - Soil Science ..... 0481
  - Wood Technology ..... 0746
- Biology
  - General ..... 0306
  - Anatomy ..... 0287
  - Animal Physiology ..... 0433
  - Biostatistics ..... 0308
  - Botany ..... 0309
  - Cell ..... 0379
  - Ecology ..... 0329
  - Entomology ..... 0353
  - Genetics ..... 0369
  - Limnology ..... 0793
  - Microbiology ..... 0410
  - Molecular ..... 0307
  - Neuroscience ..... 0317
  - Oceanography ..... 0416
  - Plant Physiology ..... 0817
  - Veterinary Science ..... 0778
  - Zoology ..... 0472
- Biophysics
  - General ..... 0786
  - Medical ..... 0760

### EARTH SCIENCES
- Biogeochemistry ..... 0425
- Geochemistry ..... 0996
- Geodesy ..... 0370
- Geology ..... 0372
- Geophysics ..... 0373
- Hydrology ..... 0388
- Mineralogy ..... 0411
- Paleobotany ..... 0345
- Paleoecology ..... 0426
- Paleontology ..... 0418
- Paleozoology ..... 0985
- Palynology ..... 0427
- Physical Geography ..... 0368
- Physical Oceanography ..... 0415

### HEALTH AND ENVIRONMENTAL SCIENCES
- Environmental Sciences ..... 0768
- Health Sciences
  - General ..... 0566
  - Audiology ..... 0300
  - Dentistry ..... 0567
  - Education ..... 0350
  - Administration, Health Care ..... 0769
  - Human Development ..... 0758
  - Immunology ..... 0982
  - Medicine and Surgery ..... 0564
  - Mental Health ..... 0347
  - Nursing ..... 0569
  - Nutrition ..... 0570
  - Obstetrics and Gynecology ..... 0380
  - Occupational Health and Safety ..... 0354
  - Oncology ..... 0992
  - Ophthalmology ..... 0381
  - Pathology ..... 0571
  - Pharmacology ..... 0419
  - Pharmacy ..... 0572
  - Public Health ..... 0573
  - Radiology ..... 0574
  - Recreation ..... 0575
  - Rehabilitaion and Therapy ..... 0382
  - Speech Pathology ..... 0460
  - Toxicology ..... 0383
- Home Economics ..... 0386

### PHYSICAL SCIENCES

#### Pure Sciences
- Chemistry
  - General ..... 0485
  - Agricultural ..... 0749
  - Analytical ..... 0486
  - Biochemistry ..... 0487
  - Inorganic ..... 0488
  - Nuclear ..... 0738
  - Organic ..... 0490
  - Pharmaceutical ..... 0491
  - Physical ..... 0494
  - Polymer ..... 0495
  - Radiation ..... 0754
- Mathematics ..... 0405
- Physics
  - General ..... 0605
  - Acoustics ..... 0986
  - Astronomy and Astrophysics ..... 0606
  - Atmospheric Science ..... 0608
  - Atomic ..... 0748
  - Condensed Matter ..... 0611
  - Electricity and Magnetism ..... 0607
  - Elementary Particles and High Energy ..... 0798
  - Fluid and Plasma ..... 0759
  - Molecular ..... 0609
  - Nuclear ..... 0610
  - Optics ..... 0752
  - Radiation ..... 0756
- Statistics ..... 0463

#### Applied Sciences
- Applied Mechanics ..... 0346
- Computer Science ..... 0984
- Engineering
  - General ..... 0537
  - Aerospace ..... 0538
  - Agricultural ..... 0539
  - Automotive ..... 0540
  - Biomedical ..... 0541
  - Chemical ..... 0542
  - Civil ..... 0543
  - Electronics and Electrical ..... 0544
  - Environmental ..... 0775
  - Industrial ..... 0546
  - Marine and Ocean ..... 0547
  - Materials Science ..... 0794
  - Mechanical ..... 0548
  - Metallurgy ..... 0743
  - Mining ..... 0551
  - Nuclear ..... 0552
  - Packaging ..... 0549
  - Petroleum ..... 0765
  - Sanitary and Municipal ..... 0554
  - System Science ..... 0790
- Geotechnology ..... 0428
- Operations Research ..... 0796
- Plastics Technology ..... 0795
- Textile Technology ..... 0994

### PSYCHOLOGY
- General ..... 0621
- Behavioral ..... 0384
- Clinical ..... 0622
- Cognitive ..... 0633
- Developmental ..... 0620
- Experimental ..... 0623
- Industrial ..... 0624
- Personality ..... 0625
- Physiological ..... 0989
- Psychobiology ..... 0349
- Psychometrics ..... 0632
- Social ..... 0451

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

COPYRIGHT PERMISSION

# THE DISSIPATION OF CHLORPYRIFOS AND CYPERMETHRIN AND THEIR METABOLITES IN ELM BARK, LITTER AND SOIL AND THEIR PERSISTENCE IN THE CONTROL OF THE NATIVE ELM BARK BEETLE

BY

HENG JIN

A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

## Acknowledgements

I would like to express my heartfelt gratitude to my advisor, Dr. G. R. Barrie Webster, for his academic directions and financial assistance which are essential to accomplish the research project, and the other members of my degree committee, Dr. Neil J. Holliday, Dr. A. Richard Westwood, and Dr. David L. Burton for their time and energy spent to provide academic advices for the research project and review and provide comments to revise my thesis.

I would also like to thank Ramon Khan, Irene L. Pines, and Terry Boyce from Forestry Branch, Manitoba Natural Resources for the assistance in insecticide application and sampling; and Phil Pines from the City of Winnipeg for the acquisition of the test elm bark beetle.

At last, I would like to thank my wife, Ming Sun, for her consistent support which is essential for me to finish the thesis.

# ABSTRACT

Experiments were carried out to study the insecticidal persistence and environmental degradation of chlorpyrifos and cypermethrin used for the control of native elm bark beetles (*Hylurgopinus rufipes*), the vector of Dutch elm disease (*Ophiostoma ulmi*) in two native American elm (*Ulmus americana*) stands near Winnipeg, Manitoba in Fall, 1991 and spring, 1992. Chlorpyrifos and cypermethrin were applied to the basal 1 metre of elm trunks. Quantitative analytical methods were developed to determine the residues of chlorpyrifos, cypermethrin, and their major metabolites in elm bark, litter, and soil.

Chlorpyrifos initial residues ranging from 388 to 444 μg/g in elm bark dissipated approximately 90% at 791 days after application. Chlorpyrifos in elm bark was still 100% effective in controlling elm bark beetles 791 days after application. The initial residues of *cis*-A, *trans*-C, *cis*-B, and *trans*-D cypermethrin in the elm bark, 28.8 to 58.9, 24.2 to 53.1, 24.9 to 34.9, 17.7 to 25.2 μg/g, respectively, dissipated by approximately 89% for *cis*-isomers and 92% for *trans*-isomers at 791 days after application. Cypermethrin was 100% effective for control of elm bark beetle for approximately 60 days after application and proved in excess of 80 % control at 364 days post application. The 100% control effectiveness of cypermethrin in field may be extended up to 627 days for the fall application and 433 days for the spring application considering the fate of affected beetles. Further field testing is required to confirm cypermethrin longevity for

beetle control. These two insecticides failed to penetrate the elm bark layer into cambium and wood tissue.

Initial chlorpyrifos residues of 187 to 916 μg/g in litter dissipated approximately 99% at 791 days after application. The residues of chlorpyrifos in soil fluctuated from 0.78 to 28.2 μg/g and were 1.04 to 2.09 μg/g at 791 days after application. The $DT_{50}$ (time for 50% dissipation) of chlorpyrifos in soil fortified in the laboratory and put under field conditions ranged from 92 to 120 days. Initial residues of *cis*-A, *trans*-C, *cis*-B, and *trans*-D cypermethrin in litter were 54.2 to 141, 41.0 to 125, 47.7 to 88.3, and 32.7 to 62.9 μg/g, respectively, and dissipated to below detection limits at 341 days after application. Total cypermethrin in soil ranged from below detection limits to 10.47 μg/g during the experiment. The $DT_{50}$ of *cis*-A, *trans*-C, *cis*-B, and *trans*-D in soil fortified in the laboratory and put under field conditions ranged from 340 to 390, 290 to 320, 360 to 390, and 280 to 290 days, respectively. Higher residue ratios of metabolites to the parent compounds were found in soil than in litter and bark. Chlorpyrifos or cypermethrin residues transferred from the treated bark surface to glass fibre filter paper pressed against it for 1 minute were well below the acceptable daily intake for human subjects.

It is suggested that cypermethrin may have potential to replace or be used in conjunction with chlorpyrifos as part of a fully integrated Dutch elm disease management program. Further research needs to be done to examine the field efficacy of cypermethrin to confirm laboratory observations.

# Contents

# LIST OF TABLES

# LIST OF FIGURES

# 1. Introduction

American or white elm (*Ulmus americana*) is widely distributed in eastern and central North America, northwestward to Manitoba and Saskatchewan, Canada, and south to the Gulf of Mexico. It is hardy and adapts to drought and variable soil conditions. Elm trees in native stands are a source of hardwood and a major component of shelter belts in the prairie provinces. The pleasing aesthetic shape of the tree has made the elm an important choice in the vast majority of urban forests in hundreds of communities in this region. Dutch elm disease has destroyed most wild and urban America elms in North America. An average annual elm loss rate of 2.4 % in Manitoban communities is attributed to Dutch elm disease (Westwood, 1991).

Dutch elm disease is caused by the fungus (*Ophiostoma (Ceratocystis) ulmi*) and is mainly spread by native elm bark beetles (*Hylurgopinus rufipes*) in the prairie provinces.

The recommended control strategies for this disease include the suppression of vector populations by sanitation and insecticide sprays, soil treatments to prevent root graft transmission, protective and therapeutic treatments with systemic fungicides, intensive surveillance and preventative or eradicative pruning on healthy trees, and the planting of resistant varieties of elm. The effective management of the native bark beetle by spraying insecticide has been a

key component in the integrated control program of Dutch elm disease in the western Canada.

At present, chlorpyrifos is a registered insecticide which has been extensively used to control elm bark beetle populations in an integrated Dutch elm disease management program in the urban areas and native elm stands in Manitoba. The insecticidal persistence of chlorpyrifos in controlling elm bark beetles at the recommended application dosage and its environmental dissipation in litter and soil had not previously been investigated in Manitoba.

It was considered advisable for us to seek an alternative insecticide which was effective in control of the elm bark beetle and, at the same time, more environmentally acceptable. Pajares and Lanier (1989) reported that, under laboratory conditions, the pyrethroid cypermethrin, when applied to infested elm wood, killed all beetles before, or shortly after they emerged; the action of cypermethrin is more rapid than that of chlorpyrifos. The lower acute mammalian toxicity of cypermethrin (251-4123 mg/kg for rats) than that of chlorpyrifos (135-163 mg/kg for rats) makes cypermethrin more attractive for use in the control of the elm bark beetle in an urban setting.

The research reported in this thesis was designed and implemented for the following objectives:

1. to determine the persistence of chlorpyrifos and cypermethrin and their metabolites in elm bark taken from the treated basal 1 metre of elm trunks.

2. to evaluate the biologically effective persistence of chlorpyrifos and cypermethrin in elm bark to control elm bark beetles.

3. to correlate the level of residues measured by chemical methods with the biological effectiveness against the beetle and so provide a chemical method to predict the effectiveness of the beetle control.

4. to evaluate the suitability of cypermethrin to substitute for chlorpyrifos in the control of elm bark beetles.

5. to study the environmental behaviour of pesticides after basal application to elm trunks to control elm bark beetles, including the dissipation of chlorpyrifos and cypermethrin and their main metabolites in soil and litter under field conditions.

6. to determine the penetration of the insecticides into the bark and wood tissue where the elm bark beetle makes its overwintering tunnels.

7. to evaluate the contact safety of the treated elm bark by measuring the insecticide availability to material in contact with the treated bark surface.

# 2. Literature Review

## *2.1 Dutch elm disease*

### 2.1.1 Host

North American species of elm, such as American (*Ulmus americana*), slippery (*U. rubra*.), and winged elms (*U. alata*), are highly susceptible to Dutch elm disease, while European elms, such as Dutch (*U. hollandica*), English (*U. procera*), and Scotch elms (*U. glabra*), are moderately resistant; and Asiatic elms, such as Chinese (*U. parvifolia*) and Siberian (*U. pumila*), are highly resistant to Dutch elm disease. Some resistant hybrids have been developed that incorporate the genetic resistance of the smaller Asiatic elms with the larger size and more pleasing shade tree form of the American elm or European elm (Tatter, 1989).

### 2.1.2 History

In 1919, elm trees in the southern Netherlands were found to have the symptoms of a sudden wilting and dying of the leaves and branches. Trees which appeared normal in early summer would wilt, lose all their leaves, and die within a matter of weeks. In other trees, the leaves on some branches in the crown became yellow and fell, and by late summer these symptoms would spread over the crown with no distinct boundaries. On shoots infected by the disease during growth, the end leaves on the withered tips often stayed after the fully grown

leaves had fallen away, giving a characteristic shepherd's crook appearance. In addition, symptomatic branches showed brown discoloration or "streaking" in the outer xylem (Spierenburg, 1921, 1922).

At the Willie Commelin Scholten Phytopathological Laboratory (1921), Schwarz (1922) isolated a fungus from discoloured current-year sapwood and described it as *Graphium ulmi* according to the coremia formed, and concluded that this fungus was the pathogen causing the disease in the elm. However, other scientists attributed the disease to bacteria, climatic or soil factors, or even mustard gas that was used in wartime (Elgersma, 1969; Heybroek *et al.*, 1982). Westerdijk (1928) proved that *G. ulmi* was indeed the causal organism. The work done by Wollenweber (1929), Betrem (1929), Roepke (1930), and Fransen (1931, 1932) and Fransen and Buisman (1935) determined that the elm bark beetles, *Scolytus scolytus* and *S. multistriatus* were vectors of the disease.

The discovery of the sexual stage of the pathogen (Buisman, 1932) resulted in a change of name of *G. ulmi* into *Ceratostomella ulmi* (Schwarz) Buisman. Later, Nannfeldt renamed the fungus as *Ophiostoma ulmi* (Buisman) Nannf. (Melin and Nannfeldt, 1934). Moreau (1952), Hunt (1956), and Upadhyay (1981) proposed the name of *Ceratocystis ulmi* (Buisman) C. Moreau for the fungus; however, considering chemotaxonomical and anamorphic characters, the name *Ophiostoma ulmi* was preferred (Hoog and Scheffer, 1984; Weijman and De Hoog, 1975). The fungus was divided into a non-aggressive and an aggressive strain

based on the performance of the fungus on the elm trees with varying degrees of resistance to the disease (Vanderplank, 1968, 1984). The aggressive strain was divided into a North American race and a Eurasian race (Brasier, 1982, 1983).

Dutch elm disease was discovered in the southern Netherlands in 1919. The disease might had existed and gone unnoticed for several years earlier. After 1919, Dutch elm disease rapidly became widespread. The disease probably reached England in 1923, though it was not identified until 1927 (Wilson and Wilson, 1928). The first infection of the disease in North America was confirmed in Cleveland, Ohio, U.S.A. in 1930 (Buisman, 1930; May, 1930). In Canada, the disease was first identified in Richelieu County, Quebec in 1944. Dutch elm disease was first detected in Selkirk, Brandon, and Winnipeg, Manitoba in 1975 and in south-eastern Saskatchewan in 1990 (Westwood, 1991). Most probably, the fungus was originally imported into North America with veneer logs from France (Beattie, 1933). The European elm bark beetle (*S. multistriatus*) was first reported in 1909 in North America (Chapman, 1910). The native elm bark beetle (*Hylurgopinus rufipes*) is also an effective vector of the disease.

### 2.1.3 General disease cycle

The fungus (*Ophiostoma ulmi*) overwinters in infected and recently killed trees, in stumps, and in recently cut brush and logs (Figure 1). The fungus is spread from infected wood to healthy trees mainly by two species of elm bark

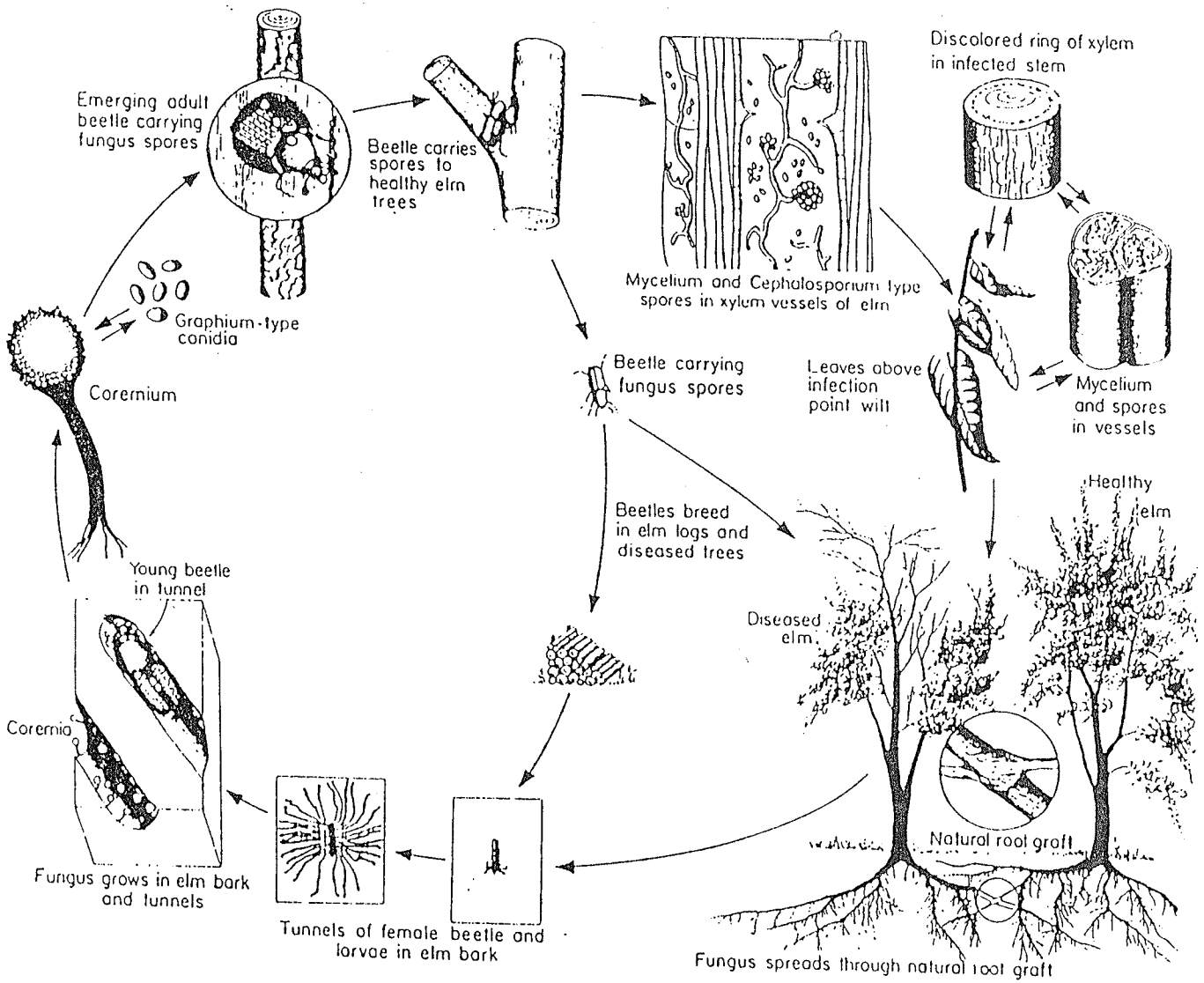



Figure 1. Disease cycle of Dutch elm disease caused by *Ceratocystis ulmi* (Tatter, 1989)

beetle, the European and native elm bark beetle in North America. Both insects lay their eggs under the bark, primarily in stressed, dead, or dying elms which may be infected with fungus. In the infected trees, the fungus produces balls of sticky spores in the beetle galleries. The bodies of the newly emerging adult elm bark beetles are contaminated with these spores when they emerge from the brood galleries.

The fungus on the bodies of insects is spread to healthy elm trees when the beetles, emerging from the brood material infected by the fungus, fly to healthy elm trees to feed on the elm branches. The European elm bark beetle feeds primarily on the bark in small twig crotches in the upper crown. The native elm bark beetle feeds primarily on large branches and sometimes on the main trunk. The fungus enters the vascular system of tree and grows progressively through the outer xylem vessels. The pathogen spreads from one vessel to another by hyphal penetration of pit membranes. In the xylem vessel, conidia may be formed which are thought to increase the rate of spread through the tree sap. As the fungus invades more vascular tissues, twigs, branches, and the entire tree wilts and dies. Wilting of the infected elm is apparently a result of interaction between fungal metabolites and the tree; blockage of the xylem vessels by the fungus itself is most unlikely because of the usually low density of the hyphae and conidia in the vessels (Dimond, 1972; Miller and Elgersma, 1976). Once a tree is dead, the fungus will continue to invade wood all over the tree until the tree begins to dry out.

The dying or dead elm tree is an attractive place for female elm bark beetles to lay eggs for the next brood. The pathogen may also invade a healthy tree through root grafts from adjacent infected trees.

### 2.1.4 The life cycle of the native elm bark beetle in the Prairie Provinces

The following information is based on the personal communication with Robbie (1989) and Westwood (1996). The native elm bark beetle primarily overwinters in the adult stage. Although overwintering larvae had been found in Minnesota and in many areas further south, there is no firm evidence suggesting larvae can survive the winter in Manitoba and Saskatchewan. Adults overwinter in the lower trunk of healthy elms.

The overwintering beetles emerge in spring (April, May, and June) and feed on the upper crown of the same elm or fly to other healthy trees where they feed on. They move to stressed, dead, or dying elms to excavate brood galleries under the bark, and begin oviposition. Larvae hatch, develop and emerge as adults after pupal stage in late July, August, or September and move to healthy trees to feed. The adults move down the healthy tree to overwinter at the base of elm trunk.

### 2.1.5 Dutch elm disease management

Various measures have been developed to prevent and manage Dutch elm disease. Measures are taken to control the fungus, or the beetles, or to increase

the resistance of the susceptible elm species. Quarantine measures can be taken to prevent the disease from spreading from infected areas to areas where the disease has not been established. A Dutch Elm Disease Act was enacted in 1980 in Manitoba to authorize the government to enter into cost-sharing agreements with municipalities to manage Dutch elm disease and to regulate the storage and transport of elm or elmwood materials. The legislative measures provided the government of Manitoba with a legal basis to implement an effective Dutch elm disease preventive and control program in the province.

A large number of elm trees are infected with Dutch elm disease through root grafts between the trees. There are very few successful curative treatments for the disease starting in the root systems. This route of infection becomes more important when diseased elm trees are left standing long enough for the fungus to spread into the root system. The preventive measures against root grafts include digging trenches between elms, or killing all the roots halfway between trees by injecting a soil sterilant such as metam-sodium into the soil. But the high cost and the technical problems of digging trenches along a road, especially with associated buried utility lines, prevent the treatment from being commonly used.

In the long run, the Dutch elm disease may be eliminated or reduced by breeding elms resistant to the Dutch elm disease. Research results have revealed that resistance of elms to Dutch elm disease is polygenically controlled and probably quantitative in nature (Heybroek, 1970; Lester and Smalley, 1972).

Research determining susceptibility and resistance has found that the characteristics of faster tylose formation, smaller diameter of xylem vessels, smaller groups of xylem vessels more widely spaced, and more summer wood may all contribute to hampering the spread of the pathogen (Bonsen *et al.*, 1985; Elgersma, 1982, 1983). Elm trees resistant to the non-aggressive strain of Dutch elm disease, but not to the aggressive strain, may be induced to resist the aggressive strain by preventive treatment with the aggressive strain (Scheffer, 1990).

*2.1.5.1 Control of the vector*

Since Dutch elm disease is spread by elm bark beetles, their suppression can play a very important role in controlling the disease when it has already established. The beetle population can be greatly reduced by spraying insecticides on the tree twig crotches or the basal elm trunk bark where the beetles feed or overwinter. Also the beetle population can be reduced by applying insecticides to logs, which are preferred breeding sites for the beetle, and using trap trees and applying a sanitation program.

Collinus *et al.* (1936) reported that beetles were killed if they fed on elm twig crotches treated with arsenical insecticides. After World War II, DDT was substituted for the arsenicals in the management of the beetle. From the mid-40s to the late-60s, DDT was the most widely recommended and often used insecticide for elm bark beetle control. When used with an efficient sanitation

program, DDT could effectively control the beetle, thus allowing Dutch elm disease losses to be limited to relatively low levels. Barger *et al.* (1973) also found that a concentration of 1.5 g/m$^2$ of DDT on elm bark would prevent feeding. But in the late-60s, the use of DDT was banned because of its long residual effect, resistance to degradation, and cumulative build-up in food chains.

It was necessary to find a substitute for DDT in the control of elm bark beetles. As early as 1949, Bromley (1950) found that methoxychlor could kill elm bark beetles effectively. Methoxychlor replaced DDT in the control of elm bark beetles because of its effectiveness in killing beetles, its lower toxicity to non-target organisms and the lack of the objectionable properties of DDT from an environmental standpoint. Barger (1976) found that Dutch elm disease rates of sprayed plots with methoxychlor in Detroit, MI, U.S.A., were lower than those on unsprayed plots. Rabaglia (1980) reported that the standard hydraulic 2% spray could provide excellent protection for 20 weeks. But Neely (1972) found no added measure of Dutch elm disease protection from spraying with methoxychlor among Illinois communities that practised sanitation. The doubt regarding its effectiveness and the possible environmental problems with methoxychlor made it necessary to look for a substitute insecticide.

Many experiments were conducted to compare the effectiveness of chlorpyrifos, carbaryl, and methoxychlor to control elm bark beetles. Buth and Ellis (1981) reported that 6% methoxychlor was as effective in reducing the number of

overwintering native elm bark beetles as a 1% chlorpyrifos solution. The toxicity and effectiveness of carbaryl against elm bark beetles were evaluated by Quattlebaum (1981), and Page and Green (1978) all of whom concluded that carbaryl is effective in the control of the elm bark beetle.

Chlorpyrifos, an organophosphorus insecticide, displays excellent efficacy to kill elm bark beetles when applied to elm bark. Phillipsen *et al.*(1986) reported that 0.5% (a.i.) chlorpyrifos is active against the beetle for at least 11 months on cut elms. Experiments conducted by Gardiner and Webb (1980) showed that spraying of the lower 2.5 metres of the trunks of living elm with chlorpyrifos until the bark is wet virtually eliminates overwintering native elm bark beetles.

Lanier *et al.* (1984) conducted an experiment to compare chlorpyrifos, carbaryl and methoxychlor in the elimination of broods in infested elm wood, exclusion of colonization of uninfested elm logs, control of beetles "landing" on treated elm bark, and prophylaxis against twig feeding. Chlorpyrifos kills 100% of the beetle broods, completely prevents colonization on uninfested logs, and killed 90 % or more of the beetles exposed for 10 seconds to bark sprayed 6 weeks earlier. Methoxychlor ranks second and carbaryl third. Pajares and Lanier (1989) tested the toxicity and longevity of four pyrethroid insecticides, fluvalinate, permethrin, cypermethrin, and esfenvalerate against the European elm bark beetle and compared the results with those of methoxychlor and chlorpyrifos for the control of this vector of Dutch elm disease. They found that the pyrethroid

insecticides are 222 - 548 times more toxic to European elm bark beetles than is methoxychlor. Cypermethrin and esfenvalerate killed all beetles contacting sample twigs and prevented twig feeding during a 18 week period of bioassays after spray. They suggested that potency and durability combined with their low risk to birds and mammals give the pyrethroids excellent potential for the control of the elm bark beetle.

*2.1.5.2 Control of the pathogen*

After the elm tree is infected by Dutch elm disease, the only method to save the tree is application of a fungicide to kill the fungus or retard the growth of the fungus. The effectiveness of injection of chemicals to control the fungus has also been tested. The tested compounds included the benzimidazole derivative carbendazim, sold under the trade name Lignasan®, thiabendazole hypophosphite, marketed as Arbotect 20-s®, Lirotect ulmi 20-s®, or Ceratotect®, tridemorph, imazalil, and fenpropimorph. The evaluation of the effectiveness of these compounds to control the fungus has shown mixed results.

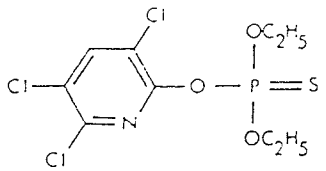
Lanier (1987) compared the effectiveness of the previously mentioned fungicides to control Dutch elm disease in elm. He found that only Arbotect® (thiabendazole) is effective as both a prophylactic and a therapeutic treatment. No other fungicide is effective therapeutically, but Fungi-sol® (benzimidazole carbamate) and $CuSO_4$ give some prophylactic protection. Marchetti *et al.* (1990) in contrast found that only carbendazim provides complete protection against the

disease; thiabendazole is not significantly different from control injected with water. The results of experiments done by Greig (1986) confirmed the efficacy of thiabendazole for suppression of the fungus by finding that all trees recovered after being treated with 0.3% thiabendazole in 20 litres per 30 cm of stem circumference, while 9 out of 10 untreated trees died. Generally, therapeutic fungicide measures are only applied to elm trees with high value because of the high cost of injection techniques.

## *2.2 Chlorpyrifos*

### 2.2.1 General information

Chlorpyrifos (*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate) is an organophosphate insecticide. This insecticide was first reported by Kenaga *et al.* (1965) and introduced by Dow Chemical Co. (now DowElanco). The chemical structure is




The acute oral $LD_{50}$ of the compound for mammals is 135 - 163 mg/kg for rats, 500 mg/kg for guinea-pigs, and 1000 - 2000 mg/kg for rabbits. Acute percutaneous $LD_{50}$ is 2000 mg/kg for rabbits. Acute inhalation $LC_{50}$ (4-h) is >0.2 ng/L for rats. The chronic NOEL (no effect level) of the chemical is 0.03 mg/kg daily for rats, and 0.01 mg/kg daily for dogs in 2-year feeding trials based on blood

plasma cholinesterase activity. The acute oral $LD_{50}$ of the insecticide is 32 mg/kg for chickens; the $LC_{50}$ for rainbow trout (96-h) is 0.003 mg/L; and for bees, the contact $LD_{50}$ is 59 ng/bee and the oral $LD_{50}$ is 250 ng/bee (Worthing 1991).

Chlorpyrifos is a broad-spectrum insecticide, showing insecticidal activity against many insect and arthropod pests. The insecticide kills the insects in such orders as Orthoptera, Diptera, Homoptera, Coleoptera, Lepidoptera, Hymenoptera, and Hemiptera (Harris and Svec, 1968) and such arachnids as spiders, mites, and ticks (Kenaga *et al.*, 1965) through contact toxicity. In addition, chlorpyrifos kills insects, in the orders as Orthoptera, Diptera, Lepidoptera (Kenaga *et al.*, 1965), Diptera, Homoptera (Harris and Svec, 1968), and Isoptera (Smith and Rust, 1991) through stomach or/and vapour contact toxicity. Chlorpyrifos has also been applied directly into water and soil to kill aquatic and soil-living pests. Chlorpyrifos has globally been employed in many pest control practices because of its versatility. Agricultural pests in crops such as corn, and cotton; horticultural pests; human health related pests such as fleas, cockroaches; and insect pests in turfgrass and ornamental trees and shrubs can all be controlled. Chlorpyrifos is formulated as a number of different commercial formulations. The most commonly available formulations are emulsifible concentrate (EC), granular (G), and wettable powder (WP).

### 2.2.2 Environmentally significant properties of chlorpyrifos

The environmental behaviour of a chemical is to a great extent determined by its physical and chemical properties, such as melting point, vapor pressure, water solubility, octanol/water partition coefficient, and partition coefficients between the aquatic compartment and soil, sediment, and the biotic compartment.

Experimentally determined melting points for chlorpyrifos have ranged from 41.5 (Brust, 1964) to 44 °C.(McDonald *et al.*, 1985b). The slight divergence in values might be related to the method of determination or to the purity of the material tested. Chlorpyrifos is of moderate volatility with a measured vapor pressure of between $1.8 \times 10^{-5}$ and $2.0 \times 10^{-5}$ mm Hg at 25 °C (Chakrabarti and Gennrich, 1987).

Chlorpyrifos is a non-polar compound which readily dissolves in many organic solvents. The water solubility of chlorpyrifos has been reported as ranging from 0.94 to 2 µg/mL (Dawson, 1989; Hummel *et al.*, 1964). The water solubility of chlorpyrifos is affected by temperature with the solubility increasing 2.9 times as the temperature increases from 10 °C to 30 °C (Bowman and Sans, 1985). The presence of dissolved electrolytes (Felsot and Dahm, 1979) decreases the solubility of chlorpyrifos in water. The experimentally determined log octanol/water partition coefficient for chlorpyrifos is 4.7 (McDonald *et al.*, 1985a) to 5.3 (Debruihn *et al.*, 1989).

The propensity for partitioning of a chemical between solid and aqueous compartments is measured by $K_d$ (mL/g) or $K_{oc}$ (mL/g) ($K_d$ adjusted for soil organic carbon content). The magnitude of the values determines what fraction of a pesticide will be most available for such processes as leaching through soil, soil surface run-off, plant uptake, and uptake by soil and aquatic organisms. The experimentally measured adsorption $K_d$ values for chlorpyrifos have ranged from as low as 13.4 mL/g (Kanazawa, 1989) to as high as 1862.0 mL/g (Sharom *et al.*, 1980) depending on the texture and organic carbon content of the tested soil, the concentration of chlorpyrifos, and soil to water ratio. The associated $K_{oc}$ sorption coefficients have ranged from 995 (Kanazawa, 1989) to 31000 mL/g (McCall, 1987). These values indicate that chlorpyrifos possesses a strong tendency to associate with a solid phase such as soil and sediment rather than to dissolve in water.

The potential for pesticide movement from water or sediment compartments to fish or other aquatic organisms is reflected by the bioconcentration factor (BCF), which is the ratio of pesticide concentration (mg/kg) in the biological compartment (e.g., fish) to the pesticide concentration (mg/L) in the environmental matrix (e.g., water) at equilibrium. The experimentally determined BCF values of chlorpyrifos varies greatly depending on the identities of the tested organisms, exposure time, exposure concentration of chlorpyrifos, exposure methods. The extreme values of

BCF for chlorpyrifos were 1 (Lal and Lal, 1987) in yeast (*Saccaromyces cerevisiae*) and 5100 (Hansen *et al.*, 1986) in Gulf toadfish (*Opsanus beta*).

### 2.2.3 Analytical methods for chlorpyrifos and its major metabolites

Many analytical methods for the determination of residues of chlorpyrifos alone, or the combination of chlorpyrifos and its major metabolites, oxon and pyridinol, in various environmental matrices have been developed. Most of the reported analytical methods involve extraction of the residues of the target analytes from the matrices with organic solvent, cleanup of the extracts by liquid-liquid partition or/and adsorbent column chromatography, and determination by gas chromatography.

Bowman and Beroza (1967) reported a method to determine the residues of chlorpyrifos and its oxon in corn silage and grass. The method involved the extraction of the residues with benzene, cleanup of the extracts with silica gel column chromatography, and determination of the residues with GC-FPD. The recoveries of chlorpyrifos and oxon in corn and grass ranged from 96 to 100% and 85 to 90%, respectively. Struble and McDonald (1973) developed methods to analyze chlorpyrifos and oxon in wheat plants and kernels using benzene extraction, silica gel column chromatography cleanup, and detection with GC-FPD. The average recoveries ranged from 85 to 95% for chlorpyrifos and from 58 to 93% for oxon.

Mourer *et al.* (1990) described a method for the determination of chlorpyrifos and pyridinol in dates. After extraction of residues with acetone, chlorpyrifos was cleaned up using Florisil and analyzed by GC-NPD. Pyridinol was derivatized with bis-(trimethylsilyl)-acetamide to form the pyridinol derivative and determined with GC- Hall electrolytic conductivity detector. The recoveries of the analytical method for chlorpyrifos and pyridinol ranged from 86 to 110%, and from 79 to 99%, respectively, in dates. Inman *et al.* (1991) reported a method to analyze the residues of chlorpyrifos and pyridinol in peppermint hay and peppermint oil. The residues of chlorpyrifos and pyridinol were extracted with mixture of hexane and 2-propanol, The residues of chlorpyrifos were cleaned up on a silica gel column and quantitated by GC-FPD. The pyridinol was separated from the extraction solvent, cleaned up on acid alumina, derivatized with N,O-bis-(trimethylsilyl)-acetamide and analyzed by GC-ECD giving average recoveries of 88% in peppermint hay and peppermint oil.

Braun (1974) developed a method for determination of chlorpyrifos, oxon, and pyridinol in vegetable tissue. Samples were extracted with acetonitrile. The extracts were partitioned between benzene and aqueous sodium carbonate to separate the chlorpyrifos and oxon from pyridinol. Both fractions were individually cleaned up on silica gel which also served to fractionate chlorpyrifos from oxon due to the hydrolysis of oxon into pyridinol. Chlorpyrifos was analyzed by GC-FPD. Residual pyridinol and pyridinol resulting from the hydrolysis of oxon in the

silica gel column were derivatized with trimethylsilyl acetamide and determined by GC-ECD. Overall recoveries from the fortified samples averaged 95% for chlorpyrifos and 85% for oxon and pyridinol.

### 2.2.4 Environmental transformation of chlorpyrifos

Like any other chemical, after chlorpyrifos is introduced into the environment, it will be transformed or degraded due to abiotic and biotic processes simultaneously acting on it. These transformation processes are of interest from the standpoints of pest control efficiency, environmental toxicity for nontarget organisms, and availability of chlorpyrifos residues for transport. A generalized pathway for its transformation in the environment is illustrated in Figure 2.

#### *2.2.4.1 Abiotic transformation processes of chlorpyrifos*

Abiotic transformation processes of chlorpyrifos in ecosystems mainly consist of hydrolytic and photolytic degradation. These abiotic processes, especially hydrolytic degradation, play an important role in the dissipation of chlorpyrifos from soil and water.

##### 2.2.4.1.1 Hydrolysis

Susceptibility to hydrolytic degradation is one of the distinctive characteristics of organophosphate insecticides. Chlorpyrifos possesses three phosphate ester bonds which can be cleaved hydrolytically: two ester bonds and

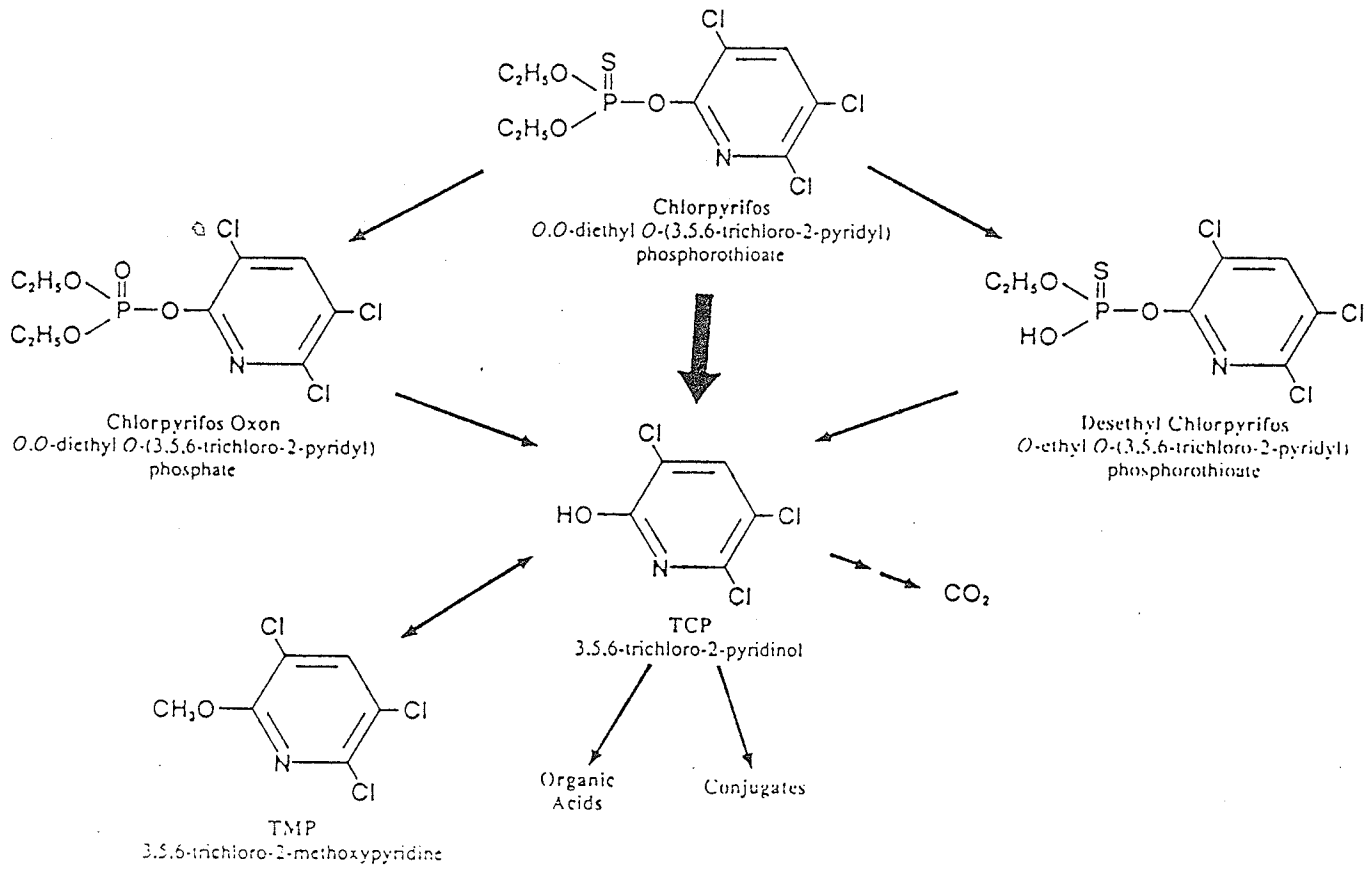



Figure 2. General transformation pathways of chlorpyrifos in the environment (Racke, 1993)

one pyridoxy ester bond. The identification of four major hydrolic products of chlorpyrifos indicates that all three ester bonds in the molecule may be hydrolyzed. A number of studies have been conducted to determine the hydrolytic dissipation rate of chlorpyrifos under various conditions. The reported hydrolytic half-life of chlorpyrifos are 0.01 days (Macalady and Wolfe, 1983) to 1925 days (Crummett, 1963; Brust, 1966) depending on the pH and temperature of the samples, initial concentration of chlorpyrifos, presence or absence of modifiers (e.g., methanol, copper ion) and the water type (e.g., distilled or natural). It was reported that copper ions (Mortland and Raman, 1967) and heterogeneous surface catalysis (Getzin, 1981a) can accelerate the hydrolytic processes of chlorpyrifos.

2.2.4.1.2 Photolysis

Direct photolysis takes place when a pesticide molecule absorbs ultraviolet radiation and then interacts with itself or reactants in the environments. Indirect photolysis may also occur when sunlight is absorbed by another environmental substance and subsequently the activated forms interact with the pesticide or produce oxygen or peroxides that do (Racke, 1993b). The reported photolytic half-life of chlorpyrifos applied on various surfaces ranged from 2.2 days (Chen *et al.*, 1984) on glass plates to 52.6 days (Walia *et al.*, 1988) on a leaf surface (*Polystichum setiferum*) depending on the light source, surface characteristics, and modifiers on the surface. The photodegradation half-life of chlorpyrifos in aqueous solution have been reported to range from 7.8 days (Meikle *et al.*, 1983) in

aqueous buffers to 29.9 days (Batzer *et al.*, 1990) in sterile, pH 7 buffered water under natural sunlight, to 108 days (Smith, 1966) in 50% aqueous methanol at pH 5 under an artificial light source (General Electric sunlamps). The air photolysis half-life of chlorpyrifos have been reported to range from 136 minutes (Klisenko and Pis'mennaya, 1979) when chlorpyrifos was exposed to an artificial light source to 2.6 days (Fontaine and Teeter, 1987) upon exposure of 265 ng/L of chlorpyrifos to a xenon lamp source (60% relative humidity, 25 °C)

*2.2.4.2 Biotic transformation processes of chlorpyrifos*

Micro-organisms play a very important role in the degradation of pesticides in the environment not only by catalysing similar metabolic reactions to mammals or plants, but also by completely mineralising many aliphatic, aromatic, and heterocyclic compounds. There are two main types of microbial degradation of organic compounds: catabolism in which the organic chemical or a portion thereof is completely degraded and the energy and nutrients derived from it are used for cell growth, and cometabolism in which the organic compounds are only partially degraded during the normal metabolic activities of the micro-organisms and the organisms do not obtain the energy and nutrients from the degradation processes. Chlorpyrifos is microbially metabolized by the cometabolism route (Racke, 1993b).

Most of the studies examining the microbial role in chlorpyrifos degradation have been conducted in soil, water, or sediment. Conclusions have been derived from the difference of degradation rates in sterilised and natural (i.e., non-sterile,

microbially viable) substrates ignoring the effect of the sterilising process on the degradation of chlorpyrifos applied after sterilization. Studies conducted in soil show significantly longer dissipation half-life under sterilised *vs.* natural conditions and have concluded that microbial activity is important in the degradation of chlorpyrifos in soil (Thiegs, 1966; Getzin and Rosefield, 1968; Getzin, 1981b; Miles *et al.*, 1983, 1984). Microbial adaptation for its catabolism has not been reported to occur in soil (Racke *et al.*, 1988; Racke and Coats, 1990), and thus any metabolism occurring is of an incidental nature. Some of the laboratory degradation studies with aqueous solutions and sediments have indicated that micro-organisms have played an important role (Schmimmel *et al.*, 1983), whereas others have shown that they play virtually no role in the degradation of chlorpyrifos in the aquatic environment (Sharom *et al.*, 1980).

### 2.2.5 Transformations in Soil

Soil is very important in the studies of environmental chemistry of pesticides, not only because some or all of most applied pesticides will enter into soil finally, no matter how the pesticides are applied, but also because pesticides in the soil can be absorbed by organisms (e.g., crop plants and earthworms) or be transferred to other environmental compartments through processes such as leaching and runoff, or to be degraded by abiotic and biotic agents or to be adsorbed by the soil components. A number of studies on the degradation of chlorpyrifos in soil have been conducted.

The persistence of chlorpyrifos in soil is of concern from both the efficacy and the environmental standpoints. The half-life of chlorpyrifos from studies conducted under laboratory and field conditions varies greatly depending on the physical and chemical properties of the tested soil, chemical concentration, application manner, and environmental factors.

A number of studies on the degradation of chlorpyrifos in soil have been conducted under controlled laboratory conditions. Thiegs (1964) first examined the degradation of chlorpyrifos in soil and found that about 50% of added chlorpyrifos disappeared in a moist soil after a 10-week incubation. Further work (Thiegs 1966) studied the degradation of chlorpyrifos in soil treated at 2.1-5.0 μg/g (a.i.) and incubated at various temperatures in the greenhouse for up to 30 weeks and estimated the degradation half-life to range from 17.0 to 58.4 days. Kuhr and Tashiro (1978) reported degradation half-life of 7-16 days in a sandy loam soil treated at 3 μg/g and seeded with grass. Bidlack (1979) examined the aerobic soil degradation of chlorpyrifos in seven different soils and investigated the effect of anaerobic conditions on the degradation. Aerobic degradation half-life of 11-141 days was noted with a mean of 63 days. Both anaerobic and aerobic/anaerobic conditions would shorten the degradation half-life of chlorpyrifos depending on the soil type. The degradation half-life of chlorpyrifos was reduced from 107 to 51-58 days in the aerobic Staccaton clay soil; but the degradation half-life was slightly increased under aerobic conditions from 11 days to 15-39 days in Commerce

loam. Miles *et al.* (1979) observed half-life of <1 and 17 weeks in a mineral and organic soil treated at 10 μg/g, respectively. Afifi and Kansouh (1980) found degradation half-life of 10-16 days in clay soil plots treated at approximately 100 μg/g initial soil concentration and incubated in a greenhouse. Chapman and Chapman (1986) examined the dissipation rates of various formulations of chlorpyrifos under various containment (open or closed) conditions and reported half-life of 31.5-49.5 days in a mineral soil and 14.1-38.5 days in an organic soil. The relative importance of formulation and containment on overall dissipation depends on soil characteristics. Dissipation is slower in closed containers than in open ones, and the formulation slightly influence dissipation rate in the mineral soil; however, the granular formulation disappear more slowly than the EC and there is little difference between open and closed containers in the organic soil. The reported half-life of chlorpyrifos was also 4 and 12 weeks for Chehalis clay loam and Sultan silt loam soils treated with 16.7 to 20 μg/g (Getzin, 1981b), 3.7 to 6.7 days in loam and loamy sand soils treated with granular chlorpyrifos (Shaaban *et al.*, 1981), 1.9 to 36.6 days in four different soils treated at 1 μg/g and incubated under different conditions (McCall *et al.*, 1984), 78.4-145.7 days (Van de Steene *et al.*, 1989; 1990) in four Belgian soils treated at 50 μg/g of technical chlorpyrifos, 3.8-43.0 days (Racke *et al.*, 1990) in several soils treated with 1 μg/g of technical chlorpyrifos and incubated in the laboratory.

Dissipation of chlorpyrifos under field conditions has been studied extensively. Harris and Svec (1968) reported that 94-96% of the initial levels of 0.67-0.78 μg/g dissipated within 1 month in surface soil planted with tobacco. McKellar *et al.* (1972) compared the persistence of EC applied in furrow and granular formulations applied to the soil surface as bands in cornfield plots in Illinois and Nebraska. Average initial residues of chlorpyrifos ranged from 2.8 to 4.5 μg/g for the furrow and 1.7-8.0 μg/g for surface application. The half-life calculated from the research results was 6.0 days in Illinois and 30.3 days in Nebraska for furrow application of the EC formulation and 12.9 days in Illinois and 42.7 days in Nebraska for band application of the granular formulation. Novozhilov *et al.* (1982) reported half-life of 5-11 weeks in the acidic Turfenpodsolic soil and 3-6 weeks in Chernozem soil treated at 0.75-5.0 kg/ha in the Chuvash region of the Soviet Union. Harris *et al.* (1988) reported half-life of 12.2 to 14.1 days in soil planted to corn in Ontario, treated at 1.1-1.25 g/m row. Harris *et al.* (1973) reported that 5.0-5.8 μg/g of chlorpyrifos remained after 1, 2, 3, and 4 months in soil planted to onion with initial residues of 18.6 μg/g. The reported half-life of chlorpyrifos in vegetable fields was 15.8-17.0 days in cabbage planted soil treated with an incorporated granular application and 17.3 days with an EC soil drench application (Szeto *et al.*, 1988); and 28-42 days, 15-18 days, and 27 days in loamy sand, sandy loam, and silt loam planted to cauliflower and treated with EC at initial soil residues from 17.1 to 26.8 μg/g (Rouchaud *et al.*, 1989), respectively.

Chlorpyrifos is also applied as a whole-tree spray or to the bark of the trunk to control orchard or forest pests.

Several studies have been done to study the dissipation of chlorpyrifos in orchard or forest soil. Oliver *et al.* ( 1987) noted initial residue levels in the upper 2.5 cm of soil beneath and between tree canopies of 0.4-0.8 and 0.2-2.3 μg/g, respectively, and average soil dissipation half-life of 1.3-4.4 days after citrus trees in Florida was sprayed with an EC formulation at 1.12 kg/ha during the growing season. Neary *et al.* (1988) studied the behaviour of chlorpyrifos in pine forest stands growing in an Astatula sand soil in Florida. They found that initial residues of 1.25 μg/g were observed in the upper 5 cm of soil, and that there was a slight increase of chlorpyrifos concentration in soil reaching 3.20 μg/g thereafter. This increase might be explained from the insecticide movement from the treated areas to soil.

### 2.2.6 Degradation in plants

In most studies, chlorpyrifos dissipated very quickly from plant foliar surfaces with dissipation half-life of several hours to weeks. Leuck *et al.* (1968) reported a dissipation half-life of 1 day from coastal Bermudagrass treated at 1.12 kg/ha (EC). Dutta and Goswami (1982) reported dissipation half-life of 1.7-2.3 days from mustard plants treated with 0.01-0.03% chlorpyrifos solution. McCall *et al.* (1984) treated corn and soybeans at 1.68 kg/ha (EC) two times and calculated a half-life of about 1.5 days on the leaves. Chlorpyrifos dissipation on soybean

and broadbean leaves was studied by Abdel-All *et al.* (1990) who observed initial residues of 13-14 μg/g following the EC application and dissipation half-life of 4.0 and 3.1 days from soybean and broadbean, respectively. The reported half-life was approximately 1 week on thatch-free bluegrass turf at 2.24 kg/ha (Kuhr and Tashiro, 1978), 7 - 14 days on annual bluegrass at 4 kg/ha (Sears and Chapman, 1979), 2.4-2.8 days on orange leaves and 2.4-3.4 days on grapefruit leaves at 5.6 or 11.2 kg/ha (Iwata *et al.*, 1983), 4 hours on both Bermudagrass and perennial ryegrass/bluegrass mixtures (Wetters *et al.*, 1985), and 2-8 hours on Kentucky bluegrass at 3% EC (Goh *et al.*, 1986).

There is much longer dissipation half-life when chlorpyrifos is applied to the bark of woody plants and trees, possibly due to surface sorption to the dead tissue layer (*vs.* living leaf epidermis) or to the higher application rates (Brady *et al.*, 1980). It took 1 month for chlorpyrifos residues to dissipate from initial residues of between 1449 and 3192 μg/g to approximately half these levels on loblolly pine trunks (0-1 cm layer) treated with 1 and 2% (EC) solutions. After the initially rapid dissipation, the rate of dissipation slowed down and 15 months later, 14-18% remained. Berisford *et al.* (1981) applied 1 and 2% solutions to pine trunks and got initial residues of 2598 to 7674 μg/g, depending on application rate. Bark dissipation half-life of 5.0-6.9 months could be calculated from results of this research. Research on dissipation of chlorpyrifos from sand pine bark treated with 2% chlorpyrifos EC drench indicated that initial residues of 24.7-40.8 μg/g in

composite tree bark and wood samples did not decline significantly over a 12 month period (Neary *et al.*, 1988;). Howell and George (1984) examined dissipation from the twigs of apple trees. The residues of 0.64-6.02 and 0.09-0.19 μg/g were observed 1 and 6 months after application, respectively, after up to three cover sprays of EC at 1.2 or 2.4 g/L. The quicker dissipation rate in surface residues on twigs might be caused by either lower application rates or the different nature of living twig surface and dead trunk bark tissue.

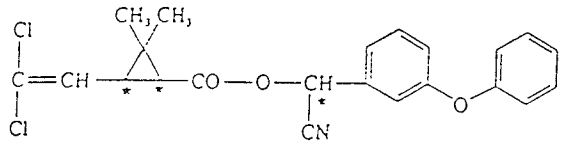
Some experiments were conducted to reveal important factors affecting the dissipation rate of chlorpyrifos on plant leaves. Veierov *et al.* (1988) studied the effect of formulation type and application rates on the persistence and penetration of chlorpyrifos into greenhouse tomato leaves. From the experimental results, the effect of application rate on the dissipation rate was demonstrated by comparing the dissipation half-life of 0.78 and 3.23 days for low-rate (0.1%) and high rate (0.5%) EC formulation spray applications. The fact that the surface residues dissipated 1.4-4.4 times more rapidly than internal leaf residues demonstrates the important role of volatility in the dissipation process. Any factors which facilitate chlorpyrifos to penetrate into leaf tissue would reduce the dissipation rate on the plant leaves. This could be demonstrated by the half-life of 11.8, 68.7-72.7, and >470 hours for EC, mineral oil, and cottonseed oil formulations, respectively. The oil formulations not only slowed the volatile loss of chlorpyrifos from the treated

plant leaves, but also increased the ability of oil-formulated chlorpyrifos to penetrate into leaf tissue (26-56%) *vs.* the EC (13%).

### *2.3 Cypermethrin*

#### 2.3.1 General information

The synthetic pyrethroid insecticide, cypermethrin, (RS)-α-cyano-3-phenoxybenzyl (1RS)-*cis-trans*-3-(2,2-dichlorovinyl)-1,1-dimethyl-cyclo-propanecarboxylate, first discovered by Elliott and co-workers (Burt *et al.*, 1974; Elliott *et al.*, 1975), was developed by Ciba-Geigy AG, ICI Agrochemicals, Mitchell Cotts, and Shell International Chemical Co. Ltd. Cypermethrin has proved to be more potent and more photostable than natural pyrethrins. Cypermethrin is a racemic mixture of eight isomers resulting from the presence of three chiral carbon atoms (*) in the molecule. The ratio of the *cis/trans* isomers in cypermethrin materials is approximately 40/60. The 1R, *cis*, alpha-S isomer of cypermethrin is one of the most potent pyrethroids available at the present time. (Casida, 1980). The chemical structure of cypermethrin is




Although cypermethrin displays very high toxicity to insects and fish, it is considered relatively safe for mammals and birds due to rapid biotransformation

and excretion by the catabolic systems in the mammal and bird. Oral toxicity values for cypermethrin vary significantly depending on such factors as carrier, *cis/trans* ratio of the sample, and species, sex, age, and degree of fasting of the test animals. Typical reported values for acute oral $LD_{50}$ are 251-4123 mg/kg for rats, 138 mg/kg for mice, >2000 mg/kg for chickens (Worthing, 1991) and 4640 mg/kg for mallard ducks (Environmental Protection Agency, 1990). Percutaneous $LD_{50}$ for rabbits is >2400 mg/kg (Worthing, 1991). Cypermethrin is a slight skin and mild eye irritant and may be a weak skin sensitizer. In 2 year feeding trials, no toxicological effect was observed in rats receiving 100 mg/kg in the diet and dogs receiving 300 mg/kg in the diet (Worthing, 1991). The acute $LC_{50}$ values of cypermethrin are 0.82 ng/L for rainbow trout (96 h), 1.78 ng/L for bluegill sunfish (96 h), and 0.26 ng/L for *Daphnia magna* (Environmental Protection Agency, 1990). Under field conditions, fish are not at risk from normal agricultural usage, probably because the chemical is adsorbed to soil or suspended organic substances in water and not easily available to fish. Cypermethrin is highly toxic to honeybees in laboratory tests, but hives are not at risk from field applications at recommended dosages (Worthing, 1991).

Cypermethrin is both a stomach poison and contact insecticide effective against a wide range of insect pests, particularly Lepidoptera in cereals, citrus, cotton, fruit, rape, soybeans, tobacco, tomatoes, vegetables, and other crops at 20-75 g a.i./ha. If applied before infestations become well established,

cypermethrin also provides protection against plant sucking Hemiptera in most crops (Worthing, 1991). In forestry applications, cypermethrin can replace lindane and is the active ingredient of insecticidal formulations (e.g., Ripcord®) intended to prevent damage to stored timber from boring insects and to suppress infestation of pine forests by bark beetles. Cypermethrin is also present in spray formulations for indoor and household usage against biting and stinging insects (Class 1992).

### 2.3.2 Environmentally Significant Properties of Cypermethrin

The experimentally determined melting point and boiling point of cypermethrin range from 60 to 80 °C, and from 170 to 195 °C, respectively, depending on the proportions of the eight isomers in the sample and determining methods (Environmental Protection Agency, 1990). The measured vapour pressures are $1.4 \times 10^{-9}$ mm Hg at 20 °C (Travis, 1990), $1.4 \times 10^{-9}$ mm Hg at room temperature (Ferraro, 1990), and $4 \times 10^{-8}$ mm Hg at 70 °C (Royal Society of Chemistry, 1983). Cypermethrin is a non-polar compound which has very large solubility in organic solvents such as acetone, hexane, and very low solubility in water. The reported water solubility is 0.005-0.01 (Stephenson, 1982), 0.004 (Wanchope, 1989), 0.041 (Coats and O'Donnel-Jeffery, 1979), 0.01-0.2 (Worthing, 1991), and 4 mg/L (Sapiets *et al.*, 1984) depending on the determining methods. The determined sorption coefficient is 2,000-16,000 (Helier and Herner, 1990), and 16000 (Travis, 1990; Ferraro, 1990). The experimentally obtained $K_{ow}$ is 40,000 (Worthing, 1991). Synthetic pyrethroid insecticides are not expected to biomagnify

through the food chain, due to their high sorption to and immobilization in soil and because they are rapidly eliminated from tissues after the end of exposure (Spehar *et al.*, 1983). The concentration factor for cypermethrin is relatively low, ranging from 3 to 7 in salmon (McLeese *et al.*, 1980).

### 2.3.3 Analytical methods for cypermethrin and its major metabolites

There are a few reported analytical methods for the determination of the residues of cypermethrin and its main metabolites in environmental matrices. Most reported methods are based on the extraction of the residues with organic solvent, cleanup with solvent-solvent partition and/or adsorption chromatography, and determination of the residues by GC with ECD.

Chapman and Harris (1978) developed an analytical method to determine the residues of cypermethrin in vegetable crops. The method involves the extraction of crop samples with acetone, partitioning into hexane, and cleanup of extracts by silica gel, or Florisil column chromatography producing average recoveries ranging from 72 to 99 % at the fortification level of 0.05 μg/g. Braun and Stanek (1982) reported an analytical method to determine residues of cypermethrin in vegetable. The cypermethrin residues were extracted with acetonitrile, partitioned into hexane, and cleaned up by Florisil column chromatography. The average recoveries from the fortified sample ranged from 94 to 105%. GC-ECD was also utilized to quantitatively analyze cypermethrin residues extracted with acetone (Harris *et al,* 1981), or with a mixture of

acetonitrile and water (Frank *et al.,* 1982) in soil, and in fruits and vegetables (Baker and Bottomley, 1982; Frank *et al.*, 1982).

Class (1992) developed analytical methods in which the residues of cypermethrin isomers and major metabolites in spruce bark and soil were extracted with dichloromethane by Soxhlet, and cleaned up on a Florisil column, and analyzed by GC-ECD, GC-MS or HPLC. In GC analysis, all eight possible cypermethrin isomers appeared in four peaks with each peak representing a pair of *cis*- or *trans*- isomers. Three of the four pairs of cypermethrin isomers were further slightly separated in normal phase HPLC. The major metabolites of cypermethrin in soil, cyclopropane carboxylic acid (CCA) and 3-phenoxybenzoic acid (PBA), were silylated with N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) and analyzed with GC-MS with EI ionization.

As an alternative method, HPLC may be used to determine the residues of cypermethrin in environmental samples. Baker and Bottomley (1982) reported an analytical method to determine the residues of cypermethrin in apple, pear, cabbage, and potato. The residues of cypermethrin were extracted with the mixture of hexane and acetone, partitioned into hexane from water, cleaned up with silica gel column chromatography, and determined with HPLC with UV resulting in detection limit of 0.2 μg/g in all the test matrices.

### 2.3.4 Transport and distribution in the environmental media

The physical and chemical properties of cypermethrin determine its mobility in the environment. Low vapour pressure and water solubility of cypermethrin cause it to be strongly adsorbed from aqueous solution by solid surfaces. These properties also greatly reduce its movement in air and water, and particularly in soil.

Kaufman *et al.* (1981) reported that virtually all the applied cypermethrin is retained in the top 2.5 cm of 10 cm soil columns, down which 5 cm of water is allowed to penetrate. Helling and Turner (1968) covered thin layer chromatographic plates with soil to study the mobility of cypermethrin in the soil and found that very little movement of cypermethrin occurred in the tested soil. However, the radio-labelled cypermethrin metabolite, 3-phenoxybenzoic acid (PBA), and the cyclopropane carboxylic acid (CCA) leached down soil columns to a level of about 8 cm. The mobility of CCA and PBA were slightly affected through adsorption by the organic matter content of the soil, but were mainly affected by soil pH value presumably because of increased dissociation of the COOH group.

Stevens and Hill (1980) conducted research on the leaching of cypermethrin in four different soil types: a clay loam, a loamy sand, a course sand, and a fen peat packed in a glass column under laboratory conditions. They concluded that more than 99% of the $^{14}C$ residues stayed within the top 5 cm in all soils after the columns had been leached with 67.5 cm water over a 10 week

period. Radioactivity in the leachate was below the limit of detection in all cases. Jackson (1977) concluded that there was little penetration of cypermethrin below the top 2-cm layer in the soil columns, even after the percolation of 1.35 metres of water.

Standen (1977) studied the percolation of distilled water through sandy loam soils containing $^{14}$C-benzyl cypermethrin from spent sheep-dip baths and reported that most (89%) of the radioactivity was retained in the top 14 cm of the columns, mainly as intact cypermethrin, with only 0.3 % of the applied radioactivity being leached out. However, most of the radioactivity was associated with fine soil particles in the leachate and could not be extracted with organic solvents. The water contained small amounts of unchanged cypermethrin and PBA. The results of research conducted by Sakata *et al.* (1986) on the leaching of radio-labelled cypermethrin with distilled water through columns of four different types of soil under laboratory conditions indicated that cypermethrin was relatively resistant to leaching after the soil columns had been leached with 3 metres of water, applied 3 mL/h for three weeks at 25 °C. But radioactivity was found in the leachate, especially in one sandy soil where, after 30 days of incubation, about 30% of the cyclopropyl label first added was collected in the leachate. The main products associated with radioactivity in the leachates were either CCA or PBA depending on the position of the label. Unchanged cypermethrin was present only in trace amounts in sand containing less than 0.1% organic matter.

### 2.3.5 Photodegradation

*2.3.5.1 In Water*

Day and Leahey (1980) studied the photodegradation of dilute aqueous solutions of cypermethrin exposed to sunlight. *Cis*- or *trans*- isomers were $^{14}C$ labelled in either the cyclopropyl- or the benzyl-ring. Cypermethrin dissolved in sterile aqueous acetonitrile at a concentration of 1 mg/L was irradiated in sunlight for 32 days. The photodegradation rate was calculated by comparing the remaining concentration in irradiated solutions with controls stored for the same length of time in the dark. At the end of the study, 89.4% of the cypermethrin remained in the samples of the irradiated benzyl label in comparison with 97.4% in the dark control. The corresponding figures for the cyclopropyl label were 92.3% and 96.8%. The photodegradation products included *cis*- and *trans*-CCA, phenoxybenzyl alcohol, aldehyde and acid, and α- cyano-3-phenoxybenzyl alcohol.

Takahashi *et al.* (1985a, 1985b) studied the photodegradation of two pairs of cypermethrin isomers (1R, *cis*-, α-RS) and (1R, *trans*-, α-RS) in aqueous solutions made from water, 2% acetone, aqueous humic acid, sea water, or natural river water (both of which had been filtered) in natural sunlight. The tested chemicals were labelled with $^{14}C$ in either the cyclopropyl, the benzyl ring, or the cyano carbon. The surfactant Tween 20® was used to disperse the isomers in the aqueous solutions to produce a 50 µg/L stabilized test suspension. The results

indicated that the degradation of the isomers was very rapid; however, a large part of the changes involved transformation to other isomers. The degradation half-life of the *cis*-isomer in river or sea water was 0.6-0.7 days, much less than 2.3-2.6 days in distilled water or humic acid solution. The most rapid change took place in the presence of acetone. Presumably the differences were attributed to photosensitization by the acetone or organic constituents of the natural waters. The main degradation products, in addition to the different isomers, were CCA, PBA, a small amount of aldehyde, and carbon dioxide.

*2.3.5.2 In soil*

Hall *et al.* (1981) studied the photodegradation of cypermethrin labelled with $^{14}C$ in either the cyclopropyl ring or the benzyl ring on soil very thinly coated on plates at a rate equivalent to about 200 g/ha in sunlight. The results indicated that 63% of the radioactivity initially applied to the irradiated plate was recovered, compared with 103% from the plate that had been kept in the dark. The half-life calculated from the data ranged from 8 to 16 days by irradiation with natural light. The main degradation products were the amide, *cis*- and *trans*- CCA, and some unidentified products in the case of the cyclopropyl label. In the case of the benzyl label, the identical products mainly included the amide analogue of cypermethrin and various phenoxybenzyl derivatives, such as the alcohol, aldehyde, and acid.

Takahashi *et al.* (1985a, 1985b) reported that photodegradation of labelled cypermethrin in 0.5 mm layers of three different soils at 1.1 ng/cm$^2$ in sunlight was

very rapid compared with that kept in the dark. The half-life ranged from 0.6 to 1.9 days with sunlight and more than 7 days in the dark. The identified products were very similar to those in the above experiment in that the main degradation products was the amide of the otherwise intact isomers. In addition, they identified smaller amounts of PBA, virtually no CCA, but occasionally small amounts of α-carbamoyl-, and α-carboxyphenoxybenzyl alcohol. In one of the soils in which degradation occurred most quickly, nearly half of the radiolabelled carbon was unextractable at the end of the exposure period.

### 2.3.6 Hydrolysis

Camilleri (1984) studied the hydrolysis of *cis*- isomer pair of enantiomers at $10^{-5}$ mol/L in dioxane-water (1:1) and found that at alkaline pH values, cypermethrin was readily degraded by ester cleavage to give CCA and PBA. The hydrolysis of the cyano group to amide could not be detected because the reaction required a much higher energy of activation.

Takahashi *et al.* (1985a) investigated the effect of pH on the hydrolysis of 1R *cis*- or *trans*- cypermethrin in abiotic buffered aqueous solutions and found that the half-life of the isomers are one or more years at acidic pH, but they are much shorter at pH 7 (2.6 days) and are a matter of minutes at pH 11. In sterilized water at pH 8, the half-life of cypermethrin was about 3 weeks at 25 °C. The *trans*-isomers were hydrolysed more readily than the *cis*-isomers.

### 2.3.7 Degradation in soil

Cypermethrin dissipates relatively quickly in soils, primarily by biological processes involving cleavage of the ester bond to produce the two major degradation products, CCA and PBA. The metabolites are themselves subsequently mineralized. There is also evidence that the amide of the intact molecule and occasionally the 4-hydroxy phenoxy analogue are formed, but these products can be further degraded (Leahey, 1979).

*2.3.7.1 Rate of degradation*

2.3.7.1.1 Laboratory studies

Roberts and Standen (1981) studied the degradation of cypermethrin in soil in the laboratory and found that the half-life of the *cis*-isomers was about 4 weeks in the unsterilized Los Palacios soils, but 10-12 weeks in the same sterilized soil. The *trans*-isomer usually displayed much shorter half-life of less than 2 weeks and less than 4 weeks on the less active soil. After one year, the amounts of unchanged cypermethrin left in the soil were below 10% of the amount applied. The residues of the *trans*-isomers are less than those of the *cis*- product at the low remaining level after such a long time. Sakata *et al.* (1986) studied the degradation of cypermethrin isomers during incubation and reported that half-life ranges from 4.1 to 17.6 days for *trans*-cypermethrin and 12.5 and 56.4 days for the *cis*-isomer under aerobic upland conditions. The degradation rate of cypermethrin greatly depends on the soil types.

Miyamoto and Mikami (1983) tested the degradation of all four of the 1R isomers of cypermethrin under laboratory conditions and found that the α-S isomers of both *cis*- and *trans*-isomers degrade much more quickly than the α-R isomers, sometimes nearly twice as fast. The *cis*-isomers also have longer half-life than the *trans*-isomers (Kaufman *et al.*, 1981; Chapman and Harris, 1981; Chapman *et al.*, 1981; Harris *et al.*, 1981). Harris *et al.* (1981) reported a substantial decrease in the 1S/1R ratio for *trans*- isomers when degradation in soil proceeded implying that 1S *trans*-isomers degraded more quickly than the 1R *trans*-isomers. But Chapman and Harris (1981) did not detect a significant difference in degradation rates between 1R and 1S isomers.

2.3.7.1.2 Field studies

Roberts and Standen (1982) demonstrated that there was little difference between the rates of degradation of cypermethrin observed in the laboratory and in the field. Their data showed that 2-4 weeks are typical half-life for the parent racemic cypermethrin during the growing season with *cis*-isomers often having twice the half-life of the *trans*-isomers. Chapman and Harris (1981) reported shorter half-life of less than 2 weeks in a mineral soil and about 3 weeks in a peat soil. Harris *et al.* (1981) reported a half-life of about 2.5 weeks for cypermethrin in Plainfield sand. The degradation of cypermethrin in soil was slowed down by high organic matter or clay content and by anaerobic conditions. These authors also found that the concentration of initial residues affected the degradation rates of

cypermethrin in soil. The degradation rates of cypermethrin in the soil with an initial concentration of 10 mg/kg was 2-3 times slower than that with an initial concentration of 0.5 mg/kg.

*2.3.7.2 Degradation pathways*

In the experiment to study the degradation pathways in soil, Roberts and Standen (1977, 1981) used either the racemic *cis*- or *trans*-isomers of cypermethrin or mixtures of the two which were $^{14}C$ radio labelled in either the benzyl or the cyclopropyl ring at a rate of 2.5 mg/kg moist soil. Three different soils were incubated under aerobic or anaerobic conditions, initially for 16 weeks and subsequently for a total of 52 weeks. The experimental results indicated that the major degradation route in all soils was hydrolysis of the ester linkage producing PBA and CCA. The main degradation products identified from the soil treated with *cis*-isomer were PBA, *cis*-CCA, plus small amounts of *trans*-CCA, and limited amounts of the 4-hydroxy derivative of cypermethrin. A similar spectrum of degradation products was detected from the *trans*-isomer treated soil, except that the *cis*-isomer was absent. The carboxylic acids formed further degraded to form carbon dioxide. A minor degradation route was ring-hydroxylation of the insecticide to form a α-cyano-3-(4-hydroxyphenoxy) benzyl ester followed by hydrolysis of the ester bond.

Sakata *et al.* (1986) studied the degradation pathways of cypermethrin in soil by incubating two Japanese soils treated with the (1R)-*cis*-(RS)-α- and (1R)-

*trans*-(RS)-α- isomers of cypermethrin which were $^{14}C$ radio labelled separately in the cyclopropyl and benzyl rings. They reported that cypermethrin degraded *via* pathways including cleavage of the ester producing CCA and PBA, or diphenyl ether bond forming the desphenoxy derivative, hydroxylation at the 4-position of the phenoxy ring, and hydrolysis of the cyano group to the amide and carboxyl groups. The main degradation route was hydrolysis of the ester linkage. The resultant products further degraded to form carbon dioxide and bound residues. More $^{14}CO_2$ was given off from the soil treated with the *trans*-isomer than with the *cis*-isomer, indicating that *trans*-isomer was more readily mineralized. Similarly, more $CO_2$ was given off from the benzyl $^{14}C$ preparation indicating that PBA was mineralized more easily than CCA. The proposed degradation pathways for cypermethrin in soil are shown in Figure 3.

### 2.3.8 Degradation within plants

#### *2.3.8.1 Degradation rate*

Ware *et al.* (1983) reported a half-life of 3.7 days for cypermethrin on the cotton leaves treated at 0.066 kg/ha EC and 4.6 days on the cotton leaves treated at 0.066 kg/ha ultra-low volume formulation. Westcott *et al.* (1987) reported that the cypermethrin residues on wheat leaves declined exponentially from initial residues of 3.74 μg/g to 0.20 μg/g 27 days after spraying when the insecticide was applied at 0.028 kg/ha to wheat. Braun *et al.* (1987) reported that cypermethrin residues on celery sprayed with 0.080 g/ha were initially 0.55 μg/g and then

decreased to 0.43, 0.13, and 0.054 μg/g by 1, 8, and 13 days after application, respectively. When lettuce was treated at the same rate, the initial residues of 0.50 μg/g decreased to 0.097 and 0.011 μg/g by 7 and 14 days after application, respectively. Furuzawa *et al.* (1981) examined the degradation rates of (1R)-*cis*- and (1R)-*trans*-cypermethrin $^{14}C$ radio labelled separately in the benzyl ring and cyclopropyl ring in cabbages at a rate of 1 μCi in 50 μL methanol per leaf by a microsyringe. The initial degradation half-life of *trans*- and *cis*-cypermethrin on and in the treated cabbage leaves were 4-5 days and 7-8 days, respectively, indicating that the *trans*-isomers degraded approximately twice as fast as the *cis*-isomers.

*2.3.8.2 Metabolic pathway*

Furuzawa *et al.* (1981) studied the metabolic pathways of (1R)-*cis*- and (1R)-*trans*- isomers on and in cabbages with $^{14}C$- preparations labelled separately in the benzyl ring and cyclopropyl ring. They reported that the metabolic pathways of both isomers of cypermethrin included epimerization to (1S)-isomers, *cis/trans*-isomerization, ester bond cleavage, hydroxylation of the phenoxy group in the alcohol moiety or the geminal methyl group in the acid moiety, hydration of the CN group to the $CONH_2$ group which subsequently hydrolyzed to the COOH group, and conjugation of the resultant carboxylic acids and alcohols with sugars. The major metabolites were glycoside conjugates of 3-(4-hydroxyphenoxy) benzoic acid and 3-phenoxybenzoic acid from the alcohol moiety, and a glycoside conjugate of 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid from the acid moiety. The isomerization proceeded more quickly from *cis*- to *trans*-

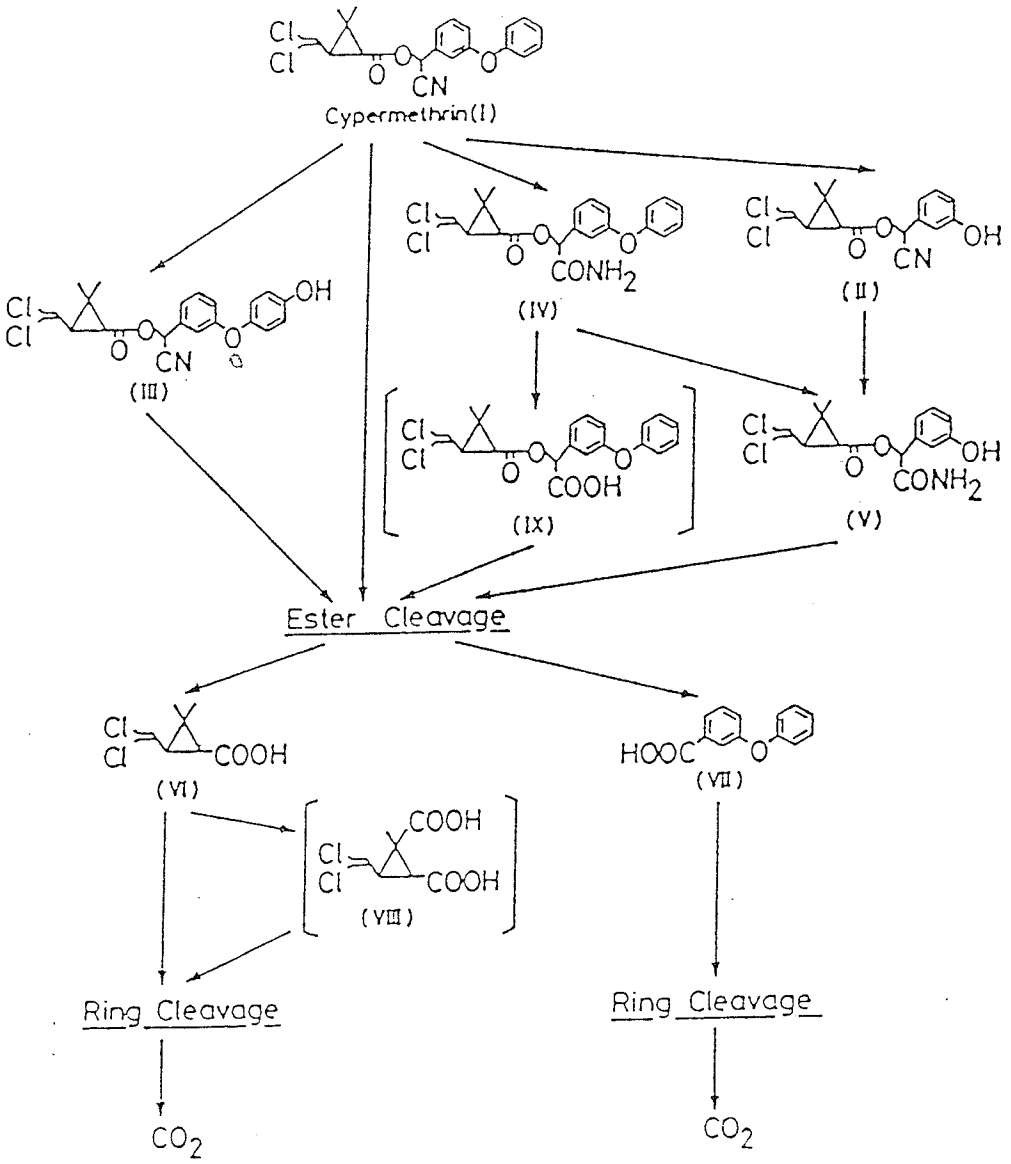



Figure 3. The proposed degradation pathways of cypermethrin in soil (Sakata et al., 1986)

isomers than from *trans*- to *cis*-isomers. The *cis/trans* isomer ratio was approximately 40/60 at equilibrium on leaves treated with *cis*-cypermethrin at the beginning. The proposed degradation pathways of *trans*- and *cis*-cypermethrin in cabbage are shown in Figure 4.

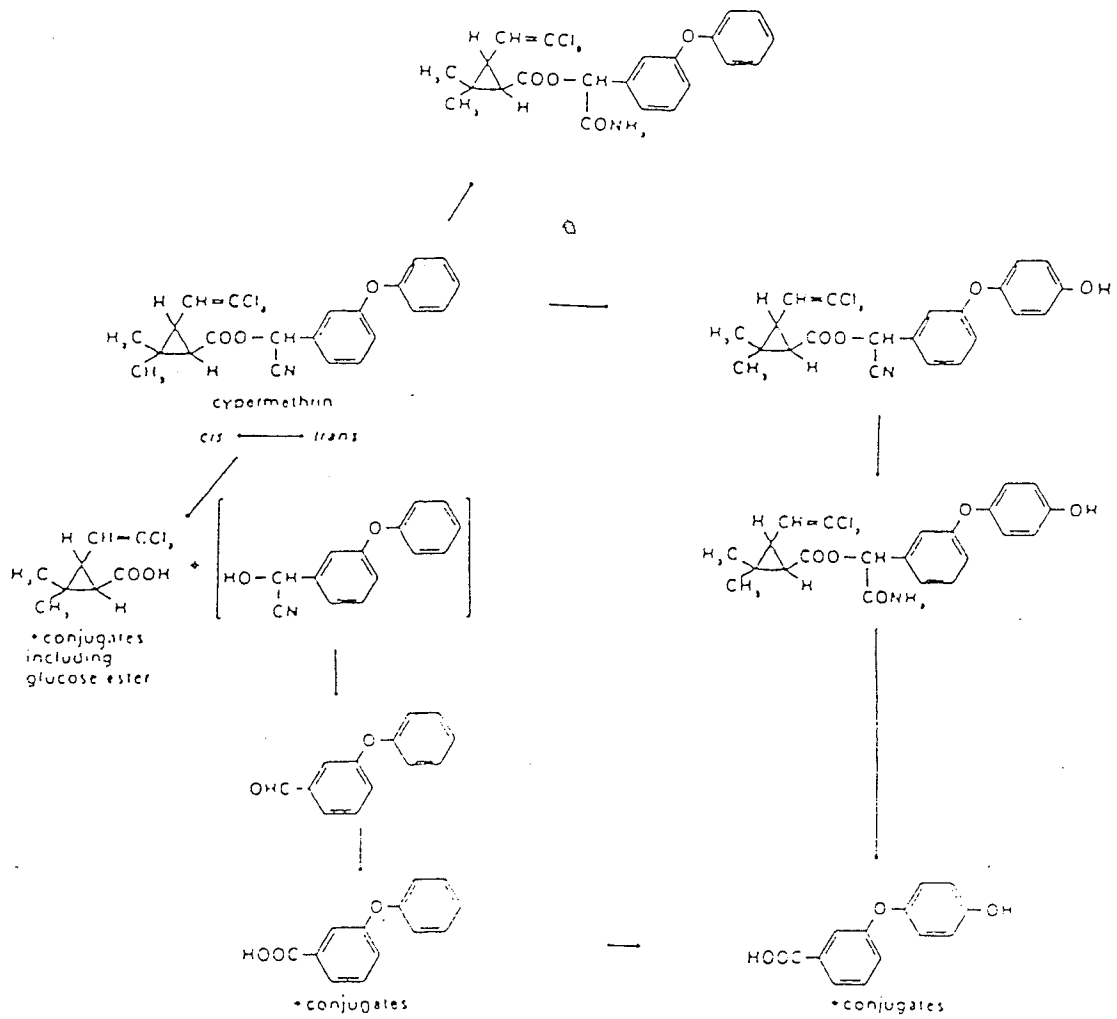



Figure 4. The proposed degradation pathways of cypermethrin in plants (Roberts and Standen, 1981)

# 3. Methods

## *3.1 Chemicals*

### 3.1.1 Reagents

Methanol, acetone, ethyl ether, water, toluene, dichloromethane, hydrochloric acid were all pesticide residue analysis grade and were purchased from Baxter (Burdick & Jackson, Muskegon, MI, U.S.A). Sodium sulphate (anhydrous) and sodium chloride (AR grade) were obtained from Mallinckrodt (Pointe-Claire, QC). Potassium hydroxide (A.C.S.), 1-methyl-3-nitro-1-nitrosoguanidine (97% purity), and α-bromo-2,3,4,5,6-pentafluoro toluene (99% purity) were from Aldrich (Milwaukee, WI, U.S.A.). Sodium carbonate (A.C.S.) was from Fisher Scientific (Nepean, ON).

### 3.1.2 Analytical Standards

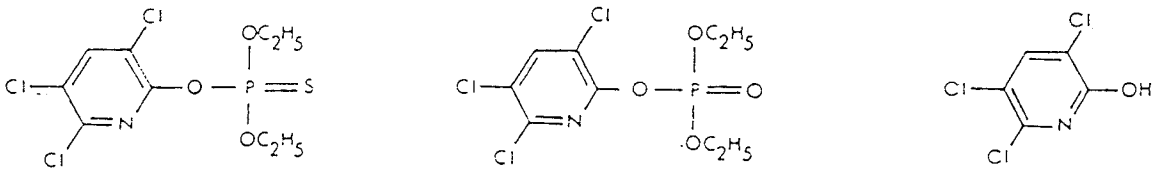
Chlorpyrifos (*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate) (99.5% purity), its oxon (*O,O*-diethyl *O*-3,5,6-trichloro-2-pyridylphosphate) (99% purity), and 3,5,6-trichloro-2-pyridinol (99.5% purity) were obtained from DowElanco (Midland, MI 48641-1706, U.S.A.). Cypermethrin *cis*-A [(RS)-α-cyano-3-phenoxybenzyl (1R) *cis*-3-(2,2-dichlorovinyl)-1,1-dimethylcyclopropanecarboxylate] (99.2% purity), cypermethrin *cis*-B [(RS)-α-cyano-3-phenoxybenzyl (1S) *cis*-3-(2,2-dichlorovinyl)-1,1-

dimethylcyclopropanecarboxylate] (98.9% purity), cypermethrin *trans*-C [(RS)-α-cyano-3-phenoxybenzyl (1R) *trans*-3-(2,2-dichlorovinyl)-1,1-dimethylcyclopropanecarboxylate] (99.4% purity), cypermethrin *trans*-D [(RS)-α-cyano-3-phenoxybenzyl (1S) *trans*-3-(2,2-dichlorovinyl)-1,1-dimethylcyclopropanecarboxylate] (99.3% purity), *cis*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylic acid (99% purity), *trans*-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylic acid (99% purity), and 3-phenoxybenzoic acid (99% purity) were all provided by ICI Agrochemicals (Jealott's Hill Research Station, Product Characterisation Group, Bracknell, Berkshire RG12 6EY, UK).

### 3.1.3 The chemical structures of target analytes

#### *3.1.3.1 Chlorpyrifos, oxon, and pyridinol*

Oxon and pyridinol are the major metabolites of chlorpyrifos.

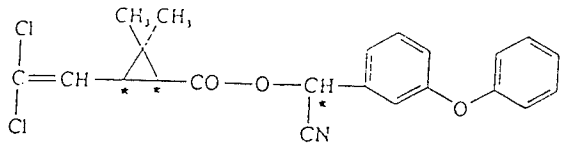


Chlorpyrifos  Oxon  Pyridinol

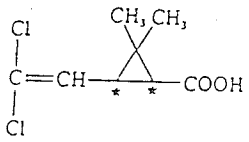
Figure 5. The chemical structures of chlorpyrifos, oxon, and pyridinol

*3.1.3.2 Cypermethrin, cis-CCA, trans-CCA, and PBA*

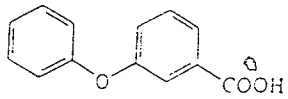
CCA and PBA are the major metabolites of cypermethrin. The chiral carbons are labelled with stars.



Cypermethrin



CCA



PBA

Figure 6. The chemical structures of cypermethrin, *cis* -CA, *trans*-CCA, and PBA

### 3.1.4 Insecticide formulations:

Dursban® 48 EC (chlorpyrifos) was the product of Dow Chemical Canada, Inc., (P.O. Box 1012, Sarnia, ON N7T 7K7). Ripcord® 40 EC (cypermethrin) was obtained from Ciba-Geigy Canada Ltd. (Agricultural Division, 6860 Century Avenue, Mississauga, ON L5N 2W5).

### 3.1.5 Preparation of diazomethane

Potassium hydroxide (2.3 g) was dissolved in 2.3 mL of water in a 125 mL conical flask; ethyl ether (25 mL) was added; and the flask was allowed to stand in

an ice bath for 15 min. Diazomethane precursor (1-methyl-nitro-nitrosoguanidine) (1.5 g) was added gradually to the flask which was slowly shaken until the reaction was complete. After the reaction had stopped, the ethyl ether layer containing the diazomethane was decanted into a container for storage. The diazomethane prepared in this way could be used for up to one week if stored in a freezer. (**Caution**: all the operations must be carried out in a fume hood because the precursor and diazomethane are mutagenic or carcinogenic).

### *3.2 Apparatus*

The apparatus used included a rotary blender (Dynamics Corporation of America, New Hartford, CT, U.S.A.); extraction apparatus consisting of 50 mL round bottom stainless steel centrifuge tubes (International Equipment Co., No. 613), stainless steel caps fitted with Teflon "O" ring gaskets (hand made by John Solomon, Winnipeg, MB), stainless steel balls of approximately 1.75 cm diameter; a shaker (Wrist Action Shaker, model 75, Burrell Corporation, Pittsburgh, PA, U.S.A.); a centrifuge (International Centrifuge, model CS, International Equipments Co., Needham Heights, MA, U.S.A.); a rotary evaporator (Büchi, Flawil, Switzerland); a hole saw (5.8 cm, d) (Canadian Tire Corporation, Ltd., Toronto,); a cordless driver drill (Makita Electric Works, Ltd., Anjo, Japan); and a gas chromatograph (Hewlett Packard 5890) equipped with auto sampler (Hewlett Packard Model 7673), DB-5 capillary column (30m x 0.32 mm), electron capture detector, and data processor [HP3365 II Chemstation (DOS Series)]. The GC

parameters were as follows: splitless injector, 200°C; oven, 100 °C for 0.5 minutes then increased to 180 °C at 30 °C/minute and kept at 180 °C for 10 minutes for chlorpyrifos and its metabolite derivatization product; and 100 °C for 0.5 minutes then increased to 230 °C at 30 °C/minute and kept at 230 °C for 10 minutes for cypermethrin isomers and their derivatized metabolite; detector, 350 °C; carrier gas, helium at 0.6 mL/minute; precolumn pressure, 110 kPa; make-up gas, 5.12% argon in methane at 60 mL/minute; purge gas, helium at 1.5 mL/minute.

The GC-MS used to confirm the analytes consisted of a gas chromatograph (Hewlett Packard 5890) equipped with splitless injector and DB-5 capillary column (60m x 0.25 mm) and ion trap detector (ITD) (Finnegan III, Model 801). The ITD settings were as follows: acquisition mode, full scan; scan range, 50 - 375 amu; scan time, 1.000 s; multiplier voltage, 1950 volts; temperature of transfer line, 285 °C; and temperature of manifold, 225 °C. The GC parameters were as follows: splitless injector, 200°C; oven, 100 °C for 6.00 minutes then increased to 250 °C at 5 °C/minute and kept at 250 °C for 3 minutes and further increased to 280 °C at 2 °C/minute and kept at 280 °C for 6 minutes; carrier gas, helium at 1 mL/minute.

### *3.3 The topography and climatic conditions of the experimental sites*

The experiments were carried out in two natival stands of American elm, Ulmus americana, near Winnipeg, Manitoba, Canada: at the Glenlea Research Station of the University of Manitoba which is adjacent to the Red River and

approximately 20 km south of the City of Winnipeg, and Beaudry Provincial Park adjacent to the Assiniboine River and approximately 20 km west of the City of Winnipeg. At the Glenlea Research Station, the elm trees used in the experiment grew along the bank of the Red River and six trees (three for chlorpyrifos treatment and three for cypermethrin treatment) for the 1991 application were subject to flooding in the spring time of each year. The elm trees in Beaudry Provincial Park were located along the bank of the Assiniboine River, but the flood waters did not reach the test trees during the experimental period. Rainwater may have accumulated on the ground when the precipitation was beyond the penetrating capability of the soil. The long term monthly average temperature and precipitation at the two experimental sites are presented at Table 1. The physical and chemical properties of soil at the two experimental sites are given at Table 2.

The amount of litter at the experimental site in Provincial Beaudry Park on June 15, August 15, October 10, 1993, and May 10, 1994 was 2.60, 1.58, 4.20, 3.80 kg (dry weight) /$m^2$, respectively.

### *3.4 Field experiment*

Forty-two healthy elm trees at each site were selected and grouped into 14 "plots" with each plot consisting of three trees. Three plots (three replicates) were allotted to chlorpyrifos treatments, three plots to cypermethrin treatments, and one plot was retained as the untreated control for the application in 1991. The

Table 1. The climatic data for the two experimental sites

| Sites | Parameters | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Glenlea (1967-1990) | Daily max T (°C) | -13 | -10 | -2.0 | 10 | 19 | 23 | 26 | 25 | 19 | 11 | 0.0 | -10 |
| | Daily min T (°C) | -24 | -21 | -12 | -2.0 | 4.5 | 10 | 13 | 12 | 6.0 | 0.0 | -9.0 | -19 |
| | Daily mean T (°C) | -18 | -15 | -7.0 | 4.0 | 12 | 17 | 20 | 18 | 12 | 6.0 | -5.0 | -15 |
| | Precipitation (mm) | 19 | 15 | 23 | 36 | 60 | 84 | 72 | 76 | 51 | 30 | 21 | 19 |
| Beaudry* (1938-1990) | Daily max T (°C) | -13 | -10 | -2.0 | 10 | 19 | 23 | 26 | 25 | 19 | 11 | -1.0 | -10 |
| | Daily min T (°C) | -24 | -21 | -13 | -2.0 | 5.0 | 10 | 13 | 11 | 6.0 | -1.0 | -10 | -20 |
| | Daily mean T (°C) | -19 | -16 | -7.0 | 4.0 | 12 | 17 | 19 | 18 | 12 | 5.0 | -5.0 | -15 |
| | Precipitation (mm) | 29 | 17 | 22 | 30 | 57 | 95 | 71 | 61 | 53 | 38 | 20 | 20 |

*: Data from the Winnipeg International Airport, approximately 20 km away from the experimental site at Beaudry Provincial Park

Table 2. The physical and chemical properties of soil at the two experimental sites

| Sites | Type | Components (%) | | | | CEC[2] | pH |
|---|---|---|---|---|---|---|---|
| | | Sand | Silt | Clay | OC[1] | | |
| Glenlea (Michalyna,1963) | St. Norbert clay | 2 | 45 | 53 | 4.2 | 45.2 | 7.6 |
| Beaudry (Michalyna, 1975) | Black Lake silt clay | 6 | 29 | 65 | 5.67 | 50.3 | 5.9 |

1. Organic Carbon
2. Cation Exchange Capacity

remaining seven plots were used for the application in 1992 with the same experimental design. The experimental plots used for 1992 application at each experimental site were located far enough from the 1991 treated plots so that the application of insecticides in 1992 would not contaminate the elm trees treated in 1991. All the treatments were arranged randomly except the check treatments which were located far enough away to avoid contamination of the check samples by insecticide drift from the treated plots. The basal 1 metre of the trunks of nine elm trees (three replicate plots) at each site was treated to run off with the label recommended dosage of 100 X aqueous dilution of 48 EC Dursban®(4.8 g a.i./L) or the effective dosage (Pajares and Lanier, 1989) of the 400 X aqueous dilution of 40 EC Ripcord® (1.0 g a.i./L) by means of back-pack sprayer on August 15, 1991 or on May 7, 1992.

### *3.5 Study of insecticide degradation in soil*

Uncontaminated soil was collected from Beaudry Provincial Park adjacent to the treatment area and air-dried and blended in the laboratory. The soil (15 g) was weighed and fortified with aqueous dilution of 48 EC Dursban® or 40 EC Ripcord® to produce a theoretical fortification concentration of 20 and 40 μg/g (w/w air-dried soil) for chlorpyrifos and 30 and 60 μg/g for cypermethrin. In total there were four treatments, two concentrations for each insecticide with three replicates and two check samples each time giving a total of 98 samples for seven times of sampling. The soil samples were wrapped in glass fibre filter paper. This paper

would not decompose but would keep the soil samples inside the paper for a long time under field conditions. The wrapped soil samples were inserted into the field soil under the litter layer at Beaudry Provincial Park on August 18, 1992 to mimic insecticide contaminated soil.

### *3.6 Sampling*

#### 3.6.1 Residue determination of chlorpyrifos and cypermethrin and their metabolites in elm bark

Samples of elm bark including the cambium layer were collected at time 0, approximately 1 hour after insecticide treatment when the treated bark surface had dried, 4, 11, 32, 62, 279, 341, 427, 627, and 791 days after application on August 15, 1991 and 0, 4, 14, 39, 74, 141, 364, 433, 532 days after the application on May 7, 1992 at the Glenlea Research Station and at Beaudry Provincial Park. Three 1.7 cm x 1.7 cm elm bark samples with cambium layer attached were taken randomly with a chisel from the insecticide-treated area of each elm tree. The nine pieces of elm bark from the three trees in each plot were combined and ground with a blender prior to extraction.

#### 3.6.2 Residue determination of chlorpyrifos, cypermethrin, and their metabolites in litter

The litter samples were taken at the same date as the elm bark samples at both experimental sites. The whole layer litter samples were collected randomly at

four points within a radius of 1 metre from each treated elm trees to give approximately 100 g dried litter sample for a plot. The litter samples taken from the 12 points around the 3 trees (a plot) were mixed well, air dried, and ground in a blender prior to extraction.

### 3.6.3 Residue determination of chlorpyrifos, cypermethrin, and their metabolites in soil

Soil samples were taken with a trowel at the same date as the elm bark and litter samples. The soil samples were taken randomly at five points within a radius of 1 metre from each treated elm tree. The sampling depth was approximately 5 cm. The soil samples taken from 15 points around the three trees in a plot produced approximately 500 g and were mixed fully in the field. As much gravel and plant tissues as possible were removed and approximately 100 g of soil was placed into a polyethylene bag. Soil was air-dried in the laboratory and well blended prior to subsampling for residue analysis.

### 3.6.4 Dissipation of chlorpyrifos and cypermethrin in soil

The soil wrapped in the glass fibre paper and embedded in the soil was collected 0, 7, 30, 58, 261, 330 days after the beginning of the experiment. There were three replicates for each treatment, the combination of the two insecticide types and the two concentrations and untreated check sample. The whole soil sample (15 g) was air-dried and analysed.

### 3.6.5 The determination of residue distribution of chlorpyrifos and cypermethrin in elm bark, cambium, and wood tissues

Samples were collected at time 0, which was approximately 1 hour after insecticide treatment when the treated bark surface had dried, 11, 62, 341, 427, and 791 days after application. Elm bark, cambium, and wood tissue were collected separately to determine the penetration of the insecticides from the treated elm bark surface at various time intervals after they were applied on the surface of the trees. This experiment was carried out only at the Glenlea Research Station and only for the 1991 application. The outer bark was collected with a 2.6 cm chisel, middle cambium with a 1.7 cm chisel, and the inner wood tissues with a 1.3 cm chisel to avoid the possible contamination of the cambium and wood layer by the insecticide. The samples of elm bark, cambium, and wood tissue were taken from five points for each insecticide. The collected sample were blended for extraction.

### 3.6.6 Determination of availability of surface residues of insecticides

Samples were taken at 0, 4, 14, 30, 74, 141, 364, 433, and 532 days after the application on May 7, 1992 at Beaudry Provincial Park to determine the availability of chlorpyrifos and cypermethrin on the insecticide treated bark surface. Samples were collected by pressing glass fibre filter paper (9 cm diameter) against the insecticide treated bark surface for 1 minute. One sample (a piece of filter paper) was taken from one of the three trees (one plot) at each sampling time.

Each treatment had three samples respectively coming from each of the three replicate plots. Samples required no further preparation before extraction.

### 3.6.7 Biological effectiveness persistence test

Bark disc samples were taken at the same time as the elm bark samples for the residue analysis. After the elm trees had been treated with chlorpyrifos or cypermethrin at Beaudry Provincial Park on August 15, 1991 or May 7, 1992, elm bark samples were taken at Beaudry Provincial Park to determine the persistence of insecticidal effectiveness of applied insecticides to kill elm bark beetles at the same time as the elm bark samples were taken for residue analysis. Elm bark disks were collected with a 5.7 cm diameter hole saw driven by a cordless drill from the treated bark of one of the three trees in each plot alternately for chlorpyrifos and cypermethrin. Each treatment had three samples, one from each of the three replicate plots.

## *3.7 Bioassay determination of insecticidal persistence*

### 3.7.1 Acquisition of test elm bark beetles

In early spring of 1992,1993, and 1994 before overwintering adult elm bark beetles had begun breeding, several piles of elm logs were placed in a wild elm stand at the Glenlea Research Station where a relatively large overwintering elm bark beetle population had been recorded. After the elm logs were colonized by elm bark beetles but before the new generation of elm beetles would appear

from the logs, the logs were transferred to two plywood boxes in the laboratory. Each box measured approximately 180 x 55 x 55 cm and in each end were two holes, diameter 15 cm, to each of which was attached a glass bottle. Two fluorescent lights (sunlight type, 60 w each) were placed over each box. The beetles emerging from the elm logs were attracted to light and collected in the bottles. The wooden container allowed the humidity within to self-regulate even after the crevices at the joints had been snugged with screws. A previously tested plastic container proved to be unsuitable. The beetles were used for bioassay within 24 hours of collection. Those beetles that could quickly climb to the top edge of a paper cylinder (28 cm deep) were selected for the bioassay tests.

### 3.7.2 Development of the elm bark beetle bioassay

Twenty elm bark beetles were introduced onto the untreated, chlorpyrifos-, or cypermethrin- treated elm bark discs that had been taken 4 days after insecticide application and encircled in transparent film strips (Canon, plastic film commonly used for overhead transparencies in photocopying machines, 21.6 cm x 2.0 cm). The time taken for appearance of bark dust from boring beetles, poisoning symptoms, and dead beetles was recorded. The poisoning symptoms were also observed. The mortality of the beetles in each treatment at various times was also measured.

### 3.7.3 Bioassay method

All the elm bark disks taken for bioassay from field elm trees were tightly encircled in 2 cm high transparent film held by rubber bands. The edges and the central hole (produced by the hole saw during sampling) of the elm bark discs were completely covered with molten paraffin wax to prevent the elm bark beetles from contacting the untreated edge of the discs. Twenty elm bark beetles were exposed to each elm bark disc in the transparent film cylinder. The ends of the transparent film cylinders were covered with petri dishes. The samples were moved into an incubator at 25°C, photoperiod of 13 hours (7 a.m.- 8 p.m.) light and 11 hours dark, and with the relative humidity elevated by exposure of an open pan of water. The mortality of test beetles for each insecticide was determined by the criterion that a beetle which could not move as far as its body length was considered dead. Mortality of tested elm bark beetles in each sample was checked after 24 hours. After the mortality had been recorded, the tested beetles were transferred onto untreated elm bark and kept in an incubator with the same conditions as the above for another 24 hours. Further mortalities for the beetles were recorded again after this 24 hour period.

## *3.8 Residue analysis*

### 3.8.1 The ball mill extraction method

This method (Solomon and Lockhart, 1977) was used to extract the residues of chlorpyrifos, cypermethrin, and their metabolites from elm bark, soil, and litter samples.

#### *3.8.1.1 Extraction*

The homogenized air-dried soil (15 g), or ground elm bark (2.5 g), or ground air-dried litter (2.5 g) was weighed into stainless steel centrifuge tubes containing two stainless steel balls. Methanol (25 mL) and 0.6 N HCl (5 mL) were added to the tube. The tightly sealed capped tubes were mounted horizontally on a wrist-action shaker and shaken for 30 minutes during which the sample was further ground by the two rolling balls. After shaking, the tubes were centrifuged for 20 minute at 2900 rpm.

#### *3.8.1.2 Liquid-liquid partition clean-up*

The methanol extraction aliquot (5 mL, equivalent to 2.5 g soil, 0.417 g litter or bark) was pipetted into separatory funnels with 50 mL of 2% sodium chloride aqueous solution, 20 mL dichloromethane and 1 mL of 2.8 N HCl. The separatory funnel was vigorously shaken for about 2 minutes. The dichloromethane phase was separated from the aqueous phase and drained through an anhydrous sodium sulphate layer into 250 mL round bottom flasks. The remaining aqueous

phase was extracted with another 20 mL of dichloromethane. The two dichloromethane extracts were combined and concentrated to approximately 0.5 mL on a rotary evaporator at 40°C and then dry nitrogen was used to remove the dichloromethane completely. The extracts were ready for derivatization of the metabolites.

*3.8.1.3 Derivatization of pyridinol*

Pyridinol which had been extracted with chlorpyrifos and oxon required derivatization for GC analysis. The pyridinol extract was quantitatively transferred from the round bottom flask into 15-mL graduated centrifuge tubes using methanol, and concentrated under dry nitrogen to dryness. Four drops of methanol and 1 mL diazomethane in ethyl ether were added to the concentrated extracts. The reagents and the extracts were mixed fully and the mixture was allowed to stand 30 minutes at room temperature. Dry nitrogen gas was used to remove the ethyl ether and methanol to dryness. Toluene (1 mL) was added and mixed fully. The mixture was diluted with toluene to an appropriate volume to facilitate analysis by gas chromatograph.

*3.8.1.4 Derivatization for CCA and PBA*

The cypermethrin metabolites are carboxylic acids which should be derivatized prior to gas chromatographic analysis. All residue extracts (cypermethrin and metabolites) were quantitatively transferred from the round bottom flask to 15 mL graduated centrifuge tubes with methanol, and concentrated

under dry nitrogen to dryness. Two drops of 30% sodium carbonate, 50 μL of 10% alpha-bromo-2,3,4,5,6-pentafluoro toluene in acetone, and 1 mL of acetone were added and mixed well; the mixture was allowed to stand 1 hour at room temperature. Dry nitrogen was used to remove acetone and water to dryness. NaCl aqueous solution (2%, 0.5 mL) and toluene (1 mL) were added to the tubes and the tubes were agitated vigorously for 1 minute. After toluene was separated from the aqueous phase, the toluene solution (1.00 μL) was taken directly for GC analysis. If required, the toluene solution was diluted with toluene to an appropriate volume for gas chromatographic analysis.

### 3.8.2 Soxhlet extraction method

Glass fibre filter paper samples taken to determine the availability of insecticide surface residue from the elm bark were put into Soxhlet thimbles and the filter paper was exhaustively extracted with 60 mL acetone for 3 hours. The extracts were concentrated on a rotary evaporator to about 0.5 mL and then dried with dry nitrogen. Toluene (1 mL) was added to the extracts. If required, the toluene solution was diluted to an appropriate volume to facilitate gas chromatographic analysis for the chlorpyrifos and oxon, or cypermethrin.

## *3.9 Gas chromatographic analysis*

The amount of analytes was determined by electron capture detection under analytic conditions stated above by comparing the peak areas from the individual sample to the standard curves made from the peak area for known

amounts of the analytes. Standard curves were produced by the computer software (HP3365 II Chemstation, DOS Series) through plotting peak areas against known amounts of analytes. The final volumes of the samples were adjusted so that all the sample peak area of the individual analytes was covered by the areas produced by the maximum standard analytes when 1.0 µL sample was injected. The peak area of the individual analytes in the samples was directly converted to residue concentration of the individual analytes through setting appropriate parameters in the computer program.

To determine the metabolites that required derivatizing prior to gas chromatographic analysis, standard solutions of the target analytes passed through the same derivatization process as real samples and standard curves were made for each group of samples to be analysed. The samples were analyzed as soon as they were prepared by injecting them into the gas chromatograph with an auto-sampler.

### *3.10 Identification*

The analytes eluting from the gas chromatograph column were identified by their retention time. The identification was further confirmed with an ion trap detector.

### *3.11 Fortification*

Recoveries of chlorpyrifos, cypermethrin (four pairs of isomers), and their metabolites were determined in triplicate at appropriate levels in the different

matrices. The fortifications were made by adding appropriate amounts of chlorpyrifos, oxon, or cypermethrin isomers in acetone, and pyridinol, *cis*-CCA, *trans*-CCA, or PBA in methanol into the homogenized, weighed ground elm bark, litter, or soil samples, or to intact glass fibre paper to give designated fortification levels in these matrices before the extracting solvent was added. The samples fortified with standards were allowed to stand for 1 hour to let the solvent evaporate. The fortified samples were then subjected to the procedures outlined above. The determinations of fortification levels of individual analytes in different matrices depended on the actual residue levels of the analytes in the samples. The range of fortification concentrations covered that of the residue concentration found in the actual samples.

# 4. Results

## *4.1 Analytical method development*

The feasibility of an analytical method for pesticide residues depends on how effectively the residues can be extracted, how low a concentration of residues can be detected; and how reproducible the method is, i.e., its accuracy, limit of detection, and precision. The fortification recoveries reflect the methodological accuracy, and the standard deviation the method's precision. The limits of detection for an individual analyte in a sample depend on the instrument sensitivity for that compound and the concentration of the interfering substances for the target analytes.

Table 3 presents the ball-mill extraction recoveries and standard deviations of chlorpyrifos, oxon, and pyridinol fortified in elm bark, litter, and soil at various levels. The table also includes the recoveries of chlorpyrifos in the glass fibre filter paper used for the determination of contact surface residues availability through Soxhlet extraction.

The recovery data for *cis*-A, *trans*-C, *cis*-B, and *trans*-D cypermethrin isomers at the fortification levels of 2, 10, and 100 μg/g in elm bark, 2, 15, and 150 μg/g in litter and 0.2, 2, and 20 μg/g in soil are tabulated in Table 4. Recovery values represented the average of three determinations at each fortification level in each of three substrates.

Table 3. The recoveries and standard deviations for chlorpyrifos, oxon, and pyridinol in elm bark, litter, soil, and glass fibre filter paper

| Analytes | Matrices | Fortified Concentrations (μg/g) | Mean (n=3) Recoveries (%) | Standard Deviations (%) |
|---|---|---|---|---|
| Chlorpyrifos | Elm bark | 500 | 97 | 3.8 |
| | | 50 | 94 | 4.2 |
| | | 5 | 97 | 5.0 |
| | Litter | 1000 | 98 | 1.5 |
| | | 50 | 97 | 1.2 |
| | | 1 | 97 | 1.9 |
| | Soil | 30 | 97 | 1.5 |
| | | 10 | 94 | 8.2 |
| | | 1 | 100 | 2.1 |
| | Filter paper* | 1100(μg/ $m^2$) | 93 | 3.5 |
| | | 0.5 (μg/ $m^2$) | 90 | 3.1 |
| Oxon | Elm bark | 5 | 91 | 4.9 |
| | | 1 | 92 | 5.3 |
| | | 0.5 | 92 | 10 |
| | Litter | 5 | 98 | 1.6 |
| | | 1 | 90 | 5.2 |
| | | 0.5 | 96 | 2.8 |
| | Soil | 1 | 98 | 3.8 |
| | | 0.5 | 95 | 5.7 |
| | | 0.1 | 99 | 2.6 |
| Pyridinol | Elm bark | 30 | 91 | 0.6 |
| | | 15 | 98 | 0.6 |
| | | 1 | 97 | 1.5 |
| | Litter | 100 | 99 | 0.4 |
| | | 10 | 96 | 2.7 |
| | | 1 | 96 | 2.7 |
| | Soil | 15 | 96 | 2.7 |
| | | 8 | 93 | 4.2 |
| | | 1 | 97 | 3.4 |

*: Contact availability test for residues on the treated elm bark

Table 4. The recoveries and standard deviations for *cis*-A, *trans*-C, *cis-B*, and *trans*-D cypermethrin isomer pairs in elm bark, litter, soil, and glass fibre filter paper

| Analytes | Matrices | Fortified Concentrations (µg/g) | Mean (n=3) Recoveries (%) | Standard Deviations (%) |
|---|---|---|---|---|
| *Cis*-A Cypermethrin | Elm Bark | 100 | 110 | 6.7 |
| | | 10 | 100 | 8.5 |
| | | 2 | 97 | 4.2 |
| | Litter | 150 | 96 | 5.7 |
| | | 15 | 110 | 5.5 |
| | | 2 | 110 | 6.1 |
| | Soil | 20 | 93 | 2.3 |
| | | 2 | 93 | 3.5 |
| | | 0.2 | 100 | 3.5 |
| | Filter Paper | 700 (µg/ $m^2$) | 92 | 5.2 |
| | | 0.1 (µg/ $m^2$) | 94 | 6.1 |
| *Trans*-C Cypermethrin | Elm Bark | 100 | 100 | 9.8 |
| | | 10 | 100 | 11 |
| | | 2 | 100 | 3.5 |
| | Litter | 150 | 93 | 7.5 |
| | | 15 | 100 | 6.5 |
| | | 2 | 100 | 7.0 |
| | Soil | 20 | 93 | 2.9 |
| | | 2 | 91 | 5.5 |
| | | 0.2 | 110 | 7.5 |
| | Filter Paper | 700 (µg/ $m^2$) | 93 | 5.5 |
| | | 0.1 (µg/ $m^2$) | 96 | 3.2 |
| *Cis*-B Cypermethrin | Elm Bark | 100 | 100 | 8.6 |
| | | 10 | 100 | 6.4 |
| | | 2 | 98 | 5.2 |
| | Litter | 150 | 91 | 2.3 |
| | | 15 | 110 | 4.1 |
| | | 2 | 100 | 5.8 |
| | Soil | 20 | 91 | 2.9 |
| | | 2 | 98 | 10 |
| | | 0.2 | 110 | 5.8 |
| | Filter Paper | 700 (µg/ $m^2$) | 94 | 5.2 |
| | | 0.1 (µg/ $m^2$) | 97 | 5.5 |
| *Trans*-D Cypermethrin | Elm Bark | 100 | 100 | 8.2 |
| | | 10 | 100 | 13 |
| | | 2 | 96 | 4.1 |
| | Litter | 150 | 94 | 11 |
| | | 15 | 110 | 4.1 |
| | | 2 | 100 | 4.2 |
| | Soil | 20 | 90 | 5.1 |
| | | 2 | 82 | 2.9 |
| | | 0.2 | 99 | 3.5 |
| | Filter Paper | 700 (µg/ $m^2$) | 94 | 4.5 |
| | | 0.1 (µg/ $m^2$) | 97 | 3.1 |

The recoveries of cypermethrin metabolites, *cis*-CCA, *trans*-CCA, and PBA at the fortification levels of 0.5, 5, and 10 μg/g in elm bark, and litter, and 0.1, 1, and 10 μg/g in soil are listed in Table 5. The recoveries of these analytes in all three matrices at all three fortification levels were quantitatively high ranging from 83 to 107%; the standard deviations calculated from the three replicates for the combination of analytes, substrates, and fortification levels ranged from 2 to 12%.

Typical gas chromatograms for the analytical standards of methylated pyridinol, oxon and chlorpyrifos, untreated control elm bark, and samples of elm bark treated with chlorpyrifos are shown in Figure 7. Typical gas chromatograms for the analytical standards, untreated litter control, and litter samples taken around the elm trees treated with chlorpyrifos are displayed in Figure 8. Typical gas chromatograms for the analytical standards, untreated control soil, and the soil sample taken around the treated elm trees with chlorpyrifos are shown in Figure 9. The retention time, the minimum detectable amount in the gas chromatograph, and the detection limits of methylated pyridinol, oxon, and chlorpyrifos in the elm bark, litter, and soil are listed in Table 6. All three figures indicate that no significant interfering peaks exist at the retention times of the target analytes.

Typical gas chromatograms of analytical standards of the four pairs of cypermethrin isomers and their three metabolites, untreated control elm bark, and elm bark samples treated with cypermethrin are shown in Figure 10. Typical gas chromatograms of analytical standard, untreated control litter, and a litter sample taken around treated elm trees area are displayed in Figure 11; and typical gas

chromatograms of analytical standards, untreated control soils, and soil samples collected around the treated elm trees are shown in Figure 12. The gas chromatograms showed seven completely separate target peaks. They were identified by retention time as *cis*-CCA, *trans*-CCA, PBA, *cis*-A cypermethrin isomers, *trans*-C cypermethrin isomers, *cis*-B cypermethrin isomers, and *trans*-D cypermethrin isomers. A total of eight cypermethrin isomers appeared in four peaks, with each peak consisting of a pair of isomers which could not be further separated on the GC. The retention times, the minimum detectable amount (2 X GC noise), and the detection limits of *cis*-CCA, *trans*-CCA, PBA, *cis-A* cypermethrin isomers, *trans*-C cypermethrin isomers, *cis*-B cypermethrin isomers, and *trans*-D cypermethrin isomers are tabulated in Table 6.

### *4.2 Dissipation of chlorpyrifos and its metabolites in elm bark*

The residues and standard deviations of chlorpyrifos, oxon, and pyridinol in elm bark taken at different intervals after chlorpyrifos treatment at the Glenlea Research Station and Beaudry Provincial Park in 1991, and 1992 applications are shown in Table 7. The initial residues of chlorpyrifos determined after 1 hour from the application ranged from 388 μg/g in the 1992 application to 444 μg/g in the 1991 application at Beaudry Provincial Park. Table 8 indicates that It took 11 - 68 days for chlorpyrifos on the elm bark to dissipate from the initial residues to half these levels. Approximately 90% of applied chlorpyrifos dissipated from the treated elm surface after 791 days from the treatments at the two sites.

Table 5. The recoveries and standard deviations for *cis*-CCA, *trans*-CCA, and PBA in elm bark, litter, and soil

| Analytes | Matrices | Fortified Concentrations (μg/g) | Mean (n=3) Recoveries (%) | Standard Deviations (%) |
|---|---|---|---|---|
| *Cis*-CCA | Elm Bark | 10 | 110 | 9.5 |
| | | 5 | 110 | 5.3 |
| | | 0.5 | 88 | 6.4 |
| | Litter | 10 | 98 | 6.7 |
| | | 5 | 110 | 6.4 |
| | | 0.5 | 96 | 3.4 |
| | Soil | 10 | 92 | 7.0 |
| | | 1 | 110 | 3.2 |
| | | 0.1 | 100 | 8.5 |
| *Trans*-CCA | Elm Bark | 10 | 94 | 2.9 |
| | | 5 | 110 | 5.9 |
| | | 0.5 | 86 | 5.5 |
| | Litter | 10 | 99 | 2.9 |
| | | 5 | 95 | 7.1 |
| | | 0.5 | 91 | 4.6 |
| | Soil | 10 | 90 | 7.6 |
| | | 1 | 110 | 2.1 |
| | | 0.1 | 91 | 3.5 |
| PBA | Elm Bark | 10 | 90 | 5.8 |
| | | 5 | 95 | 2.3 |
| | | 0.5 | 92 | 6.1 |
| | Litter | 10 | 88 | 5.5 |
| | | 5 | 100 | 4.2 |
| | | 0.5 | 99 | 5.7 |
| | Soil | 10 | 83 | 6.9 |
| | | 1 | 84 | 2.0 |
| | | 0.1 | 100 | 4.2 |

Table 6. The retention times, minimum detectable amounts, and detection limits of the target analytes in different matrices

| Analytes | Retention Time (Minutes) | Minimum Detectable Amount (ng) | Detection Limit (µg/g) | | |
|---|---|---|---|---|---|
| | | | Elm bark | Litter | Soil |
| Chlorpyrifos | 11.6 | 0.002 | 0.024 | 0.024 | 0.004 |
| Oxon | 10.8 | 0.004 | 0.048 | 0.048 | 0.008 |
| Pyridinol | 5.4 | 0.002 | 0.024 | 0.024 | 0.004 |
| *Cis*-CCA | 5.9 | 0.002 | 0.024 | 0.024 | 0.002 |
| *Trans*-CCA | 6.0 | 0.002 | 0.024 | 0.024 | 0.002 |
| PBA | 9.5 | 0.004 | 0.048 | 0.048 | 0.005 |
| *Cis*-A Cypermethrin | 20.6 | 0.015 | 0.180 | 0.180 | 0.018 |
| *Trans*-C Cypermethrin | 21.2 | 0.015 | 0.180 | 0.180 | 0.018 |
| *Cis*-B Cypermethrin | 21.6 | 0.015 | 0.180 | 0.180 | 0.018 |
| *Trans*-D Cypermethrin | 21.9 | 0.015 | 0.180 | 0.180 | 0.018 |

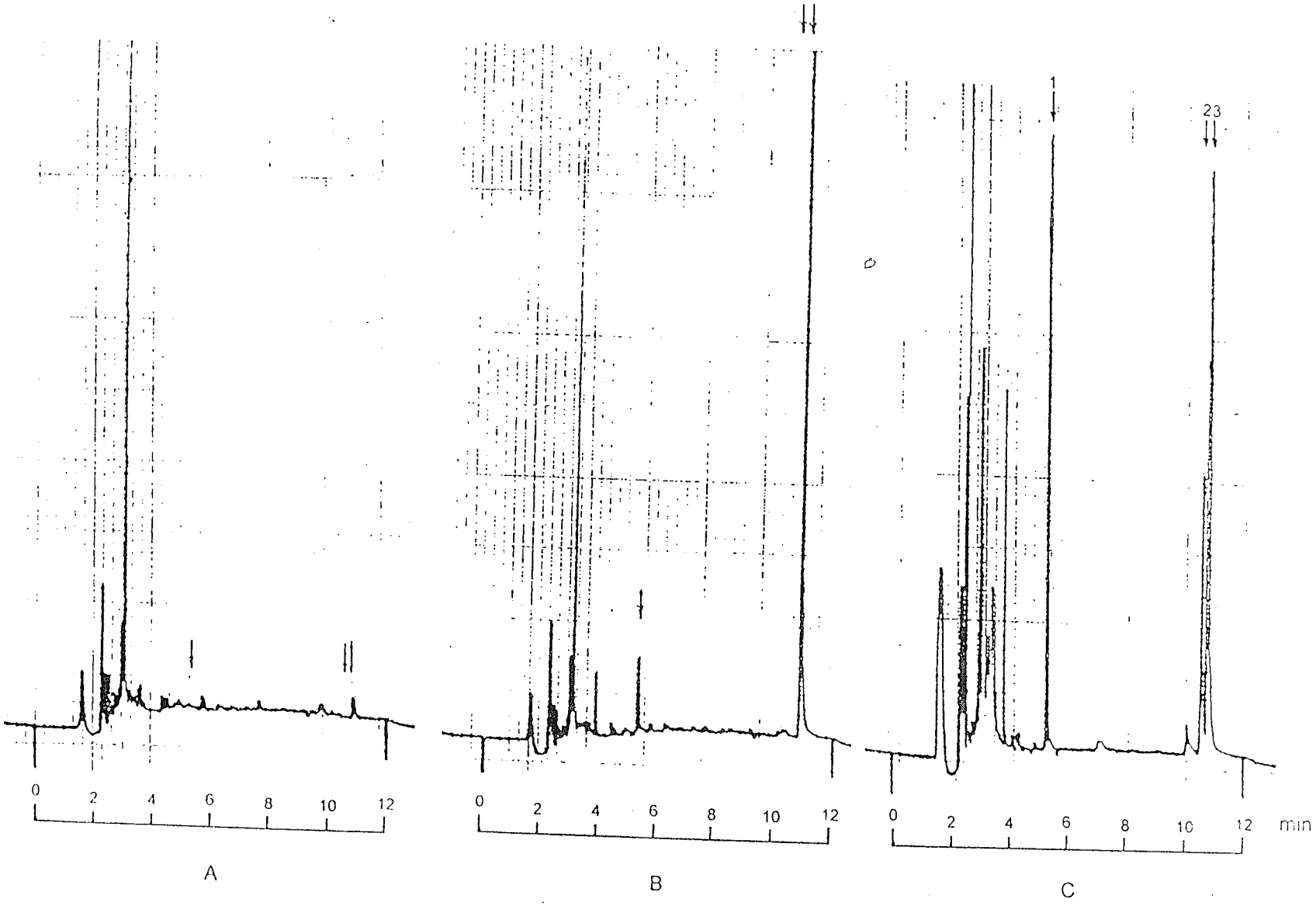



Figure 7 Typical GC-ECD chromatograms of untreated elm bark control (A), treated elm bark sample(B), and standards(C) of pyridinol (1), oxon (2), and chlorpyrifos (3) (arrowed from left to right)

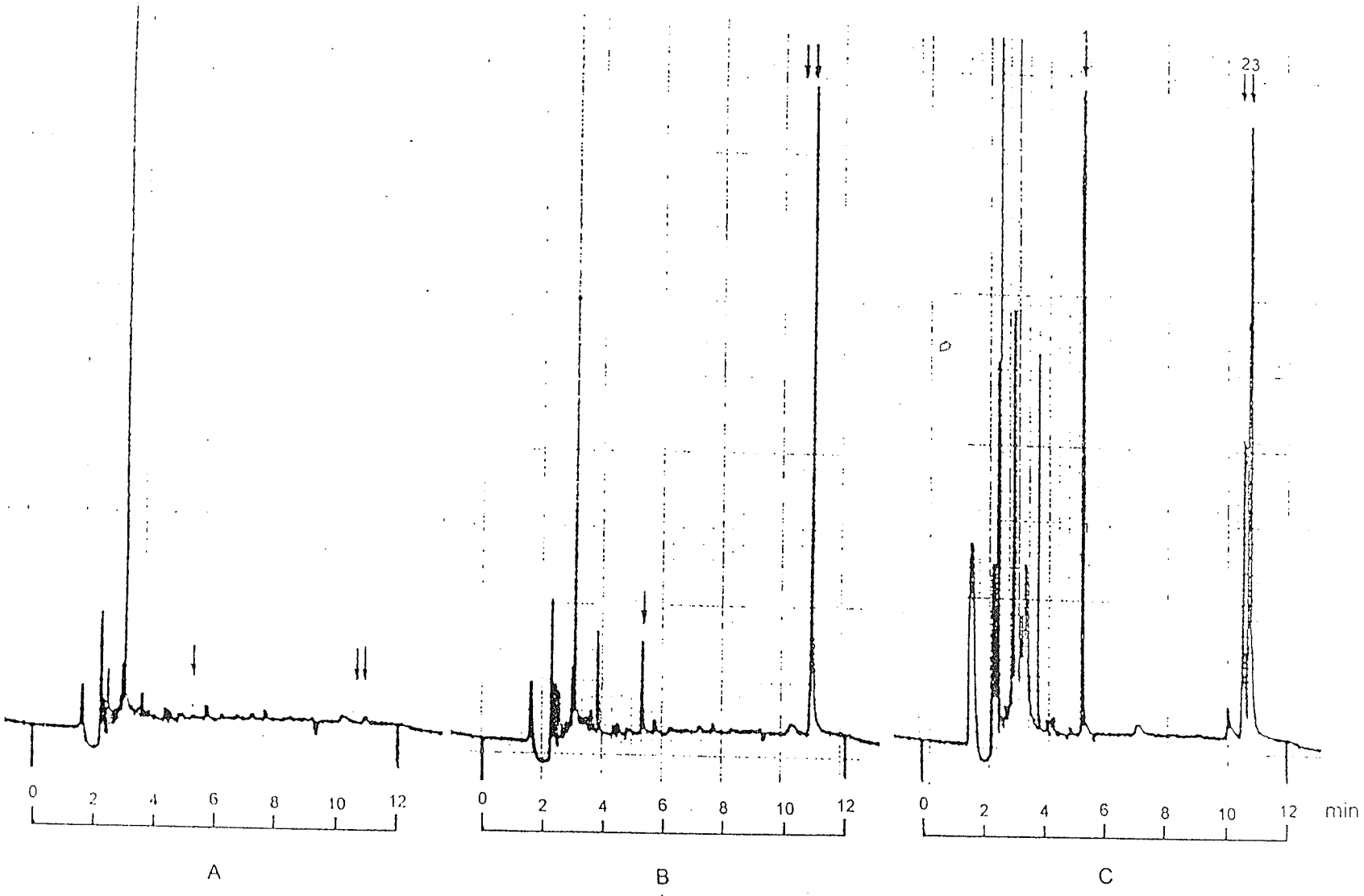



Figure 8 Typical GC-ECD chromatograms of untreated litter control (A), treated litter sample(B), and standards(C) of pyridinol (1), oxon (2), and chlorpyrifos (3) (arrowed from left to right)

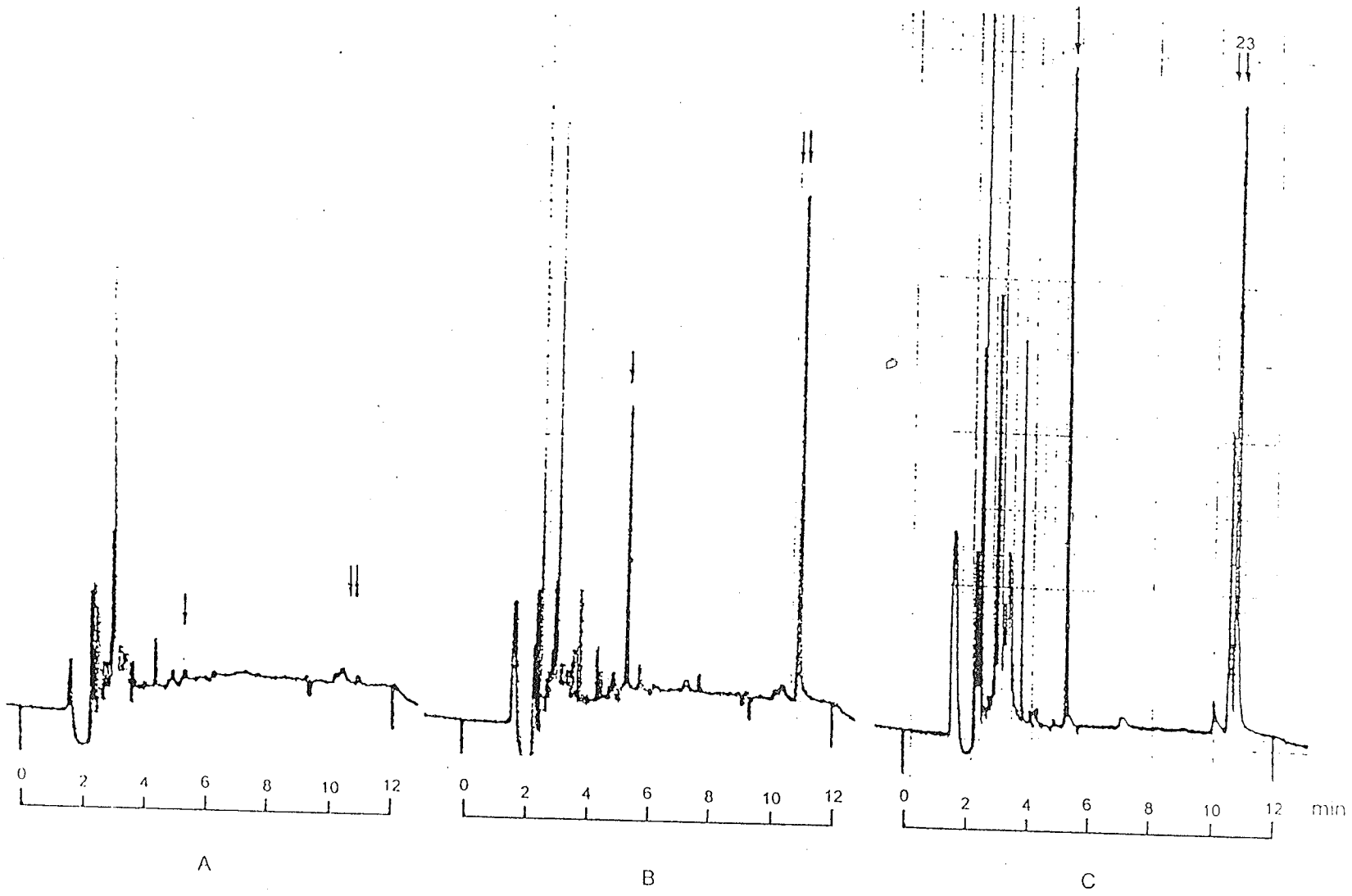



Figure 9 Typical GC-ECD chromatograms of untreated soil control (A), treated soil sample(B), and standards(C) of pyridinol (1), oxon (2), and chlorpyrifos (3) (arrowed from left to right)

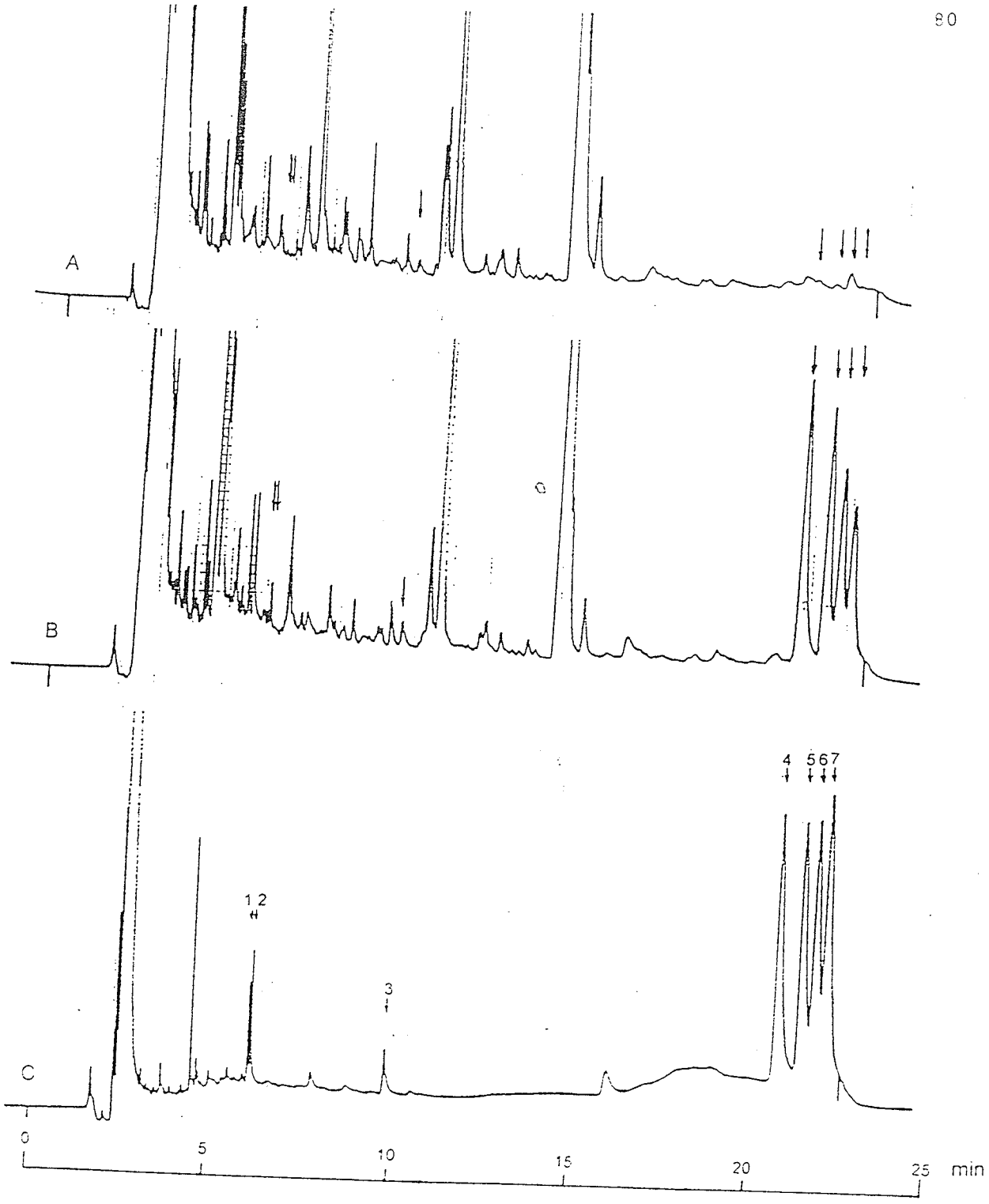



Figure 10 Typical GC-ECD chromatograms of untreated elm bark control (A), treated elm bark sample(B), and standards(C) of *cis* CCA (1), *trans* CCA(2), PBA(3), *cis* A (4), *trans* C (5), *cis* B (6), and *trans* D (7) cypermethrin isomer pairs(arrowed from left to right)

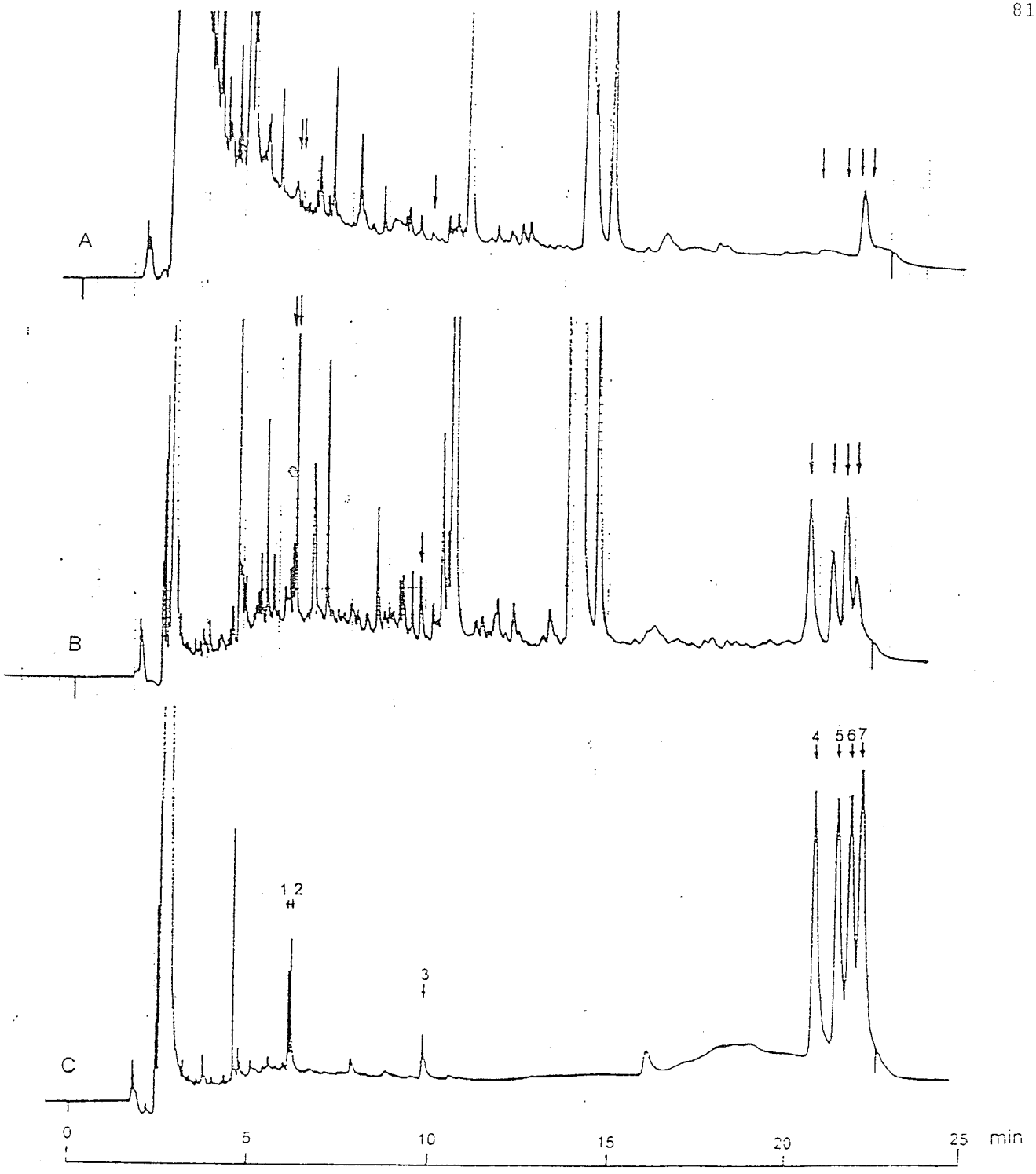



Figure 11 Typical GC-ECD chromatograms of untreated litter control (A), treated litter sample(B), and standards(C) of *cis* CCA (1), *trans* CCA (2), PBA (3), *cis* A (4), *trans* C (5), *cis* B (6), and *trans* D (7) cypermethrin isomer pairs(arrowed from left to right)

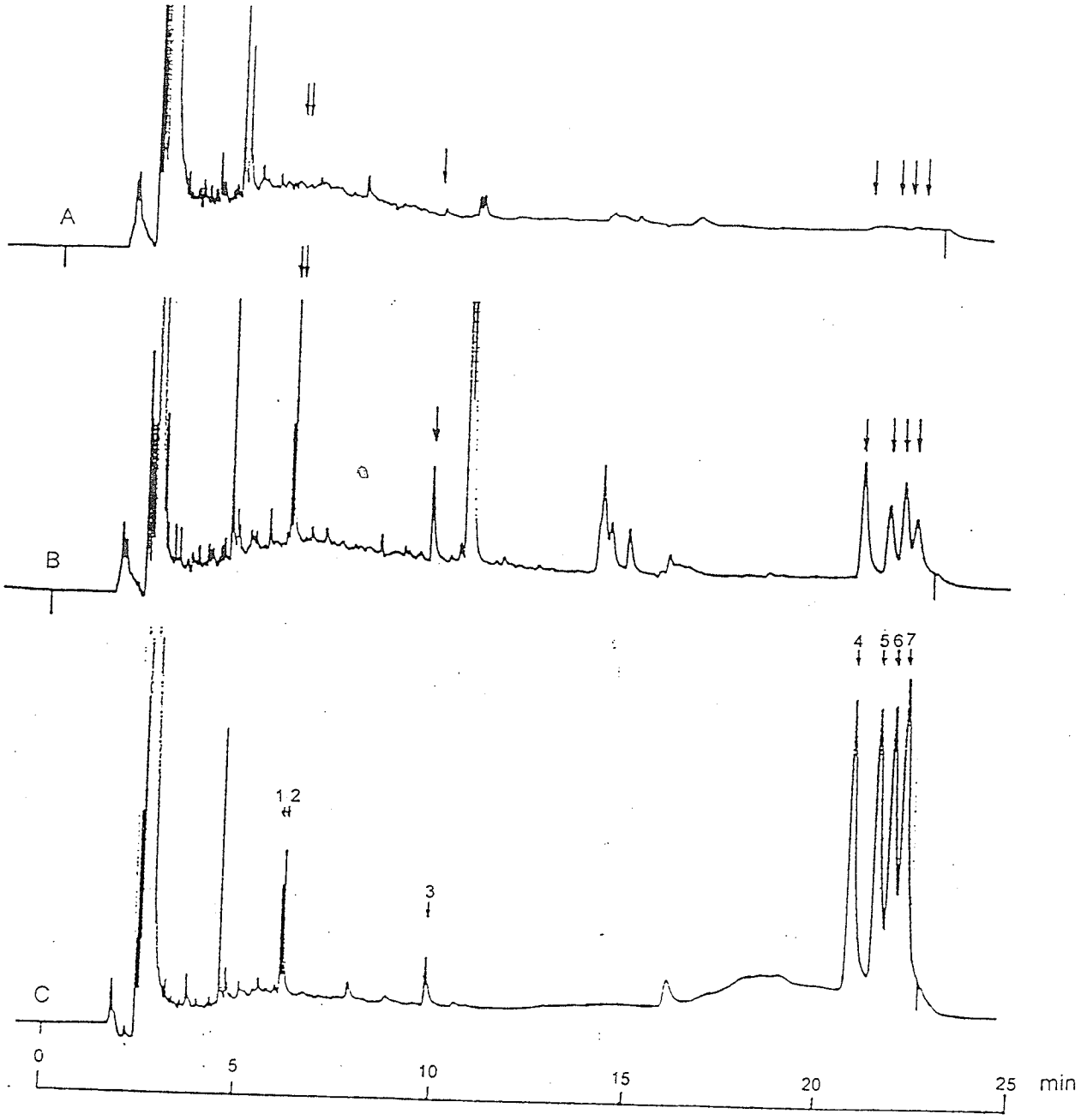



Figure 12 Typical GC-ECD chromatograms of untreated soil control (A), treated soil sample(B), and standards(C) of *cis* CCA (1), *trans* CCA (2), PBA (3), *cis* A (4), *trans* C(5), *cis* B (6), and *trans* D (7) cypermethrin isomer pairs(arrowed from left to right)

Table 7. The residues and standard deviations (n=3) of chlorpyrifos, oxon, and pyridinol in elm bark at both experimental sites and application times

| Sampling Date | Days after Application | Chlorpyrifos | | | | Oxon | | | | Pyridinol | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Glenlea | | Beaudry | | Glenlea | | Beaudry | | Glenlea | | Beaudry | |
| | | Residues (μg/g) | SD (μg/g) | Residues (μg/g) | SD (μg/g) | Residues (μg/g) | SD (μg/g) | Residues (μg/g) | SD (μg/g) | Residues (μg/g) | SD (μg/g) | Residues (μg/g) | SD (μg/g) |
| 91.08.15* | 0 | 433 | 43.1 | 444 | 59.9 | ND | 0 | ND | 0 | 3.71 | 0.61 | 3.91 | 0.40 |
| 91.08.19 | 4 | 289 | 32.2 | 294 | 21.2 | ND | 0 | ND | 0 | 6.40 | 0.68 | 4.23 | 0.11 |
| 91.08.26 | 11 | 288 | 24.1 | 273 | 26.3 | ND | 0 | ND | 0 | 10.1 | 2.78 | 6.18 | 1.89 |
| 91.09.19 | 32 | 243 | 30.9 | 247 | 85.4 | ND | 0 | ND | 0 | 21.2 | 6.40 | 6.89 | 1.38 |
| 91.10.16 | 62 | 219 | 36.6 | 177 | 36.4 | ND | 0 | ND | 0 | 20.1 | 3.81 | 3.56 | 1.01 |
| 92.05.20 | 279 | 125 | 10.5 | 119 | 37.9 | ND | 0 | ND | 0 | 15.9 | 2.89 | 5.89 | 0.89 |
| 92.07.21 | 341 | 67.4 | 6.80 | 59.4 | 16.5 | ND | 0 | ND | 0 | 8.31 | 3.67 | 2.56 | 0.18 |
| 92.10.15 | 427 | 57.3 | 1.70 | 56.3 | 2.80 | ND | 0 | ND | 0 | 3.09 | 0.90 | 0.71 | 0.57 |
| 93.05.03 | 627 | 46.7 | 11.8 | 44.3 | 10.3 | ND | 0 | ND | 0 | 6.08 | 2.02 | 4.48 | 0.69 |
| 93.10.14 | 791 | 41.3 | 6.40 | 39.7 | 10.7 | ND | 0 | ND | 0 | 8.80 | 2.49 | 3.59 | 1.01 |
| 92.05.07* | 0 | 408 | 48.6 | 388 | 127 | ND | 0 | ND | 0 | 3.89 | 0.48 | 2.45 | 0.70 |
| 92.05.11 | 4 | 246 | 66.1 | 237 | 28.3 | ND | 0 | ND | 0 | 3.71 | 0.89 | 2.50 | 0.31 |
| 92.05.21 | 14 | 228 | 11.1 | 173 | 14.3 | ND | 0 | ND | 0 | 18.1 | 6.01 | 9.91 | 1.09 |
| 92.06.15 | 30 | 198 | 33.3 | 155 | 27.0 | ND | 0 | ND | 0 | 14.2 | 3.31 | 5.68 | 0.51 |
| 92.07.20 | 74 | 164 | 25.1 | 111 | 28.1 | ND | 0 | ND | 0 | 5.40 | 2.78 | 1.78 | 0.89 |
| 92.09.25 | 141 | 117 | 12.9 | 67.7 | 13.0 | ND | 0 | ND | 0 | 4.56 | 0.38 | 1.80 | 0.38 |
| 93.05.06 | 364 | 69.3 | 16.4 | 54.6 | 11.2 | ND | 0 | ND | 0 | 20.1 | 6.01 | 8.19 | 1.38 |
| 93.07.14 | 433 | 62.0 | 16.8 | 54.3 | 8.00 | ND | 0 | ND | 0 | 13.2 | 4.78 | 6.18 | 1.01 |
| 93.10.21 | 532 | 46.8 | 9.90 | 42.4 | 3.80 | ND | 0 | ND | 0 | 11.9 | 4.79 | 3.80 | 0.72 |

*: Application time of chlorpyrifos

Figures 13, 14, 15, and 16 show the chlorpyrifos dissipation in elm bark at the time after treatment and the corresponding chlorpyrifos residues at both experimental sites and both application times. Chlorpyrifos dissipated very quickly in the elm bark. The dissipation rate of chlorpyrifos during the later period was much slower than during the earlier period.

The chlorpyrifos oxygen analogue, oxon, could not be detected during the experimental period at either site or application time. The residues of pyridinol in the elm bark fluctuated with time after treatment with a low initial concentration and then an increase in residues with time. The residues of pyridinol detected in the elm bark ranged from 0.71 to 21.2 μg/g. Higher residue concentrations of pyridinol were found at the Glenlea Research Station than at the Beaudry Provincial Park.

### *4.3 Dissipation of cypermethrin isomers and their metabolites in elm bark*

Tables 9 and 10 show the residues and standard deviations of four pairs of cypermethrin isomers and their major metabolites in elm bark in 1991, and 1992 applications at the Glenlea Research Station and Beaudry Provincial Park. The initial cypermethrin residues in the bark were higher in the 1991 application than in the 1992 application. Table 11 listed the DT50 and DT90 values of the four pairs of cypermethrin isomers in elm bark. It took 31 - 140 days for cypermethrin to dissipate from the initial residues to half initial levels depending on the treatments and isomers. The residues of cis-isomers dissipated approximately 89% after 791

Table 8. The $DT_{50}$ and $DT_{90}$ of chlorpyrifos in elm bark

| Treatment | $DT_{50}$ (Days) | $DT_{90}$ (Days) |
|---|---|---|
| Glenlea, 1991 | 68 | 730 |
| Glenlea, 1992 | 27 | 570* |
| Beaudry, 1991 | 43 | 630 |
| Beaudry, 1992 | 11 | 560* |

*: The values are estimated based on the residue concentrations in the samples of the last two times.

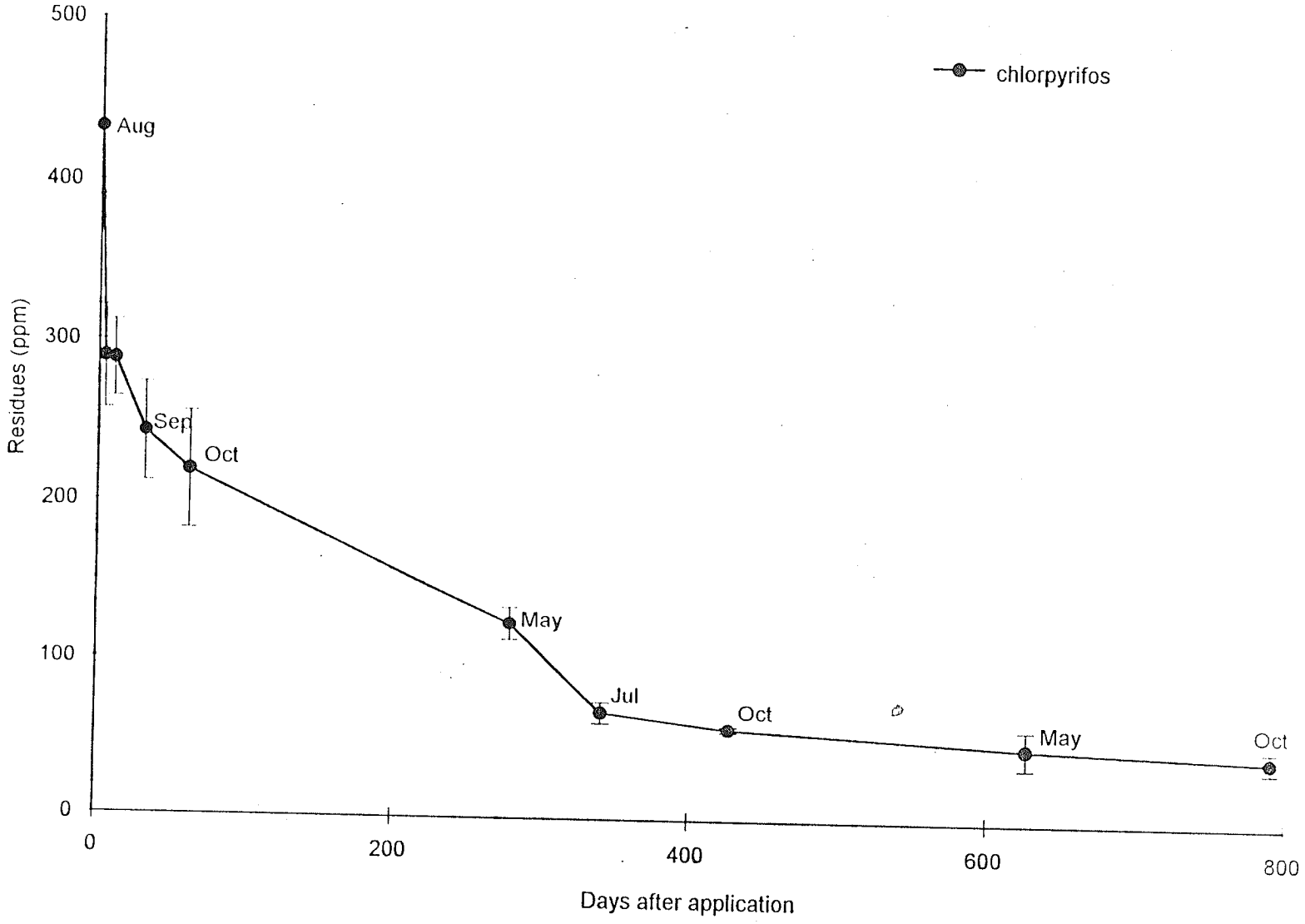



Figure 13. Dissipation of chlorpyrifos in elm bark at the Glenlea Research Station for the 1991 application

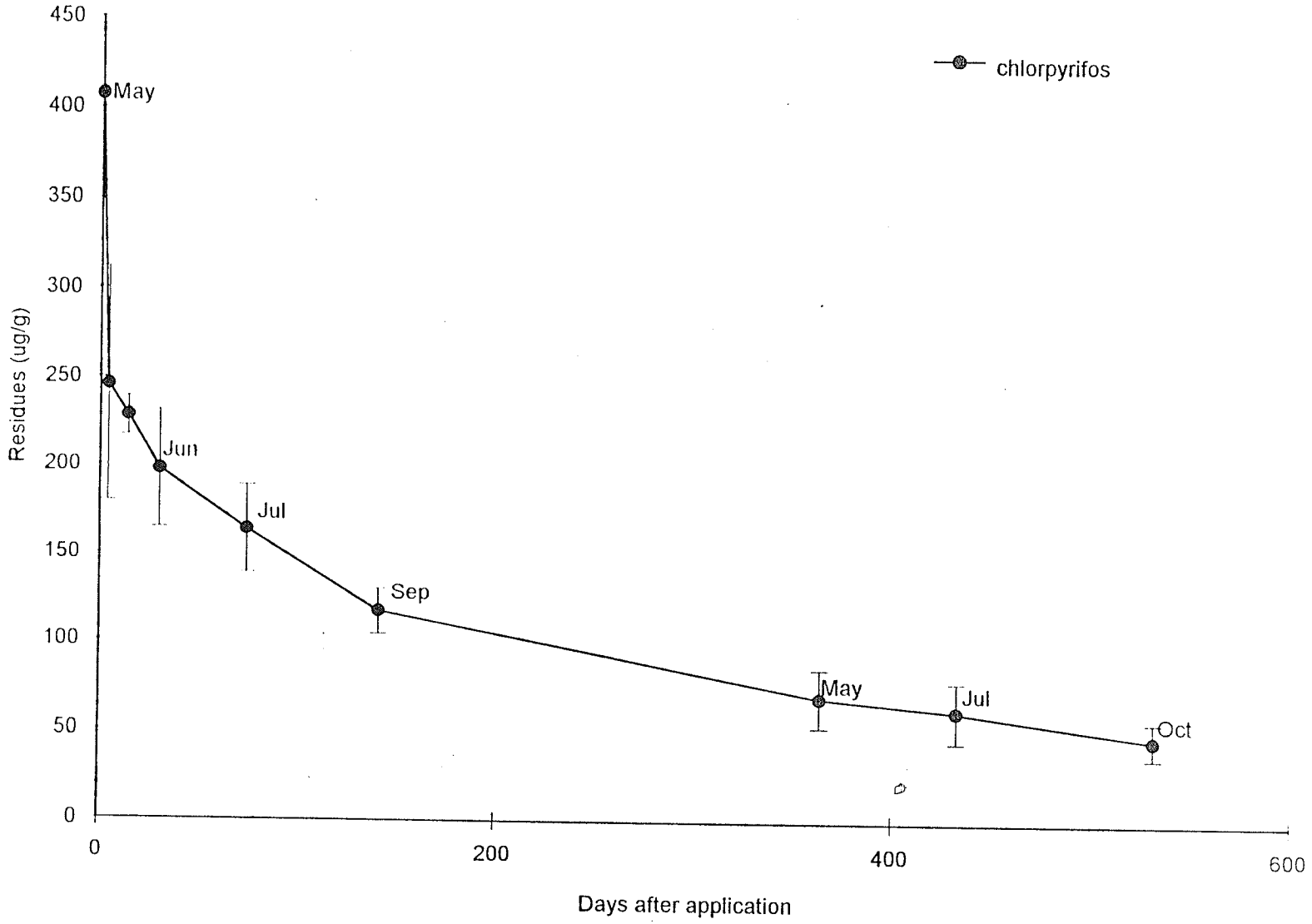



Figure 14. Dissipation of chlorpyrifos in elm bark at the Glenlea Research Station for the 1992 application

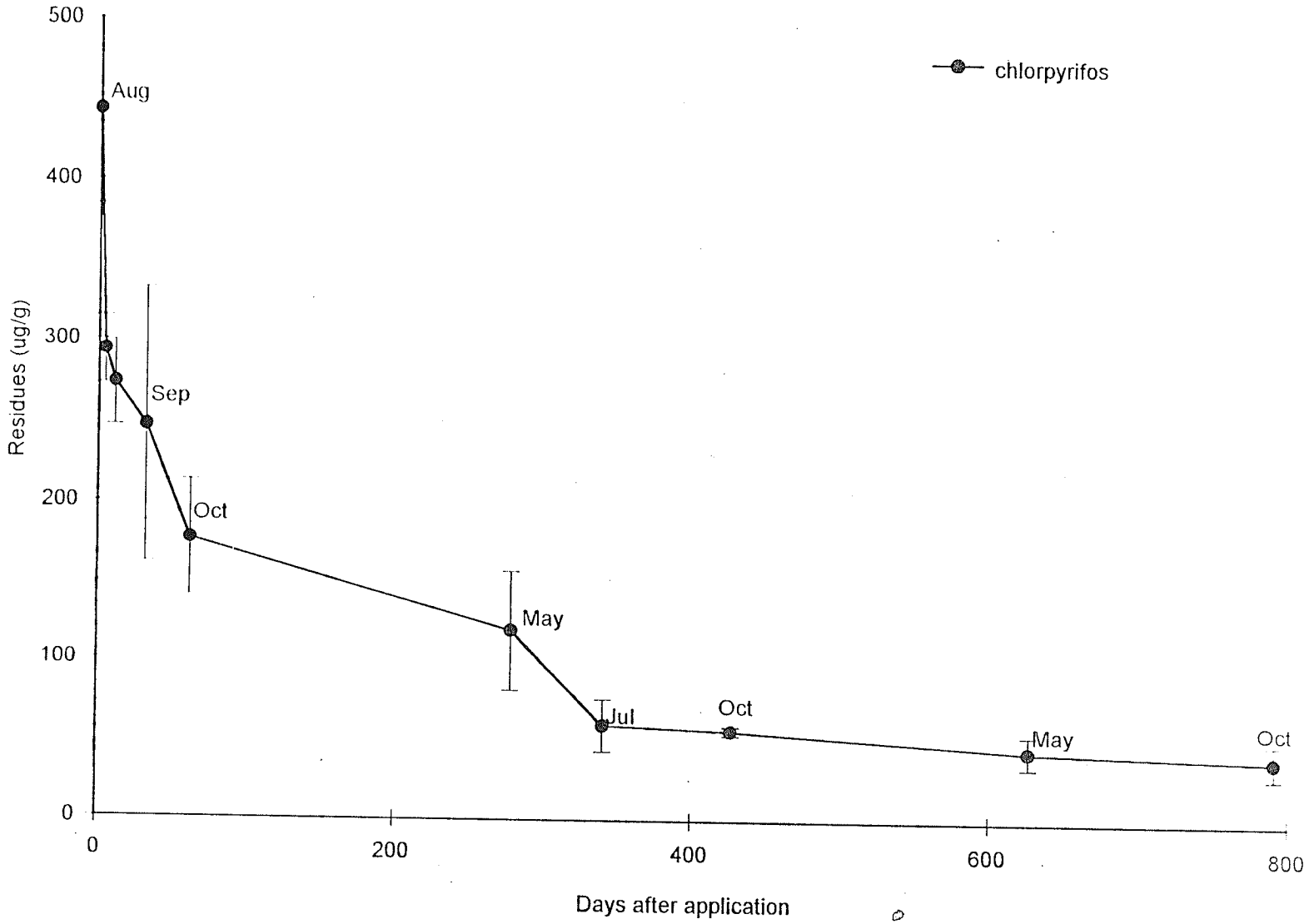



Figure 15. Dissipation of chlorpyrifos in elm bark at the Beaudry Provincial Park for the 1991 application

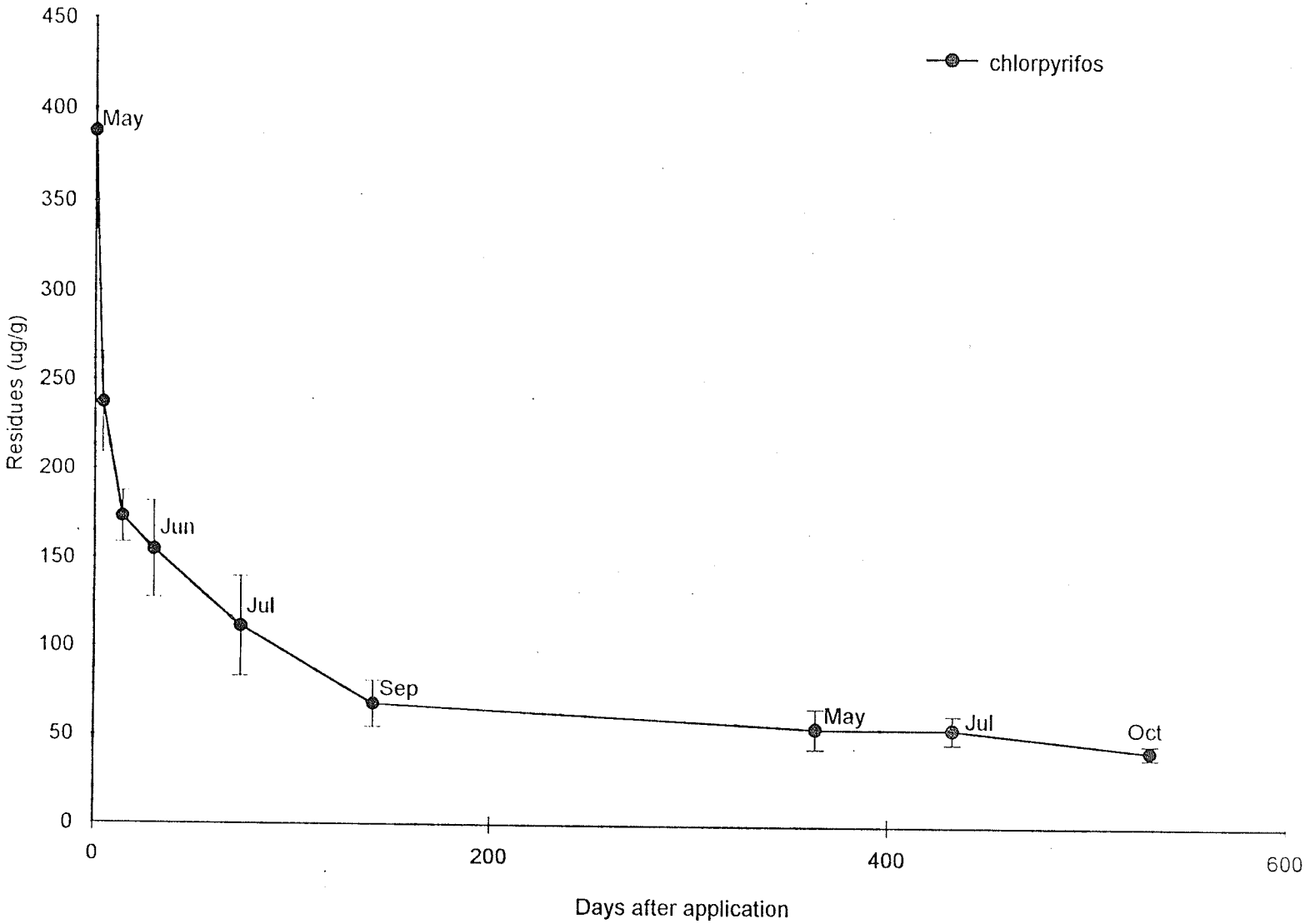



Figure 16. Dissipation of chlorpyrifos in elm bark at the Beaudry Provincial Park for the 1992 application

days from the 1991 application, and approximately 85% after 532 days in 1992 application; while the residues of *trans*-isomers degraded approximately 92% after 791 days from the 1991 application and approximately 91% after 532 days from the 1992 application seemingly indicating that *trans* -isomers dissipated more readily than their *cis* -counterparts. Compared to their parent compounds, the residues of the metabolites in elm bark were negligible. The residues of PBA were normally higher than the sum of both *cis*-CCA and *trans*-CCA, probably indicating that CCA dissipates more readily from the target area. The concentration of *trans*-CCA was higher than *cis*-CCA

Figures 17, 18, 19, and 20 show the cypermethrin isomers dissipation in the elm bark. The experimental data indicate that the residues of four pairs of cypermethrin isomers dissipate more quickly initially and then slow down thereafter.

### *4.4 Dissipation of chlorpyrifos and its metabolites in litter*

The residues and standard deviations of chlorpyrifos, oxon, and pyridinol in litter at different time intervals after treatment are shown in Table 12. The initial residues of chlorpyrifos in litter ranged from 828 to 916 μg/g in the August application and from 187 to 505 μg/g in the May application. Approximately 99% of the initial residues of chlorpyrifos in litter dissipated after 791 days in the 1991 application and after 532 days in the 1992 application. Higher residues of chlorpyrifos in litter were found in 1991 than in the 1992 application. No oxon was

Table 9. The residues and standard deviations (n=3) of four pairs of cypermethrin isomers and their major metabolites in elm bark at the Glenlea Research Station at both application times

| Sampling Date | Days after Application | *Cis*-CCA Residues (µg/g) | SD (µg/g) | *Trans*-CCA Residues (µg/g) | SD (µg/g) | PBA Residues (µg/g) | SD (µg/g) | *Cis*-A Residues (µg/g) | SD (µg/g) | *Trans*-C Residues (µg/g) | SD (µg/g) | *Cis*-B Residues (µg/g) | SD (µg/g) | *Trans*-D Residues (µg/g) | SD (µg/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 91.08.15* | 0 | 0.22 | 0.08 | 1.06 | 0.40 | 1.10 | 0.46 | 52.1 | 16.9 | 47.1 | 13.4 | 35.2 | 7.74 | 24.4 | 5.72 |
| 91.08.19 | 4 | 0.35 | 0.08 | 1.10 | 0.33 | 1.41 | 0.62 | 36.7 | 10.1 | 34.2 | 16.3 | 24.8 | 8.12 | 17.2 | 4.11 |
| 91.08.26 | 11 | 0.62 | 0.50 | 1.80 | 1.07 | 2.20 | 0.53 | 34.5 | 9.31 | 30.1 | 9.51 | 22.9 | 6.26 | 15.9 | 7.15 |
| 91.09.19 | 32 | 0.79 | 1.03 | 1.14 | 0.20 | 3.39 | 0.80 | 30.2 | 7.33 | 27.2 | 7.50 | 20.5 | 4.63 | 14.7 | 4.13 |
| 91.10.16 | 62 | 0.35 | 0.28 | 0.75 | 0.50 | 2.07 | 1.75 | 23.1 | 2.21 | 20.6 | 1.94 | 15.1 | 2.05 | 12.6 | 2.64 |
| 92.05.20 | 279 | 0.34 | 0.07 | 0.55 | 0.15 | 1.47 | 0.81 | 18.4 | 3.74 | 18.6 | 3.50 | 12.2 | 2.04 | 11.5 | 1.14 |
| 92.07.21 | 341 | 0.90 | 0.67 | 0.65 | 0.14 | 2.01 | 1.30 | 14.4 | 4.90 | 13.4 | 2.79 | 9.20 | 1.71 | 8.82 | 2.12 |
| 92.10.15 | 427 | 0.05 | 0.01 | 0.03 | 0.02 | 0.10 | 0.02 | 14.0 | 2.52 | 10.6 | 2.76 | 9.04 | 2.11 | 5.52 | 1.14 |
| 93.05.03 | 627 | 0.04 | 0.01 | 0.09 | 0.05 | 0.33 | 0.14 | 7.20 | 1.10 | 6.30 | 1.95 | 5.17 | 0.63 | 3.71 | 1.09 |
| 93.10.14 | 791 | 0.03 | 0.01 | 0.05 | 0.02 | 0.06 | 0.00 | 5.50 | 1.02 | 4.10 | 1.72 | 4.03 | 0.72 | 2.19 | 0.81 |
| 92.05.07* | 0 | ND | 0.00 | ND | 0.00 | ND | 0.00 | 33.4 | 10.0 | 28.1 | 9.21 | 30.3 | 10.8 | 20.5 | 8.07 |
| 92.05.11 | 4 | 0.34 | 0.19 | 0.55 | 0.26 | 1.30 | 0.58 | 22.5 | 9.78 | 20.5 | 8.93 | 21.5 | 7.75 | 14.8 | 8.45 |
| 92.05.21 | 14 | 0.55 | 0.07 | 0.71 | 0.15 | 2.14 | 0.45 | 20.1 | 8.01 | 17.3 | 0.13 | 18.3 | 9.12 | 12.9 | 2.71 |
| 92.06.15 | 30 | 0.42 | 0.07 | 0.84 | 0.07 | 1.34 | 0.63 | 16.1 | 3.46 | 14.2 | 1.75 | 13.5 | 2.27 | 10.3 | 2.42 |
| 92.07.20 | 74 | 0.59 | 0.15 | 1.09 | 0.36 | 1.81 | 0.89 | 14.1 | 2.47 | 9.38 | 1.31 | 11.6 | 3.01 | 8.02 | 0.50 |
| 92.09.25 | 141 | 0.24 | 0.16 | 0.24 | 0.25 | 0.99 | 1.11 | 11.8 | 1.91 | 6.07 | 0.91 | 7.61 | 1.63 | 5.07 | 0.76 |
| 93.05.06 | 364 | 0.12 | 0.08 | 0.20 | 0.16 | 1.29 | 0.70 | 9.11 | 0.78 | 5.51 | 1.12 | 7.68 | 0.94 | 4.23 | 0.58 |
| 93.07.14 | 433 | 0.03 | 0.01 | 0.03 | 0.01 | 0.10 | 0.11 | 6.58 | 1.01 | 3.82 | 0.18 | 5.42 | 0.94 | 2.98 | 0.24 |
| 93.10.21 | 532 | 0.02 | 0.01 | 0.01 | 0.01 | 0.08 | 0.09 | 5.57 | 1.02 | 3.01 | 0.73 | 4.71 | 0.91 | 2.02 | 0.41 |

*: Application time of cypermethrin

Table 10. The residues and standard deviations (n=3) of four pairs of cypermethrin isomers and their major metabolites in elm bark at Beaudry Provincial Park at both application times

| Sampling Date | Days after Application | *Cis*-CCA Residues (μg/g) | SD (μg/g) | *Trans*-CCA Residues (μg/g) | SD (μg/g) | PBA Residues (μg/g) | SD (μg/g) | *Cis*-A Residues (μg/g) | SD (μg/g) | *Trans*-C Residues (μg/g) | SD (μg/g) | *Cis*-B Residues (μg/g) | SD (μg/g) | *Trans*-D Residues (μg/g) | SD (μg/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 91.08.15* | 0 | 0.26 | 0.13 | 0.66 | 0.26 | 0.92 | 0.13 | 57.9 | 4.18 | 53.1 | 4.51 | 34.9 | 3.78 | 25.2 | 1.89 |
| 91.08.19 | 4 | 0.31 | 0.08 | 0.62 | 0.08 | 0.88 | 0.15 | 38.6 | 10.4 | 35.4 | 11.2 | 25.9 | 7.28 | 19.1 | 4.56 |
| 91.08.26 | 11 | 0.44 | 0.08 | 0.79 | 0.26 | 1.63 | 0.62 | 35.9 | 16.9 | 34.2 | 17.3 | 23.9 | 6.56 | 17.8 | 5.61 |
| 91.09.19 | 32 | 0.40 | 0.13 | 1.01 | 0.08 | 2.29 | 0.76 | 31.2 | 9.01 | 30.0 | 9.56 | 20.6 | 11.6 | 14.8 | 9.32 |
| 91.10.16 | 62 | 0.26 | 0.01 | 0.40 | 0.10 | 1.06 | 0.13 | 21.1 | 4.78 | 20.8 | 4.44 | 14.9 | 3.41 | 12.8 | 2.56 |
| 92.05.20 | 279 | 0.34 | 0.07 | 0.50 | 0.13 | 0.76 | 0.13 | 16.4 | 3.89 | 16.9 | 4.89 | 11.1 | 2.56 | 9.51 | 1.67 |
| 92.07.21 | 341 | 0.52 | 0.04 | 0.72 | 0.09 | 0.89 | 0.68 | 13.7 | 0.78 | 15.3 | 4.56 | 10.9 | 2.78 | 9.32 | 2.89 |
| 92.10.15 | 427 | 0.05 | 0.03 | 0.01 | 0.02 | 0.06 | 0.04 | 13.4 | 3.71 | 13.5 | 0.67 | 9.72 | 0.81 | 7.28 | 0.13 |
| 93.05.03 | 627 | 0.03 | 0.02 | 0.05 | 0.02 | 0.21 | 0.10 | 7.21 | 0.18 | 7.78 | 0.61 | 5.28 | 0.22 | 4.29 | 0.42 |
| 93.10.14 | 791 | 0.03 | 0.00 | 0.03 | 0.01 | ND | 0.00 | 5.53 | 0.91 | 4.18 | 0.82 | 4.02 | 0.67 | 2.01 | 0.38 |
| 92.05.07* | 0 | ND | 0.00 | ND | 0.00 | ND | 0.00 | 28.8 | 4.31 | 24.2 | 3.89 | 24.9 | 5.21 | 17.7 | 2.78 |
| 92.05.11 | 4 | 0.25 | 0.13 | 0.50 | 0.13 | 0.38 | 0.22 | 19.9 | 7.48 | 18.5 | 4.58 | 17.2 | 7.64 | 13.1 | 2.46 |
| 92.05.21 | 14 | 0.42 | 0.19 | 0.50 | 0.25 | 1.64 | 0.25 | 17.4 | 3.78 | 16.4 | 1.76 | 14.6 | 1.63 | 12.2 | 1.11 |
| 92.06.15 | 30 | 0.38 | 0.01 | 0.50 | 0.13 | 0.63 | 0.22 | 17.0 | 0.76 | 15.4 | 4.38 | 12.8 | 3.28 | 11.1 | 3.02 |
| 92.07.20 | 74 | 0.42 | 0.07 | 0.63 | 0.13 | 0.67 | 0.32 | 14.2 | 1.01 | 11.0 | 1.01 | 11.1 | 1.01 | 9.58 | 1.27 |
| 92.09.25 | 141 | 0.11 | 0.01 | 0.12 | 0.02 | 0.27 | 0.03 | 13.7 | 3.62 | 8.91 | 2.51 | 10.1 | 2.08 | 6.46 | 1.51 |
| 93.05.06 | 364 | 0.07 | 0.05 | 0.11 | 0.10 | 0.34 | 0.39 | 8.58 | 2.63 | 6.89 | 1.72 | 7.31 | 2.17 | 5.37 | 1.43 |
| 93.07.14 | 433 | 0.03 | 0.01 | 0.03 | 0.03 | 0.08 | 0.07 | 4.89 | 0.74 | 3.58 | 0.64 | 4.23 | 0.57 | 2.58 | 0.48 |
| 93.10.21 | 532 | 0.03 | 0.02 | 0.04 | 0.02 | 0.10 | 0.12 | 3.87 | 1.18 | 2.27 | 0.72 | 3.74 | 1.02 | 1.47 | 0.57 |

*: Application time of cypermethrin

detected during the experimental period. The residues of pyridinol in litter fluctuated from 1 to 67 μg/g.

Figures 21, 22, 23, and 24 show chlorpyrifos dissipation in litter at both experimental sites and both application times. The experimental data indicated that chlorpyrifos in litter dissipated rapidly in the initial four days after application, and then the dissipation rate slowed down. The $DT_{50}$ and $DT_{90}$ of chlorpyrifos in litter for the whole experimental period at both sites and both application times are presented in Table 13 indicating that it took 3.9 - 59 days for the initial residues of chlorpyrifos in litter to dissipate by 50 % and 55 - 310 days by 90 %.

### *4.5 Dissipation of cypermethrin isomers and their metabolites in litter*

The residues of the four pairs of cypermethrin isomers and their major metabolites in litter at different time intervals after insecticide treatment at two experimental are shown in Tables 14 and 15. Compared to the initial residues of chlorpyrifos, the initial residues of cypermethrin in litter were much lower due to lower application rates. The initial residues of *cis*-A, *trans*-C, *cis*-B, and *trans*-D in litter ranged from 106 to 142, from 92.6 to 125, from 67.3 to 88.3, and from 48.3 to 62.9 μg/g, respectively, in the August application and from 54.2 to 70.3, from 41.0 to 54.3, from 47.7 to 62.8, and 32.7 to 40.8 μg/g, respectively, in the May application indicating that the initial residues of cypermethrin in the May application

Table 11. The $DT_{50}$ and $DT_{90}$ of four pairs of cypermethrin isomers in elm bark

| Treatment | Analytes | $DT_{50}$ (Days) | $DT_{90}$ (days) |
|---|---|---|---|
| Glenlea, 1991 | *Cis*-A | 50 | 820* |
| | *Trans*-C | 49 | 750 |
| | *Cis*-B | 48 | 860* |
| | *Trans*-D | 140 | 760 |
| Glenlea, 1992 | *Cis*-A | 28 | 750* |
| | *Trans*-C | 32 | 560* |
| | *Cis*-B | 25 | 770* |
| | *Trans*-D | 31 | 530 |
| Beaudry, 1991 | *Cis*-A | 39 | 770 |
| | *Trans*-C | 43 | 740 |
| | *Cis*-B | 48 | 860* |
| | *Trans*-D | 75 | 750 |
| Beaudry, 1992 | *Cis*-A | 71 | 630* |
| | *Trans*-C | 63 | 520 |
| | *Cis*-B | 39 | 790* |
| | *Trans*-D | 94 | 510 |

*: The values are estimated based on the residue concentrations in the samples of the last two times.

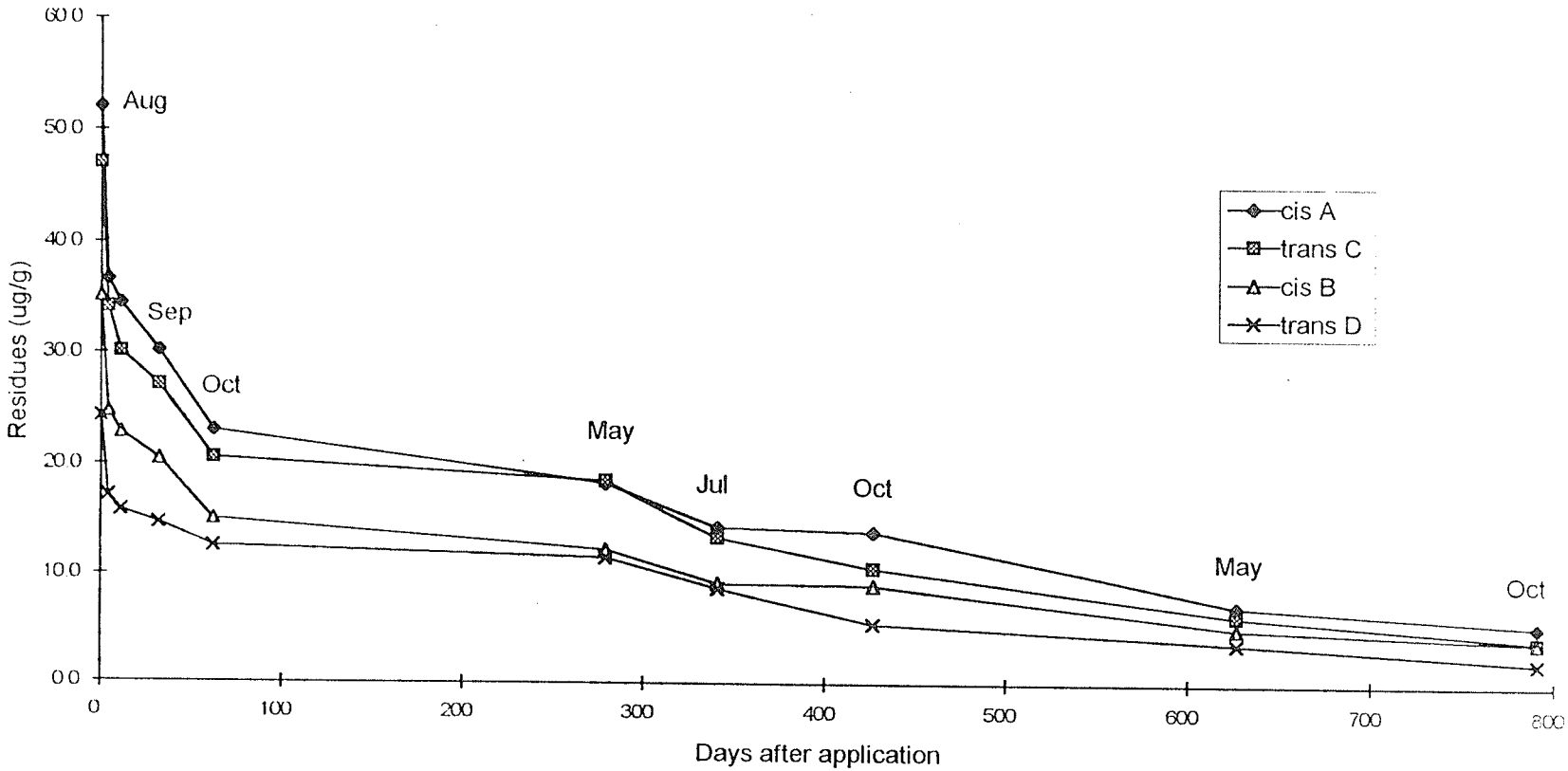



Figure 17. Dissipation of four pairs of cypermethrin isomers in elm bark at the Glenlea Research Station for the 1991 application

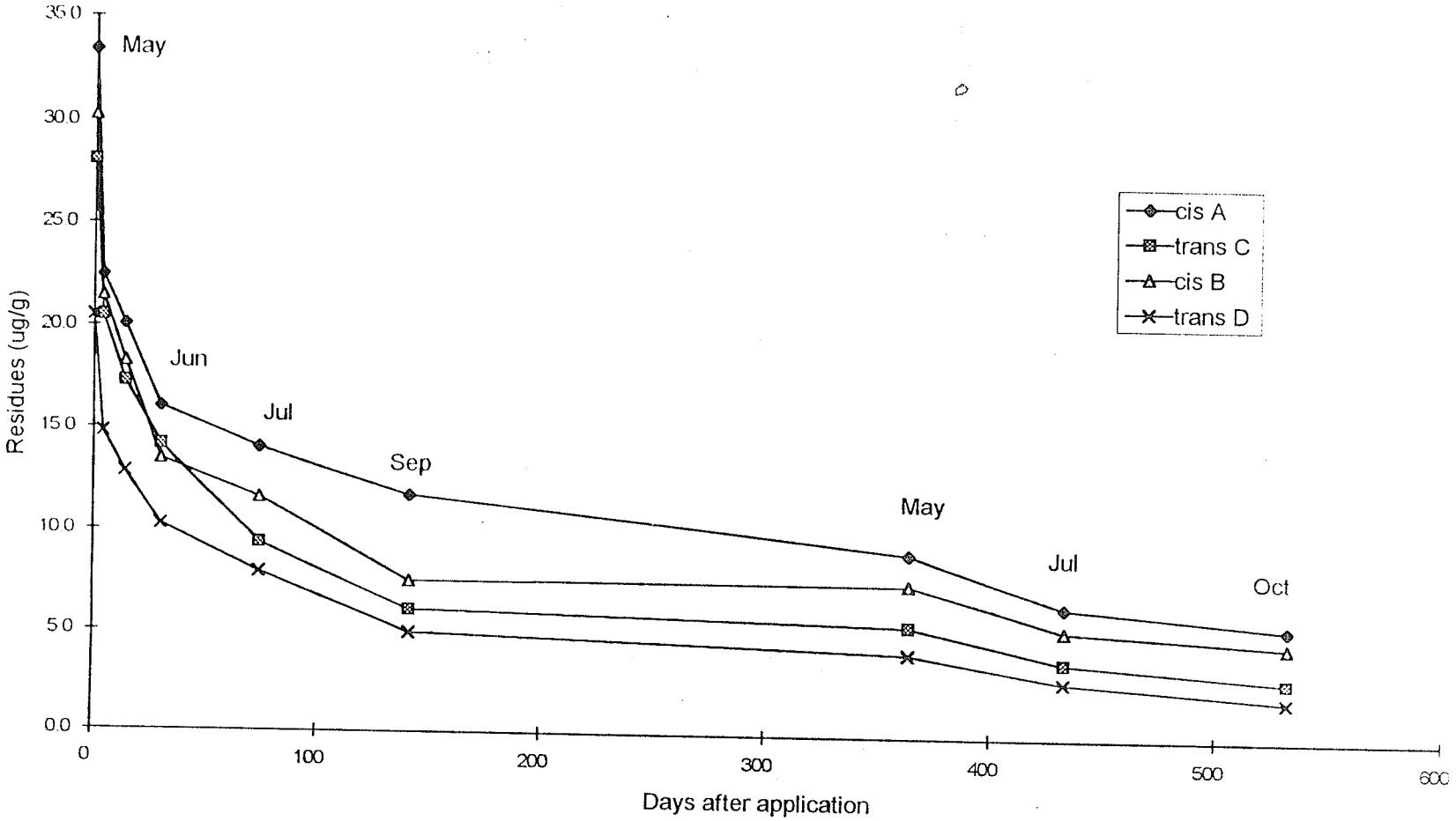



Figure 18. Dissipation of four pairs of cypermethrin isomers in elm bark at the Glenlea Research Station for the 1992 application

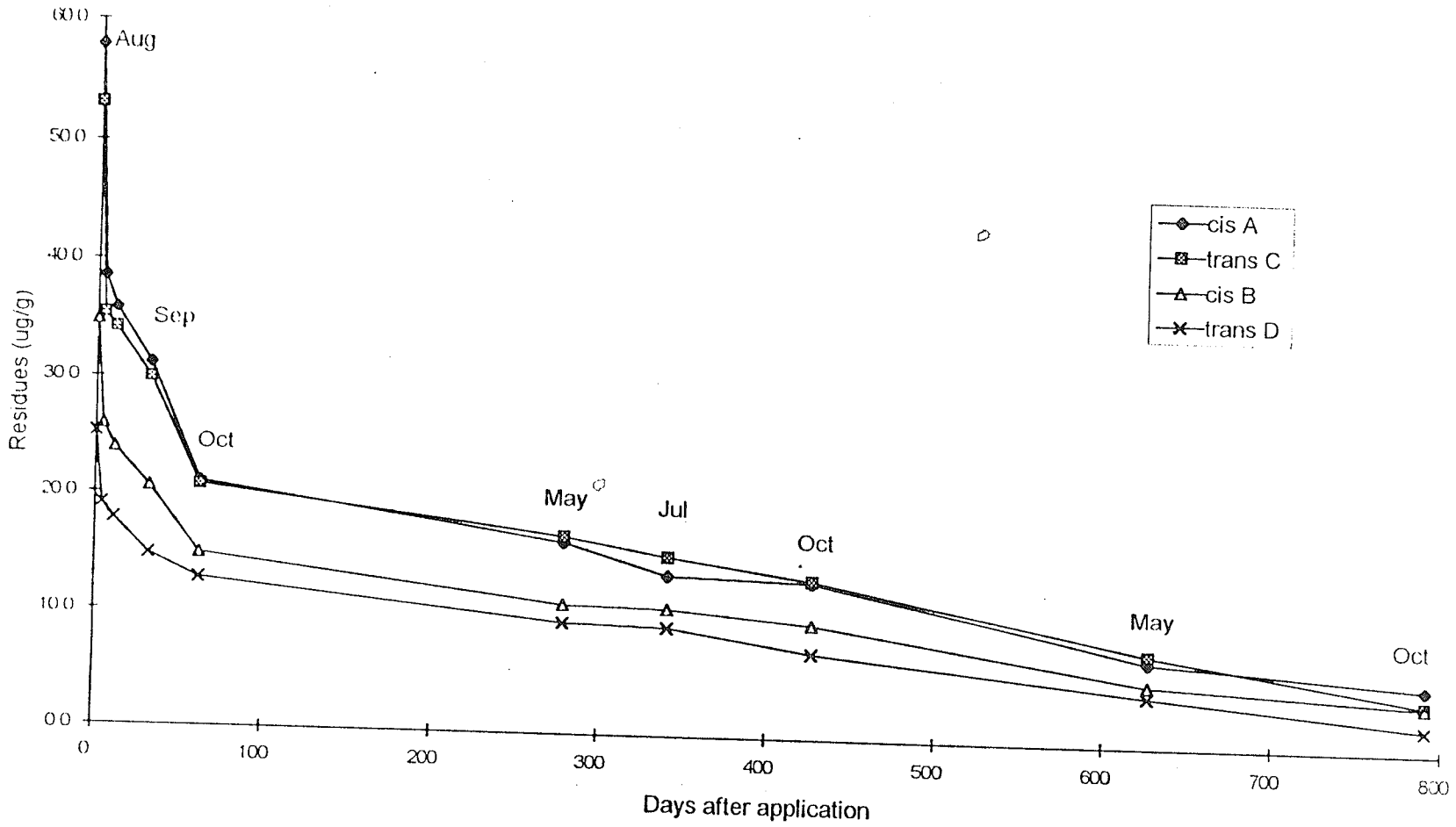



Figure 19. Dissipation of four pairs of cypermethrin isomers in elm bark at Beaudry Provincial Park for the 1991 application

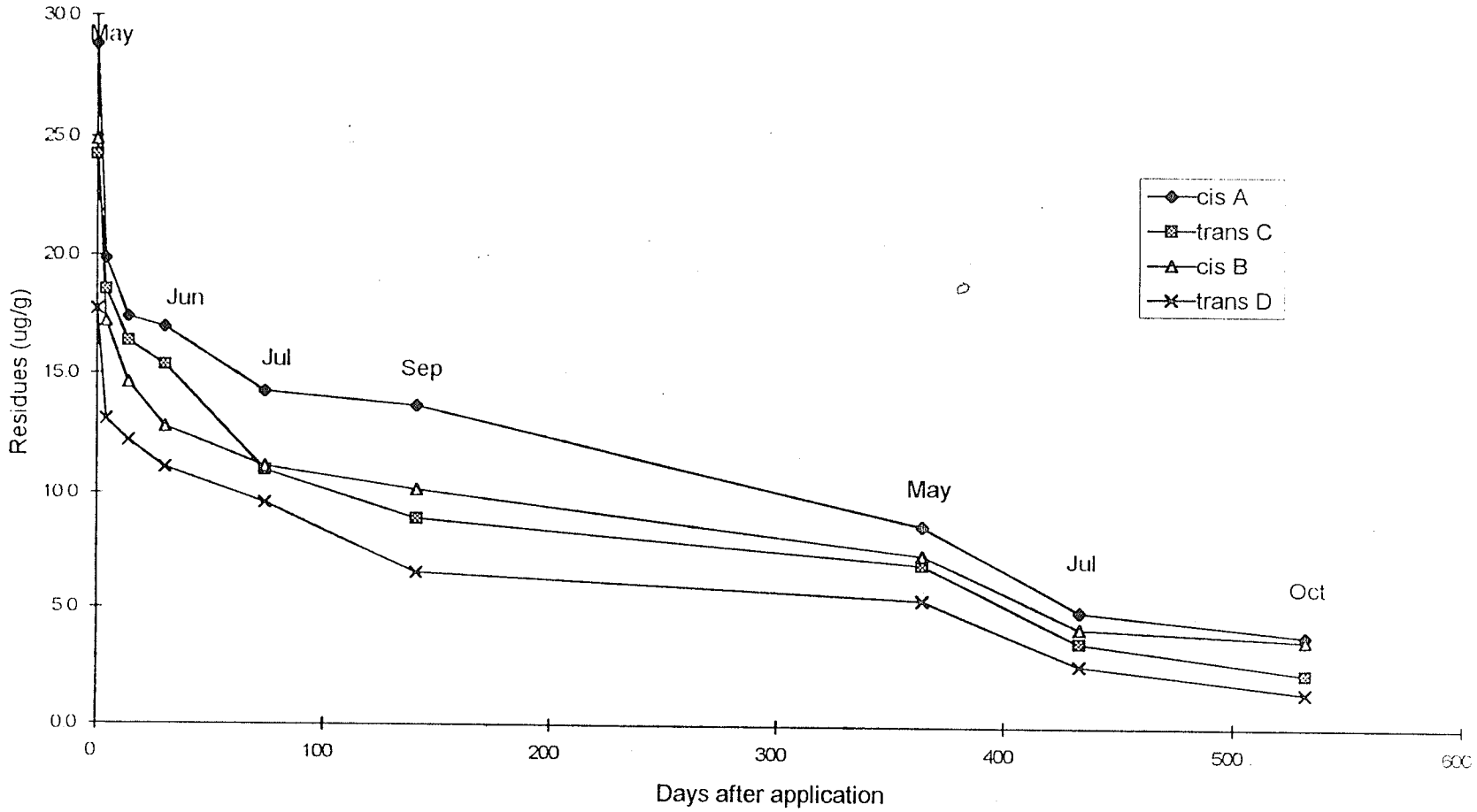



Figure 20. Dissipation of four pairs of cypermethrin isomers in elm bark at Beaudry Provincial Park for the 1992 application

Table 12. The residues and standard deviations (n=3) of chlorpyrifos, oxon, and pyridinol in litter at both experimental sites and application times

| Sampling Date | Days after Application | Chlorpyrifos | | | | Oxon | | | | Pyridinol | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Glenlea | | Beaudry | | Glenlea | | Beaudry | | Glenlea | | Beaudry | |
| | | Residues (μg/g) | SD (μg/g) | Residues (μg/g) | SD (μg/g) | Residues (μg/g) | SD (μg/g) | Residues (μg/g) | SD (μg/g) | Residues (μg/g) | SD (μg/g) | Residues (μg/g) | SD (μg/g) |
| 91.08.15* | 0 | 916 | 330 | 828 | 105 | ND | 0 | ND | 0 | 48.0 | 2.71 | 56.4 | 4.56 |
| 91.08.19 | 4 | 650 | 54.0 | 610 | 11.7 | ND | 0 | ND | 0 | 56.5 | 0.78 | 52.6 | 0.58 |
| 91.08.26 | 11 | 532 | 116 | 481 | 49.2 | ND | 0 | ND | 0 | 29.7 | 1.11 | 40.3 | 0.49 |
| 91.09.19 | 32 | 499 | 63.1 | 461 | 5.09 | ND | 0 | ND | 0 | 23.6 | 0.28 | 47.3 | 3.18 |
| 91.10.16 | 62 | 199 | 7.18 | 193 | 2.51 | ND | 0 | ND | 0 | 21.0 | 0.37 | 20.3 | 0.09 |
| 92.05.20 | 279 | 174 | 109 | 141 | 17.6 | ND | 0 | ND | 0 | 27.6 | 18.5 | 19.7 | 2.28 |
| 92.07.21 | 341 | 17.8 | 12.1 | 16.6 | 9.89 | ND | 0 | ND | 0 | 3.41 | 2.49 | 1.58 | 0.27 |
| 92.10.15 | 427 | 10.8 | 2.01 | 9.81 | 5.11 | ND | 0 | ND | 0 | 1.78 | 0.38 | 1.68 | 0.56 |
| 93.05.03 | 627 | 5.18 | 1.48 | 8.78 | 3.02 | ND | 0 | ND | 0 | 5.68 | 0.78 | 8.09 | 0.89 |
| 93.10.14 | 791 | 4.78 | 1.11 | 7.49 | 4.02 | ND | 0 | ND | 0 | 1.38 | 1.47 | 3.89 | 0.92 |
| 92.05.07* | 0 | 187 | 86.9 | 505 | 72.3 | ND | 0 | ND | 0 | 44.1 | 16.0 | 66.6 | 27.2 |
| 92.05.11 | 4 | 126 | 42.0 | 247 | 101 | ND | 0 | ND | 0 | 16.3 | 7.38 | 11.3 | 3.06 |
| 92.05.21 | 14 | 122 | 37.7 | 142 | 84.1 | ND | 0 | ND | 0 | 59.5 | 15.2 | 27.4 | 21.1 |
| 92.06.15 | 30 | 122 | 125 | 66.0 | 52.7 | ND | 0 | ND | 0 | 26.5 | 27.4 | 14.8 | 7.48 |
| 92.07.20 | 74 | 79.3 | 5.71 | 38.2 | 10.8 | ND | 0 | ND | 0 | 5.61 | 0.71 | 2.71 | 0.21 |
| 92.09.25 | 141 | 35.6 | 23.2 | 7.82 | 3.21 | ND | 0 | ND | 0 | 2.38 | 1.53 | 1.47 | 0.19 |
| 93.05.06 | 364 | 12.3 | 6.48 | 10.6 | 6.58 | ND | 0 | ND | 0 | 16.9 | 8.18 | 5.12 | 1.59 |
| 93.07.14 | 433 | 7.89 | 4.19 | 5.31 | 1.02 | ND | 0 | ND | 0 | 7.11 | 2.17 | 2.14 | 0.78 |
| 93.10.21 | 532 | 4.22 | 1.19 | 5.09 | 4.68 | ND | 0 | ND | 0 | 4.28 | 1.49 | 2.27 | 1.13 |

*: Application time of chlorpyrifos

were lower than in August application. The residues of cypermethrin isomers in litter were below the detection limit at 341 days for the 1991 application at both experimental sites and at 433 days at Beaudry Provincial Park and at 532 days at the Glenlea Research Station for the 1992 application. The residue concentrations of the metabolites in litter were very low compared to their parent compounds.

Figures 25, 26, 27, and 28 show the cypermethrin isomer pair dissipation in litter at different sites and application times. The $DT_{50}$ and $DT_{90}$ values of the four pairs of cypermethrin isomers in litter during the whole experimental period are shown in Table 16. It took 8.8 - 29 days for the initial residues of cypermethrin isomers to dissipate by 50 % and 95 - 300 days by 90 %.

### *4.6 The residues of chlorpyrifos and its metabolites in soil*

The residues of chlorpyrifos, oxon, and pyridinol at different time intervals after application in soil at both sites and both application times are shown in Table 17. The residues of chlorpyrifos in soil fluctuated with time after treatment ranging from 1.04 to 28.2 μg/g at both sites in the August application and from 0.78 to 11.8 μg/g in the May application. Chlorpyrifos residues in soil increased first with time and then decreased. The chlorpyrifos residues in soil remained at 1.04 to 2.09

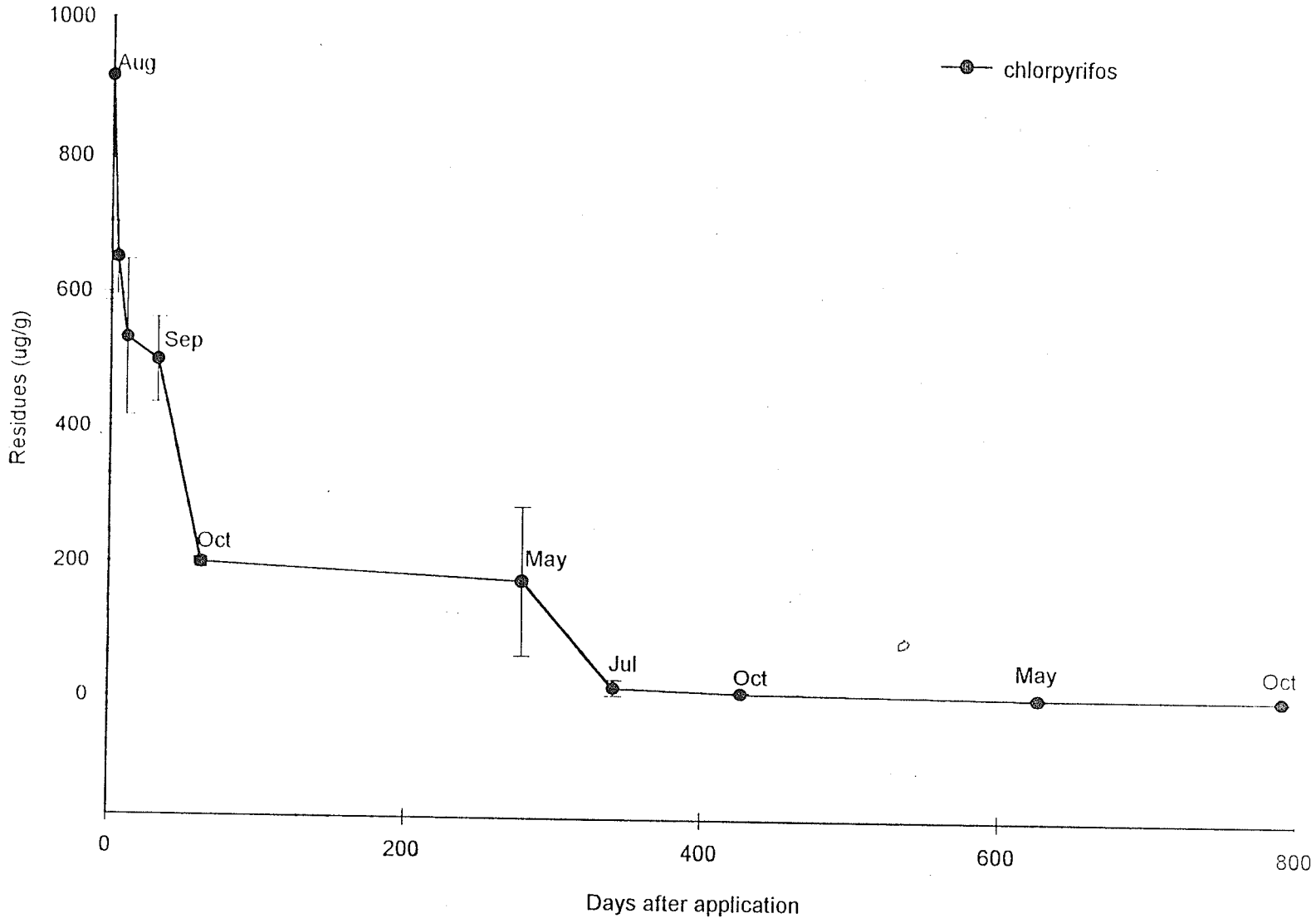



Figure 21. Dissipation of chlorpyrifos in litter at the Glenlea Research Station for the 1991 application

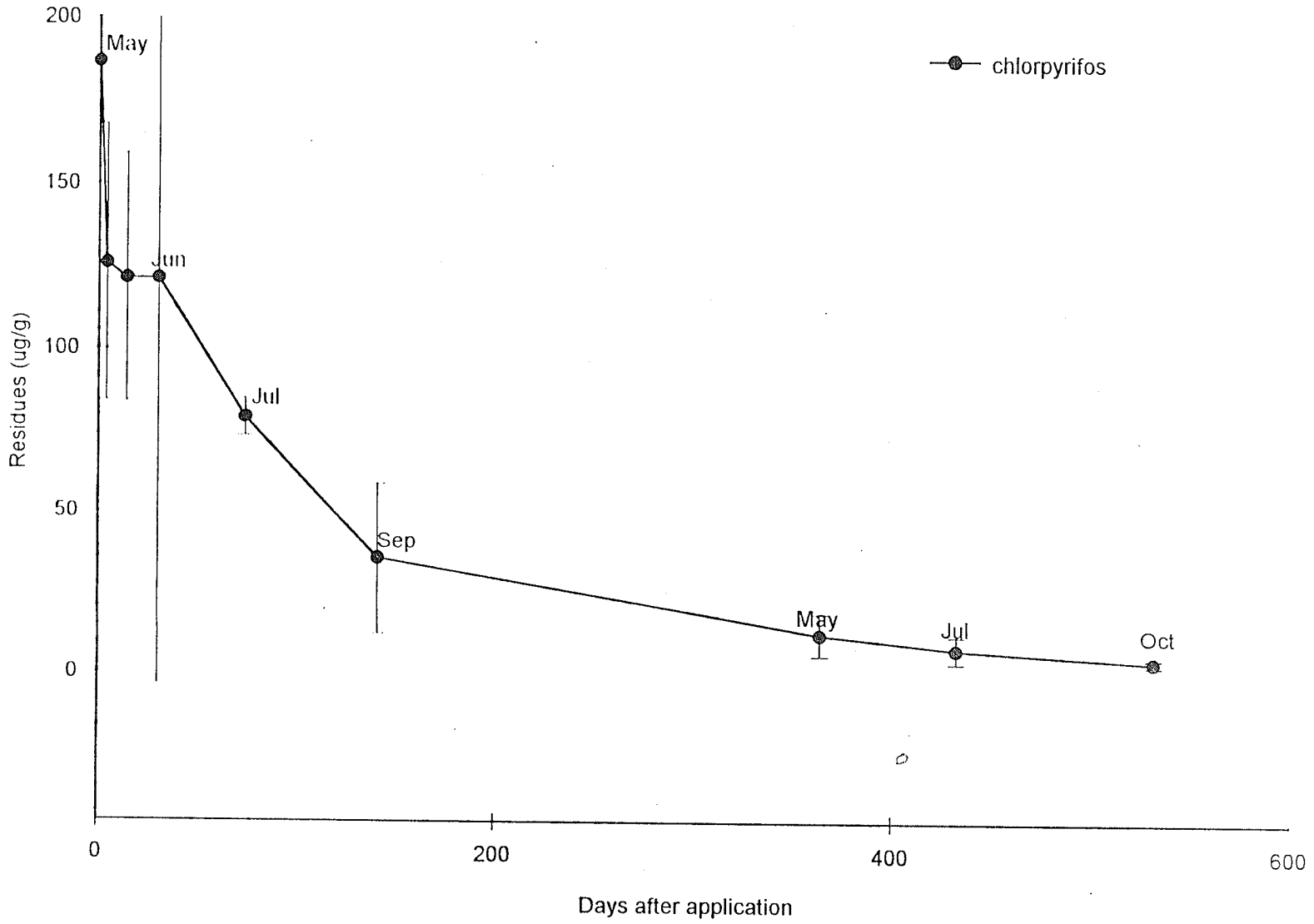



Figure 22. Dissipation of chlorpyrifos in litter at the Glenlea Research Station for the 1992 application

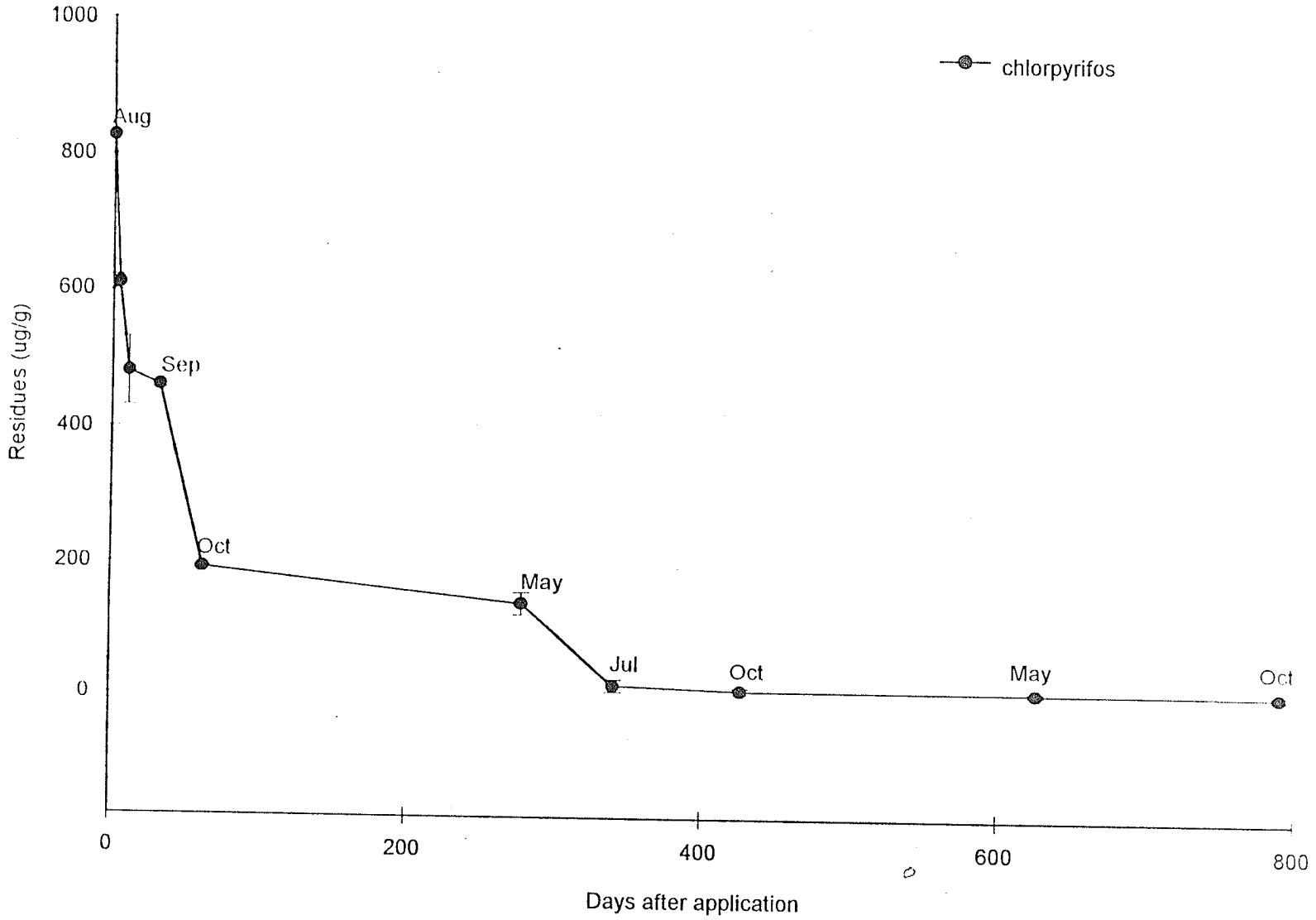



Figure 23. Dissipation of chlorpyrifos in litter at the Beaudry Provincial Park for the 1991 application

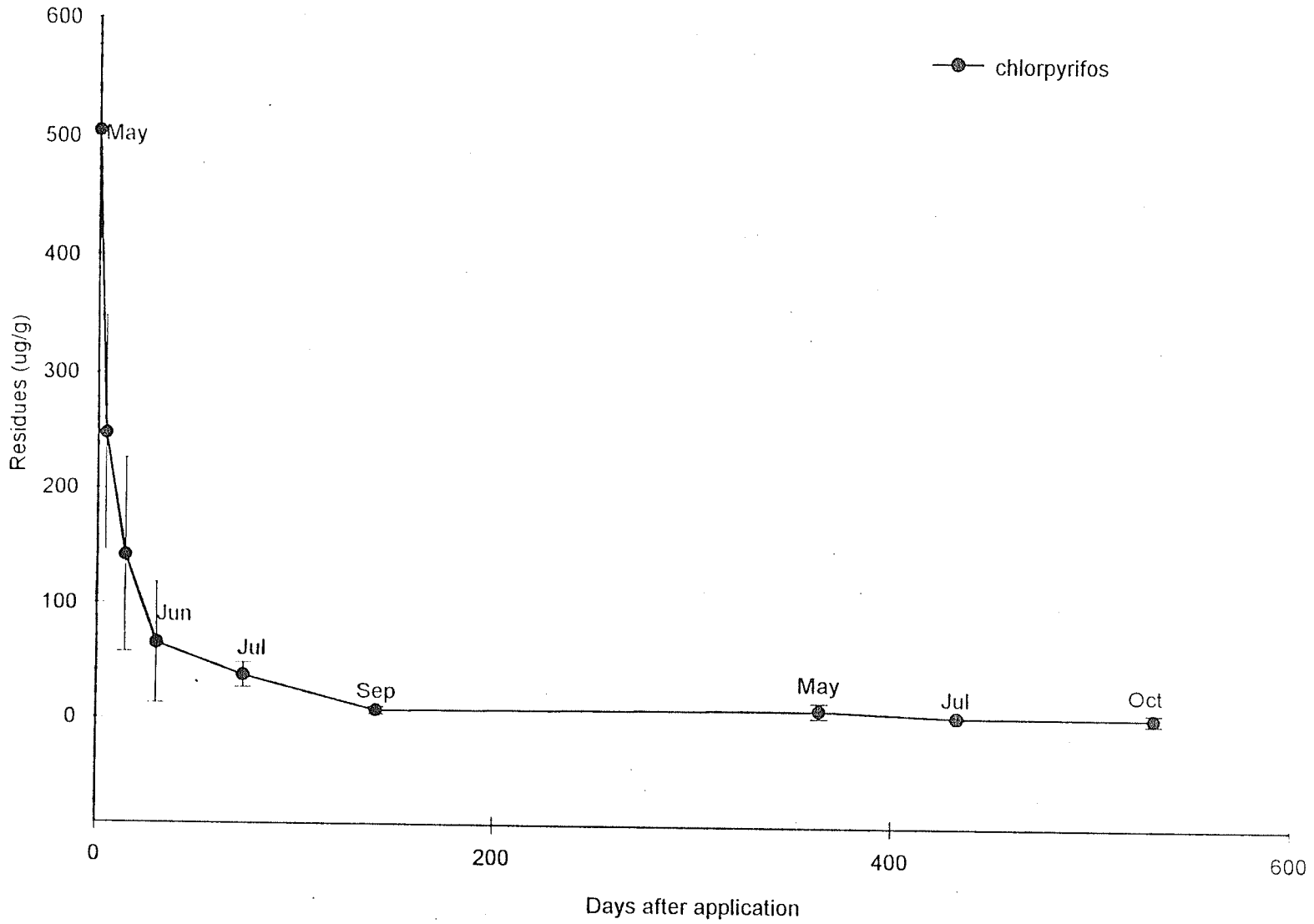



Figure 24. Dissipation of chlorpyrifos in litter at the Beaudry Provincial Park for the 1992 application

Table 13. The $DT_{50}$ and $DT_{90}$ of chlorpyrifos in litter

| Treatment | $DT_{50}$ (Days) | $DT_{90}$ (Days) |
|---|---|---|
| Glenlea, 1991 | 36 | 310 |
| Glenlea, 1992 | 59 | 300 |
| Beaudry, 1991 | 37 | 310 |
| Beaudry, 1992 | 3.9 | 55 |

Table 14. The residues and standard deviations (n=3) of four pairs of cypermethrin isomers and their major metabolites in litter at the Glenlea Research Station at both application times

| Sampling Date | Days after Application | *Cis*-CCA Residues (µg/g) | SD (µg/g) | *Trans*-CCA Residues (µg/g) | SD (µg/g) | PBA Residues (µg/g) | SD (µg/g) | *Cis*-A Residues (µg/g) | SD (µg/g) | *Trans*-C Residues (µg/g) | SD (µg/g) | *Cis*-B Residues (µg/g) | SD (µg/g) | *Trans*-D Residues (µg/g) | SD (µg/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 91.08.15* | 0 | 1.08 | 0.09 | 2.75 | 0.32 | 2.16 | 0.61 | 142 | 18.2 | 125 | 13.8 | 88.3 | 6.78 | 62.9 | 5.18 |
| 91.08.19 | 4 | 0.76 | 0.19 | 2.38 | 0.19 | 2.92 | 0.32 | 93.2 | 12.9 | 83.4 | 21.9 | 57.4 | 12.5 | 42.2 | 9.09 |
| 91.08.26 | 11 | 0.32 | 0.05 | 1.08 | 0.18 | 0.76 | 0.19 | 67.4 | 4.3 | 54.8 | 4.71 | 38.1 | 9.12 | 26.8 | 1.01 |
| 91.09.19 | 32 | 2.33 | 0.76 | 10.51 | 5.06 | 0.70 | 1.97 | 50.3 | 8.9 | 27.5 | 4.89 | 21.1 | 3.38 | 16.5 | 2.78 |
| 91.10.16 | 62 | 1.94 | 0.32 | 5.78 | 1.08 | 2.92 | 0.28 | 33.6 | 2.1 | 20.8 | 2.56 | 19.1 | 1.67 | 9.89 | 0.76 |
| 92.05.20 | 279 | 0.67 | 0.38 | 0.87 | 0.53 | 0.54 | 0.11 | 18.3 | 10.6 | 13.8 | 9.12 | 14.3 | 9.08 | 7.39 | 5.58 |
| 92.07.21 | 341 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.0 | ND | 0.0 | ND | 0.0 | ND | 0.0 |
| 92.10.15 | 427 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.0 | ND | 0.0 | ND | 0.0 | ND | 0.0 |
| 92.05.07* | 0 | ND | 0.00 | ND | 0.00 | ND | 0.00 | 54.2 | 24.5 | 41.0 | 16.6 | 47.7 | 18.6 | 32.7 | 16.3 |
| 92.05.11 | 4 | 0.79 | 0.33 | 1.11 | 0.36 | 1.00 | 0.15 | 38.1 | 2.01 | 28.5 | 1.47 | 31.6 | 0.89 | 20.4 | 1.89 |
| 92.05.21 | 14 | 1.35 | 0.89 | 1.54 | 0.98 | 1.31 | 0.57 | 32.7 | 8.12 | 24.5 | 6.76 | 30.0 | 6.47 | 19.6 | 4.35 |
| 92.06.15 | 30 | 0.92 | 0.47 | 1.42 | 0.84 | 1.31 | 0.77 | 24.6 | 10.1 | 17.0 | 6.35 | 23.6 | 7.86 | 13.9 | 4.38 |
| 92.07.20 | 74 | 0.25 | 0.11 | 0.31 | 0.09 | 0.35 | 0.07 | 12.2 | 5.67 | 7.17 | 2.78 | 10.7 | 5.17 | 5.08 | 2.27 |
| 92.09.25 | 141 | 0.05 | 0.03 | 0.04 | 0.03 | 0.09 | 0.03 | 4.03 | 3.47 | 1.25 | 1.58 | 4.78 | 1.58 | 1.17 | 0.11 |
| 93.05.06 | 364 | 0.16 | 0.21 | 0.01 | 0.02 | 0.09 | 0.01 | 2.94 | 0.38 | 0.87 | 0.18 | 2.48 | 0.25 | 0.87 | 1.62 |
| 93.07.14 | 433 | 0.03 | 0.01 | ND | 0.00 | ND | 0.00 | 1.48 | 0.82 | ND | 0.0 | 1.08 | 0.49 | ND | 0.0 |
| 93.10.21 | 532 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.0 | ND | 0.0 | ND | 0.0 | ND | 0.0 |

*: Application time of cypermethrin

Table 15. The residues and standard deviations (n=3) of four pairs of cypermethrin isomers and their major metabolites in litter at Beaudry Provincial Park at both application times

| Sampling Date | Days after Application | *Cis*-CCA Residues (μg/g) | SD (μg/g) | *Trans*-CCA Residues (μg/g) | SD (μg/g) | PBA Residues (μg/g) | SD (μg/g) | *Cis*-A Residues (μg/g) | SD (μg/g) | *Trans*-C Residues (μg/g) | SD (μg/g) | *Cis*-B Residues (μg/g) | SD (μg/g) | *Trans*-D Residues (μg/g) | SD (μg/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 91.08.15* | 0 | 0.43 | 0.19 | 0.86 | 0.19 | 1.62 | 0.32 | 106 | 18.0 | 92.6 | 17.1 | 67.3 | 13.3 | 48.3 | 9.31 |
| 91.08.19 | 4 | 0.65 | 0.16 | 1.67 | 0.09 | 1.67 | 0.09 | 60.6 | 25.8 | 49.2 | 20.8 | 33.1 | 17.9 | 25.4 | 11.0 |
| 91.08.26 | 11 | 0.65 | 0.01 | 1.84 | 1.19 | 3.94 | 2.77 | 58.8 | 11.6 | 48.6 | 10.1 | 33.6 | 8.62 | 25.4 | 5.09 |
| 91.09.19 | 32 | 1.78 | 0.28 | 8.75 | 1.96 | 5.35 | 1.33 | 45.4 | 7.71 | 37.0 | 6.28 | 24.2 | 8.79 | 19.1 | 3.38 |
| 91.10.16 | 62 | 0.54 | 0.34 | 1.84 | 0.47 | 1.19 | 0.25 | 21.5 | 15.0 | 17.9 | 12.4 | 15.2 | 9.08 | 11.0 | 8.57 |
| 92.05.20 | 279 | 0.51 | 0.14 | 0.53 | 0.16 | 0.73 | 0.34 | 14.7 | 1.22 | 8.64 | 0.46 | 6.63 | 1.67 | 3.71 | 0.46 |
| 92.07.21 | 341 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.0 | ND | 0.0 | ND | 0.0 | ND | 0.0 |
| 92.10.15 | 427 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.0 | ND | 0.0 | ND | 0.0 | ND | 0.0 |
| 92.05.07* | 0 | ND | 0.00 | ND | 0.00 | ND | 0.00 | 70.3 | 15.8 | 54.3 | 12.6 | 62.8 | 15.4 | 40.8 | 7.41 |
| 92.05.11 | 4 | 4.94 | 1.43 | 9.12 | 0.47 | 7.98 | 2.11 | 49.5 | 8.78 | 29.4 | 17.3 | 33.3 | 21.4 | 24.7 | 13.5 |
| 92.05.21 | 14 | 1.45 | 0.23 | 1.65 | 0.41 | 1.21 | 0.29 | 35.1 | 18.3 | 27.7 | 4.08 | 32.2 | 5.78 | 19.6 | 4.0 |
| 92.06.15 | 30 | 0.66 | 0.65 | 0.80 | 0.60 | 1.49 | 0.91 | 22.4 | 1.77 | 16.5 | 2.04 | 20.9 | 1.58 | 12.9 | 1.58 |
| 92.07.20 | 74 | 0.25 | 0.06 | 0.28 | 0.03 | 0.35 | 0.07 | 11.0 | 3.38 | 7.61 | 2.68 | 9.43 | 3.08 | 5.31 | 1.72 |
| 92.09.25 | 141 | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 3.33 | 1.56 | 1.23 | 1.04 | 3.04 | 0.93 | 1.43 | 0.47 |
| 93.05.06 | 364 | 0.02 | 0.00 | 0.02 | 0.00 | 0.08 | 0.02 | 2.04 | 3.38 | 0.45 | 0.76 | 1.31 | 2.22 | 0.67 | 1.23 |
| 93.07.14 | 433 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0 | ND | 0 | ND | 0.0 | ND | 0.0 |
| 93.10.21 | 532 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0 | ND | 0 | ND | 0.0 | ND | 0.0 |

*: Application time of cypermethrin

μg/g after 791 days following the 1991 application and at 0.78 to 1.38 μg/g after 532 days following the 1992 application. There was no significant difference of chlorpyrifos residues in soil between two sites. The initial residues of chlorpyrifos in soil following the 1992 application were much lower than 1991 application.

No oxon was detected in the soil during the experimental period. The residues of pyridinol in soil ranged from 0.22 to 10.7 μg/g at both sites in 1991 application and from 0.21 to 6.01 μg/g after the 1992 application. The fact that the ratios between pyridinol and chlorpyrifos at each sampling time were higher in soil than in elm bark and litter probably means that chlorpyrifos was hydrolysed into pyridinol more quickly in soil than in bark or litter.

### *4.7 The residues of cypermethrin isomers and their metabolites in soil*

The residue concentrations and standard deviations of the four pairs of cypermethrin isomers and their major metabolites at each sampling time in soil at both sites and both application times are shown in Tables 18 and 19. At the beginning of cypermethrin treatment, initial residues were low ranging from undetected to 0.53 μg/g. Then the cypermethrin residues in soil increased. The cypermethrin residues began to decline after the residue level reached their peak values in soil at 69 or 74 days after application. The highest residue levels of *cis*-A, *trans*-C, *cis*-B, and *trans*-D in soil during the whole experiment were 3.68, 3.10,

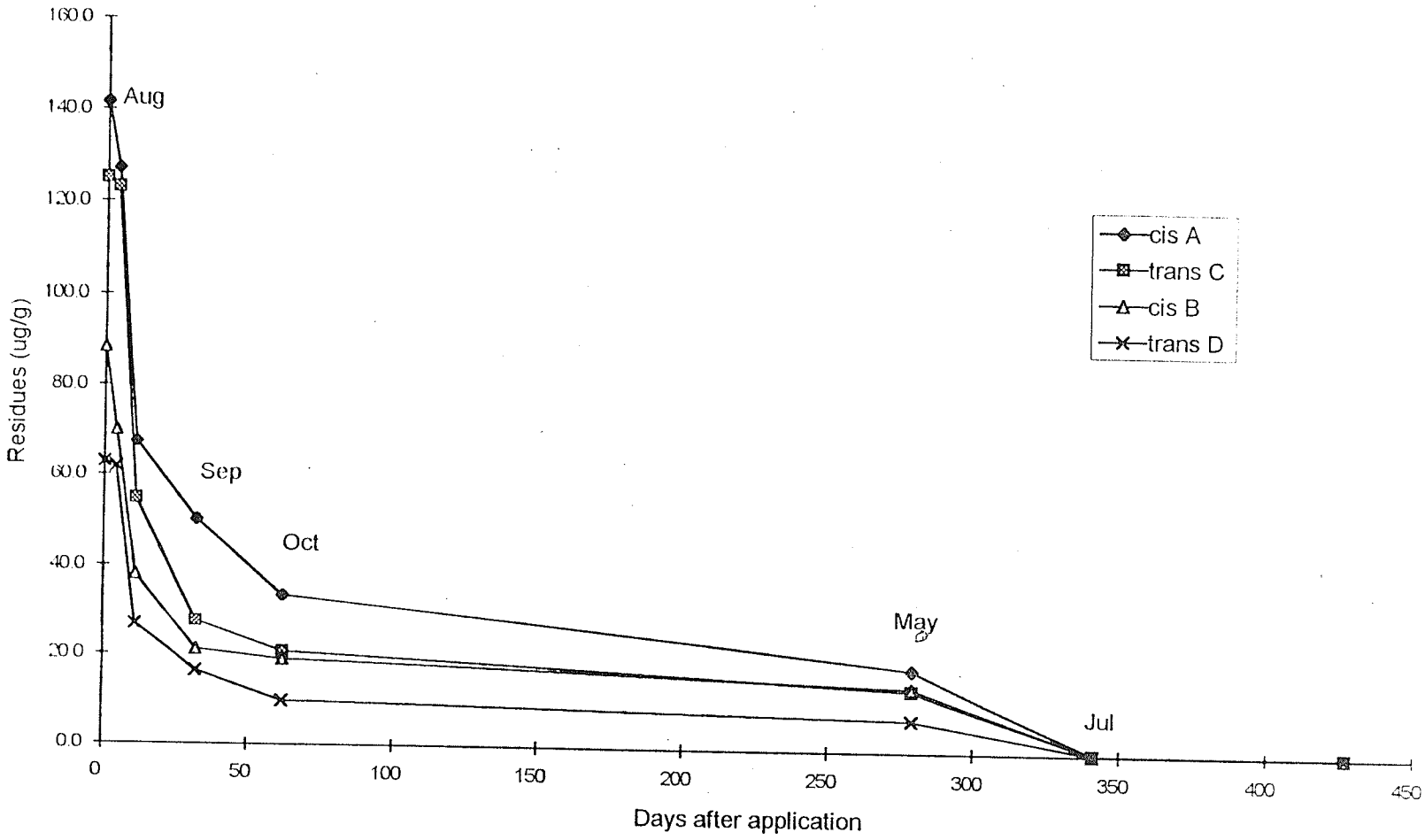



Figure 25. Dissipation of four pairs of cypermethrin isomers in litter at the Glenlea Research Station for the 1991 application

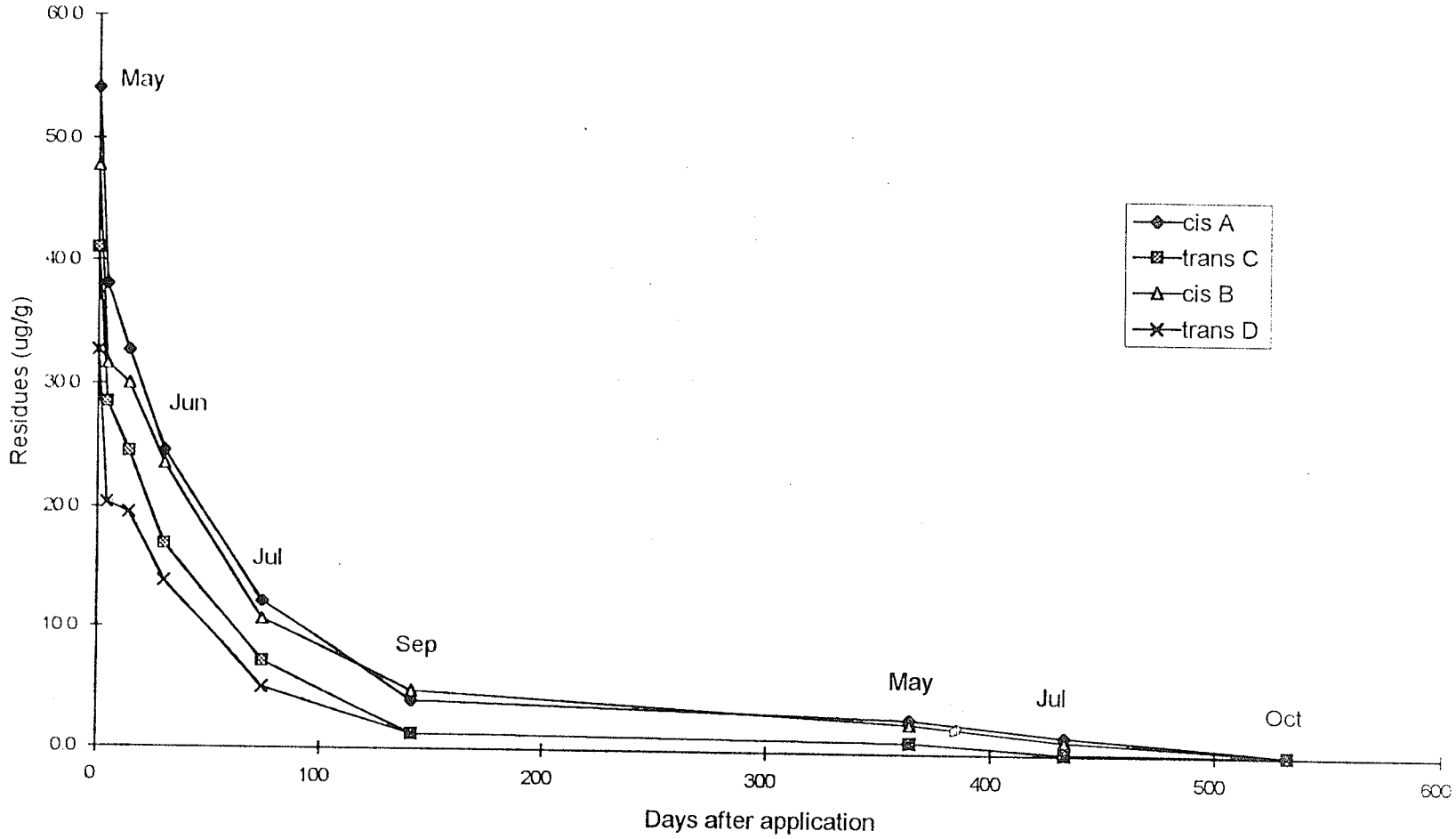



Figure 26. Dissipation of four pairs of cypermethrin isomers in litter at the Glenlea Research Station for the 1992 application

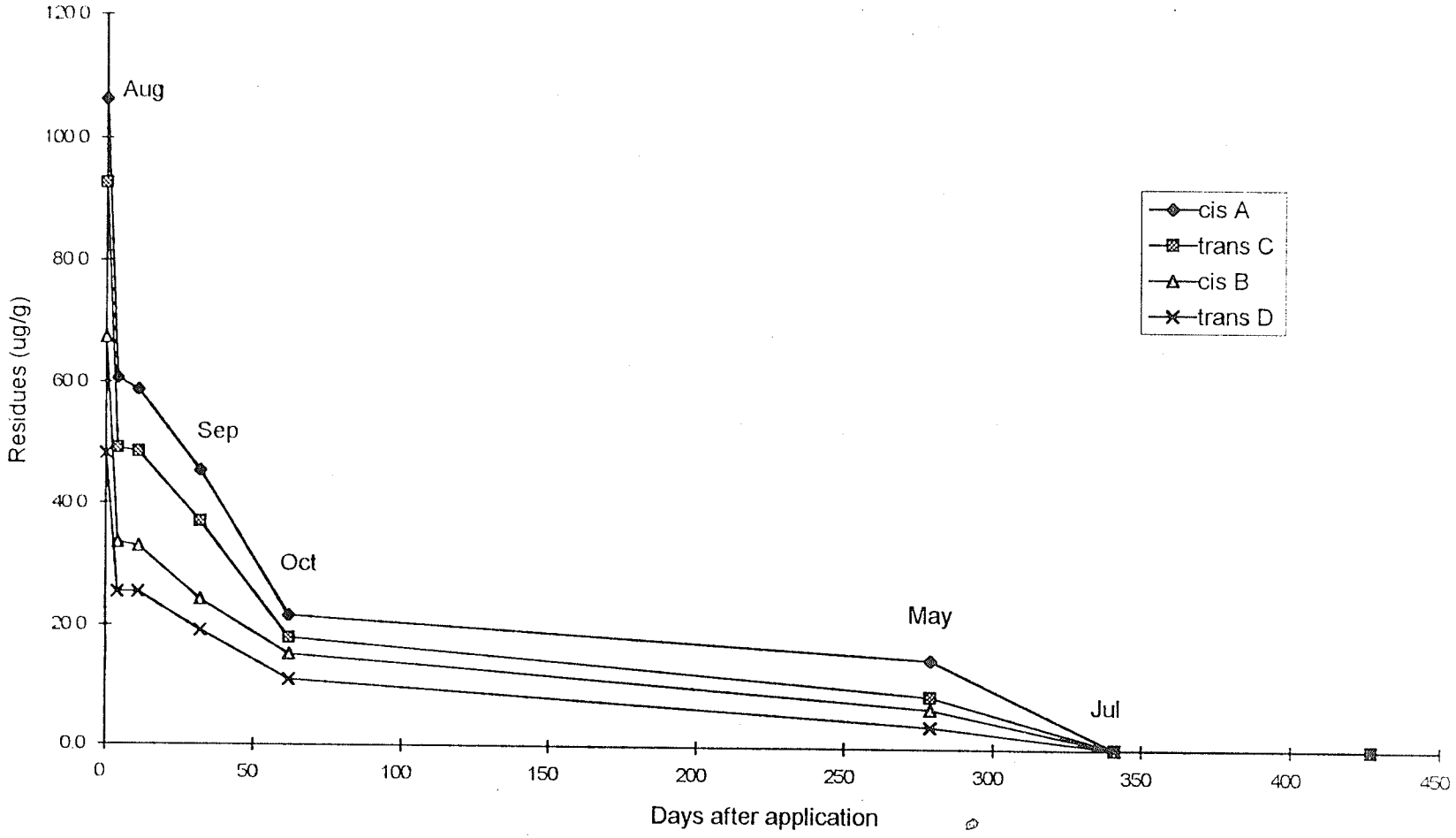



Figure 27. Dissipation of four pairs of cypermethrin isomers in litter at Beaudry Provincial Park for the 1991 application

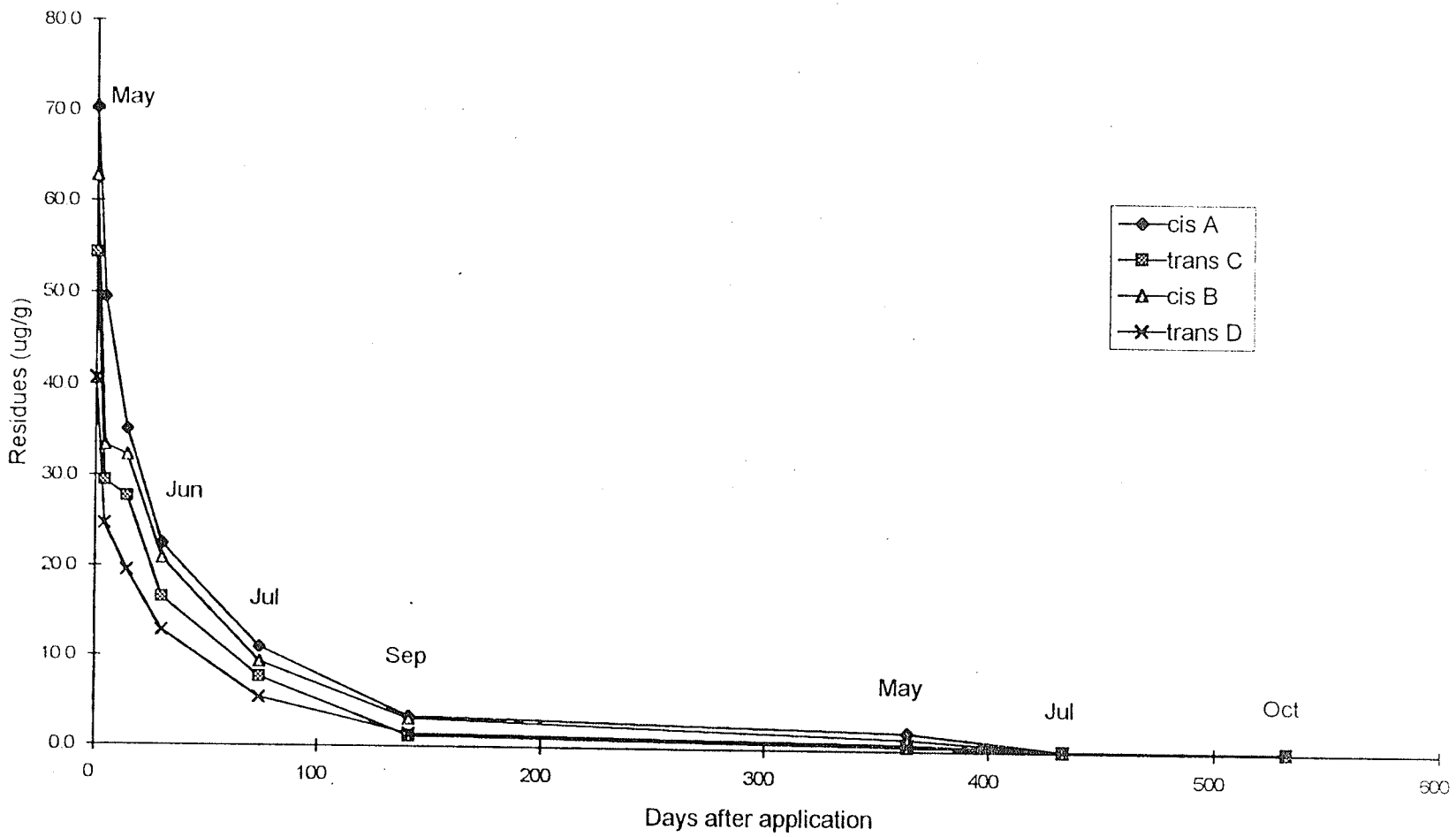



Figure 28. Dissipation of four pairs of cypermethrin isomers in litter at Beaudry Provincial Park for the 1992 application

Table 16. The $DT_{50}$ and $DT_{90}$ of four pairs of cypermethrin isomers in litter

| Treatment | Analytes | $DT_{50}$ (Days) | $DT_{90}$ (Days) |
|---|---|---|---|
| Glenlea, 1991 | *Cis*-A | 10 | 290 |
| | *Trans*-C | 9.1 | 290 |
| | *Cis*-B | 8.8 | 300 |
| | *Trans*-D | 8.9 | 290 |
| Glenlea, 1992 | *Cis*-A | 25 | 130 |
| | *Trans*-C | 23 | 110 |
| | *Cis*-B | 29 | 140 |
| | *Trans*-D | 23 | 110 |
| Beaudry, 1991 | *Cis*-A | 20 | 300 |
| | *Trans*-C | 15 | 260 |
| | *Cis*-B | 11 | 280 |
| | *Trans*-D | 15 | 250 |
| Beaudry, 1992 | *Cis*-A | 14 | 110 |
| | *Trans*-C | 15 | 97 |
| | *Cis*-B | 15 | 110 |
| | *Trans*-D | 12 | 95 |

Table 17. The residues and standard deviations (n=3) of chlorpyrifos, oxon, and pyridinol in soil at both experimental sites and application times

| Sampling Date | Days after Application | Chlorpyrifos | | | | Oxon | | | | Pyridynol | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Glenlea | | Beaudry | | Glenlea | | Beaudry | | Glenlea | | Beaudry | |
| | | Residues (µg/g) | SD (µg/g) | Residues (µg/g) | SD (µg/g) | Residues (µg/g) | SD (µg/g) | Residues (µg/g) | SD (µg/g) | Residues (µg/g) | SD (µg/g) | Residues (µg/g) | SD (µg/g) |
| 91.08.15 | 0 | 20.4 | 0.71 | 28.2 | 4.48 | ND | 0 | ND | 0 | 7.01 | 0.91 | 9.33 | 1.21 |
| 91.08.19 | 4 | 16.8 | 0.48 | 20.5 | 1.78 | ND | 0 | ND | 0 | 6.32 | 0.27 | 9.42 | 0.92 |
| 91.08.26 | 11 | 23.6 | 0.46 | 14.6 | 0.76 | ND | 0 | ND | 0 | 8.17 | 0.18 | 9.38 | 0.67 |
| 91.09.19 | 32 | 17.8 | 0.28 | 5.41 | 0.45 | ND | 0 | ND | 0 | 9.04 | 0.87 | 5.27 | 0.76 |
| 91.10.16 | 62 | 27.3 | 4.17 | 9.44 | 0.89 | ND | 0 | ND | 0 | 10.7 | 1.57 | 6.12 | 0.37 |
| 92.05.20 | 279 | 9.91 | 4.91 | 14.4 | 7.01 | ND | 0 | ND | 0 | 4.28 | 2.01 | 10.5 | 2.68 |
| 92.07.21 | 341 | 8.78 | 2.34 | 12.0 | 8.54 | ND | 0 | ND | 0 | 4.04 | 0.67 | 6.54 | 4.04 |
| 92.10.15 | 427 | 3.01 | 3.18 | 4.45 | 1.13 | ND | 0 | ND | 0 | 1.14 | 0.78 | 2.68 | 1.67 |
| 93.05.03 | 627 | 3.56 | 1.08 | 5.07 | 1.48 | ND | 0 | ND | 0 | 0.87 | 0.48 | 2.17 | 0.71 |
| 93.10.14 | 791 | 1.04 | 0.27 | 2.09 | 1.31 | ND | 0 | ND | 0 | 0.22 | 0.11 | 0.49 | 0.32 |
| 92.05.07 | 0 | 1.01 | 0.51 | 3.81 | 3.61 | ND | 0 | ND | 0 | 0.31 | 0.18 | 1.01 | 0.81 |
| 92.05.11 | 4 | 1.89 | 0.58 | 3.53 | 3.22 | ND | 0 | ND | 0 | 0.91 | 0.11 | 1.27 | 1.01 |
| 92.05.21 | 14 | 1.67 | 0.49 | 3.04 | 0.71 | ND | 0 | ND | 0 | 1.29 | 0.29 | 2.67 | 0.89 |
| 92.06.15 | 30 | 3.68 | 0.57 | 6.18 | 4.01 | ND | 0 | ND | 0 | 2.38 | 1.01 | 6.01 | 2.28 |
| 92.07.20 | 74 | 4.42 | 1.72 | 4.79 | 2.03 | ND | 0 | ND | 0 | 1.68 | 0.51 | 3.12 | 1.79 |
| 92.09.25 | 141 | 3.94 | 1.61 | 11.8 | 10.7 | ND | 0 | ND | 0 | 1.91 | 0.42 | 3.58 | 2.88 |
| 93.05.06 | 364 | 2.01 | 0.67 | 2.61 | 0.19 | ND | 0 | ND | 0 | 1.02 | 0.57 | 1.67 | 0.27 |
| 93.07.14 | 433 | 1.18 | 0.38 | 1.37 | 0.18 | ND | 0 | ND | 0 | 0.41 | 0.10 | 0.79 | 0.14 |
| 93.10.21 | 532 | 0.78 | 0.31 | 1.38 | 0.21 | ND | 0 | ND | 0 | 0.21 | 0.02 | 0.37 | 0.0 |

Table 18. The residues and standard deviations (n=3) of four pairs of cypermethrin isomers and their major metabolites in soil at the Glenlea Research Station at both application times

| Sampling Date | Days after Application | *Cis*-CCA Residues (µg/g) | SD (µg/g) | *Trans*-CCA Residues (µg/g) | SD (µg/g) | PBA Residues (µg/g) | SD (µg/g) | *Cis*-A Residues (µg/g) | SD (µg/g) | *Trans*-C Residues (µg/g) | SD (µg/g) | *Cis*-B Residues (µg/g) | SD (µg/g) | *Trans*-D Residues (µg/g) | SD (µg/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 91.08.15* | 0 | ND | 0.00 | ND | 0.00 | ND | 0.00 | 0.53 | 0.07 | 0.35 | 0.15 | 0.27 | 0.07 | 0.19 | 0.03 |
| 91.08.19 | 4 | 0.02 | 0.01 | 0.03 | 0.00 | 0.12 | 0.02 | 1.19 | 0.06 | 1.01 | 0.06 | 0.73 | 0.07 | 0.59 | 0.00 |
| 91.08.26 | 11 | 0.06 | 0.01 | 0.21 | 0.01 | 0.23 | 0.01 | 3.02 | 0.18 | 2.56 | 0.09 | 1.81 | 0.07 | 1.24 | 0.11 |
| 91.09.19 | 32 | 0.30 | 0.03 | 1.17 | 0.12 | 0.50 | 0.07 | 1.85 | 0.38 | 1.20 | 0.31 | 1.04 | 0.31 | 0.68 | 0.19 |
| 91.10.16 | 62 | 0.28 | 0.01 | 0.67 | 0.02 | 0.44 | 0.02 | 2.36 | 0.23 | 1.42 | 0.09 | 1.42 | 0.27 | 0.87 | 0.10 |
| 92.05.20 | 279 | 1.28 | 0.78 | 0.52 | 0.27 | 0.20 | 0.09 | 1.92 | 1.04 | 0.71 | 0.30 | 1.67 | 0.93 | 0.47 | 0.18 |
| 92.07.21 | 341 | 0.24 | 0.12 | 0.20 | 0.09 | 0.16 | 0.03 | 1.00 | 1.15 | 0.51 | 0.59 | 0.47 | 0.49 | 0.32 | 0.29 |
| 92.10.15 | 427 | 0.21 | 0.02 | 0.05 | 0.01 | 0.01 | 0.02 | 1.23 | 0.26 | 0.42 | 0.02 | 1.04 | 0.73 | ND | 0.00 |
| 93.05.03 | 627 | 0.10 | 0.01 | 0.04 | 0.01 | 0.08 | 0.02 | 0.72 | 0.15 | 0.24 | 0.05 | 0.66 | 0.24 | ND | 0.00 |
| 93.10.14 | 791 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.00 |
| 92.05.07* | 0 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.00 |
| 92.05.11 | 4 | 0.01 | 0.02 | 0.01 | 0.01 | 0.03 | 0.05 | 0.17 | 0.30 | 0.14 | 0.24 | 0.14 | 0.24 | 0.14 | 0.25 |
| 92.05.21 | 14 | 0.04 | 0.07 | 0.02 | 0.04 | 0.02 | 0.03 | 0.17 | 0.30 | 0.11 | 0.18 | 0.16 | 0.27 | 0.11 | 0.18 |
| 92.06.15 | 30 | 0.12 | 0.16 | 0.11 | 0.10 | 0.09 | 0.12 | 0.44 | 0.53 | 0.23 | 0.23 | 0.41 | 0.50 | 0.21 | 0.22 |
| 92.07.20 | 74 | 0.11 | 0.05 | 0.09 | 0.03 | 0.03 | 0.02 | 0.49 | 0.10 | 0.30 | 0.02 | 0.45 | 0.07 | 0.29 | 0.03 |
| 92.09.25 | 141 | 0.01 | 0.00 | 0.01 | 0.00 | 0.04 | 0.01 | 0.37 | 0.06 | ND | 0.00 | 0.51 | 0.14 | ND | 0.00 |
| 93.05.06 | 364 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.21 | 0.09 | ND | 0.00 | 0.11 | 0.04 | ND | 0.00 |
| 93.07.14 | 433 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.00 |
| 93.10.21 | 532 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.00 |

*: Application time of cypermethrin

Table 19. The residues and standard deviations (n=3) of four pairs of cypermethrin isomers and their major metabolites in soil at Beaudry Provincial Park at both application times

| Sampling Date | Days after Application | *Cis*-CCA Residues (µg/g) | SD (µg/g) | *Trans*-CCA Residues (µg/g) | SD (µg/g) | PBA Residues (µg/g) | SD (µg/g) | *Cis*-A Residues (µg/g) | SD (µg/g) | *Trans*-C Residues (µg/g) | SD (µg/g) | *Cis*-B Residues (µg/g) | SD (µg/g) | *Trans*-D Residues (µg/g) | SD (µg/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 91.08.15* | 0 | ND | 0.00 | ND | 0.00 | ND | 0.00 | 0.33 | 0.05 | 0.25 | 0.06 | 0.12 | 0.01 | 0.12 | 0.03 |
| 91.08.19 | 4 | 0.03 | 0.01 | 0.06 | 0.01 | 0.14 | 0.02 | 2.67 | 0.61 | 2.25 | 0.58 | 1.66 | 0.55 | 1.20 | 0.30 |
| 91.08.26 | 11 | 0.07 | 0.01 | 0.15 | 0.01 | 0.21 | 0.02 | 3.68 | 0.19 | 3.10 | 0.20 | 2.19 | 0.11 | 1.50 | 0.12 |
| 91.09.19 | 32 | 0.24 | 0.01 | 0.88 | 0.11 | 0.64 | 0.15 | 3.50 | 0.86 | 2.24 | 0.15 | 2.04 | 0.44 | 1.33 | 0.29 |
| 91.10.16 | 62 | 0.14 | 0.01 | 0.27 | 0.02 | 0.35 | 0.06 | 1.59 | 0.27 | 0.92 | 0.20 | 0.92 | 0.30 | 0.58 | 0.09 |
| 92.05.20 | 279 | 0.59 | 0.28 | 0.35 | 0.16 | 0.29 | 0.05 | 2.40 | 0.90 | 0.96 | 0.40 | 2.03 | 0.35 | 0.79 | 0.32 |
| 92.07.21 | 341 | 0.56 | 0.39 | 0.74 | 0.56 | 0.65 | 0.20 | 2.96 | 1.14 | 1.58 | 0.68 | 1.52 | 1.14 | 1.06 | 0.46 |
| 92.10.15 | 427 | 0.25 | 0.13 | 0.17 | 0.13 | 0.12 | 0.02 | 1.81 | 0.56 | 0.46 | 0.17 | 1.69 | 0.62 | 0.15 | 0.13 |
| 93.05.03 | 627 | 0.11 | 0.03 | 0.05 | 0.01 | 0.14 | 0.13 | 0.85 | 0.16 | 0.26 | 0.06 | 0.93 | 0.19 | 0.06 | 0.00 |
| 93.10.14 | 791 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.00 | ND | 0.00 |
| 92.05.07* | 0 | ND | 0.00 | ND | 0.00 | ND | 0.00 | 0.37 | 0.32 | 0.19 | 0.17 | 0.44 | 0.40 | 0.25 | 0.22 |
| 92.05.11 | 4 | 0.03 | 0.02 | 0.02 | 0.02 | 0.06 | 0.06 | 0.32 | 0.28 | 0.21 | 0.19 | 0.23 | 0.20 | 0.19 | 0.17 |
| 92.05.21 | 14 | 0.06 | 0.04 | 0.05 | 0.02 | 0.05 | 0.02 | 0.50 | 0.15 | 0.35 | 0.08 | 0.40 | 0.15 | 0.33 | 0.09 |
| 92.06.15 | 30 | 0.14 | 0.07 | 0.05 | 0.01 | 0.09 | 0.05 | 0.57 | 0.21 | 0.35 | 0.10 | 0.55 | 0.27 | 0.35 | 0.07 |
| 92.07.20 | 74 | 0.05 | 0.08 | 0.07 | 0.07 | 0.04 | 0.05 | 0.40 | 0.41 | 0.19 | 0.18 | 0.38 | 0.38 | 0.13 | 0.12 |
| 92.09.25 | 141 | 0.05 | 0.04 | 0.02 | 0.01 | 0.05 | 0.03 | 1.05 | 0.56 | 0.21 | 0.19 | 1.22 | 0.70 | 0.08 | 0.15 |
| 93.05.06 | 364 | 0.02 | 0.01 | 0.01 | 0.01 | 0.03 | 0.01 | 0.72 | 0.28 | 0.15 | 0.13 | 0.69 | 0.26 | 0.12 | 0.10 |
| 93.07.14 | 433 | 0.01 | 0.01 | 0.01 | 0.01 | 0.03 | 0.03 | 0.17 | 0.13 | ND | 0.00 | 0.13 | 0.10 | ND | 0.00 |
| 93.10.21 | 532 | 0.02 | 0.02 | 0.01 | 0.01 | ND | 0.00 | 0.17 | 0.21 | ND | 0.00 | 0.13 | 0.13 | ND | 0.00 |

*: Application time of cypermethrin

2.19, and 1.50 μg/g, respectively. No residues of all four pairs of cypermethrin isomers in soil could be detected after 791 days from the 1991 application and 532 days from the 1992 application. *Cis*-isomers persisted longer in soil than *trans*-isomers. No dissipation half-life of cypermethrin isomers could be calculated because cypermethrin residue concentrations in soil fluctuated from time to time. The residues of *cis*-CCA, *trans*-CCA, and PBA in soil were very low compared to their parent compounds with the highest levels of 1.28, 0.67, and 0.64 μg/g, respectively.

### *4.8 Chlorpyrifos degradation in laboratory prepared soil*

Table 20 shows the degradation of two concentrations of chlorpyrifos in soil fortified in the laboratory, wrapped in glass fibre filter paper, and embedded in soil under the litter layer in the field. The detected initial concentration of chlorpyrifos in fortified soil was 18 μg/g for the lower level and 39 μg/g for the higher level, a little lower than their theoretical fortification concentrations of 20 μg/g and 40 μg/g, respectively. The initial residues of chlorpyrifos in the soil sample dissipated 93% after 422 days from beginning time for both concentrations. No oxon was detected within the entire experimental period. The residue concentrations of the pyridinol in soil fluctuated from 0.5 μg/g to 1.4 μg/g for the lower level and from 1.0 μg/g to 3.1 μg/g for the higher level.

Table 20. The residues and standard deviations (n=3) of chlorpyrifos, oxon, and pyridinol in artificially fortified soil

| Concentration | Sampling Time | Days after Application | Chlorpyrifos Residues (μg/g) | Chlorpyrifos SD (μg/g) | Oxon Residues (μg/g) | Oxon SD (μg/g) | Pyridinol Residues (μg/g) | Pyridinol SD (μg/g) |
|---|---|---|---|---|---|---|---|---|
| Low | 92.08.18* | 0 | 18.0 | 2.31 | ND | 0.00 | 0.71 | 0.18 |
| | 92.08.25 | 7 | 16.9 | 0.89 | ND | 0.00 | 1.18 | 0.09 |
| | 92.09.27 | 30 | 13.6 | 3.49 | ND | 0.00 | 1.29 | 0.21 |
| | 92.10.15 | 58 | 10.9 | 1.22 | ND | 0.00 | 1.38 | 0.18 |
| | 93.05.06 | 261 | 4.71 | 0.79 | ND | 0.00 | 1.37 | 0.81 |
| | 93.07.14 | 330 | 2.33 | 0.32 | ND | 0.00 | 1.11 | 0.10 |
| | 93.10.14 | 422 | 1.18 | 0.21 | ND | 0.00 | 0.51 | 0.11 |
| High | 92.08.18* | 0 | 39.0 | 2.18 | ND | 0.00 | 1.51 | 0.20 |
| | 92.08.25 | 7 | 29.8 | 2.39 | ND | 0.00 | 2.19 | 0.11 |
| | 92.09.27 | 30 | 30.0 | 5.19 | ND | 0.00 | 2.58 | 0.59 |
| | 92.10.15 | 58 | 21.5 | 4.58 | ND | 0.00 | 3.11 | 0.58 |
| | 93.05.06 | 261 | 9.71 | 1.68 | ND | 0.00 | 2.78 | 0.21 |
| | 93.07.14 | 330 | 6.01 | 1.09 | ND | 0.00 | 2.01 | 0.18 |
| | 93.10.14 | 422 | 2.68 | 0.48 | ND | 0.00 | 1.02 | 0.12 |

*: The time when the samples were inserted into soil in the field.

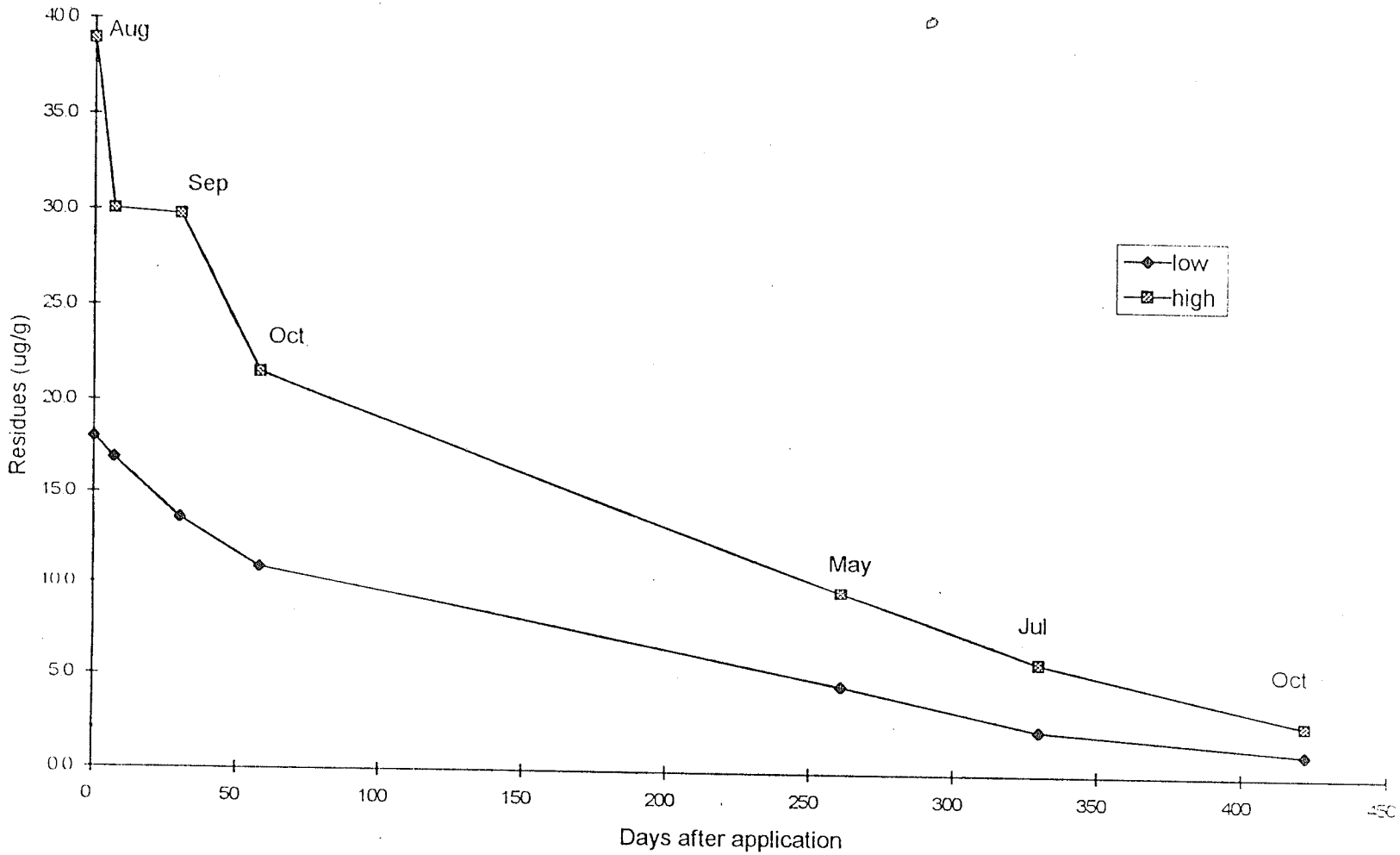



Figure 29. The degradation of chlorpyrifos in the artificially fortified soil

The degradation graphs are shown in Figure 29 for lower and higher initial fortified concentrations. The $DT_{50}$ and $DT_{90}$ of chlorpyrifos at lower and higher initial fortified concentrations are listed in Table 22. Chlorpyrifos dissipated quite slowly in the soil with $DT_{50}$ of 120 days for the lower level and 92 days for the higher level.

### *4.9 Cypermethrin isomer degradation in laboratory prepared soil*

Table 21 shows the degradation of two concentrations of cypermethrin in soil spiked with 40 EC Ripcord® aqueous solution in the laboratory, wrapped in glass fibre filter paper, and embedded in soil under the litter layer in the field. The detected initial concentrations of *cis*-A, *trans*-C, *cis*-B, and *trans*-D cypermethrin isomers in fortified soil were 8.71, 6.10, 7.31, and 5.31 μg/g, respectively, for the lower level and 16.2, 12.1, 13.5, and 10.3 μg/g, respectively, for the higher level. The initial residues of *cis*- cypermethrin isomers in the soil sample dissipated 56 to 59 %, however, the initial residues of *trans*- cypermethrin isomers dissipated 66 to 75 % after 422 days from beginning time. The residue concentrations of CCA and PBA were very low compared to their parent compounds ranging from 0.01 to 0.41 μg/g within the whole experimental period.

The degradation graphs of cypermethrin isomer pairs in soil are shown in Figures 30 for the lower concentration and Figure 31 for the higher concentration. The $DT_{50}$ and $DT_{90}$ of four cypermethrin isomer pairs in the fortified soil at two concentrations are displayed in Table 22. The $DT_{50}$ of *cis*- A, *trans*- C, *cis*- B, and

Table 21. The residues and standard deviations (n=3) of four pairs of cypermethrin isomers and their major metabolites in artificially fortified soil

| Sampling Time | Days after Application | *Cis*-CCA Residues (μg/g) | SD (μg/g) | *Trans*-CCA Residues (μg/g) | SD (μg/g) | PBA Residues (μg/g) | SD (μg/g) | *Cis*-A Residues (μg/g) | SD (μg/g) | *Trans*-C Residues (μg/g) | SD (μg/g) | *Cis*-B Residues (μg/g) | SD (μg/g) | *Trans*-D Residues (μg/g) | SD (μg/g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 92.08.18* | 0 | 0.04 | 0.02 | 0.04 | 0.01 | 0.05 | 0.02 | 8.71 | 0.91 | 6.10 | 0.51 | 7.31 | 0.71 | 5.31 | 0.51 |
| 92.08.25 | 7 | 0.02 | 0.01 | 0.02 | 0.02 | 0.05 | 0.02 | 8.49 | 0.67 | 6.01 | 0.29 | 7.09 | 0.58 | 5.18 | 0.52 |
| 92.09.27 | 30 | 0.03 | 0.00 | 0.04 | 0.00 | 0.07 | 0.02 | 8.42 | 0.22 | 5.87 | 0.58 | 6.80 | 0.39 | 5.09 | 0.29 |
| 92.10.15 | 58 | 0.04 | 0.01 | 0.05 | 0.01 | 0.10 | 0.05 | 8.01 | 0.29 | 5.59 | 0.22 | 6.81 | 0.28 | 5.01 | 0.11 |
| 93.05.06 | 261 | 0.07 | 0.03 | 0.09 | 0.03 | 0.29 | 0.13 | 7.02 | 1.28 | 3.71 | 0.81 | 6.01 | 1.19 | 2.91 | 0.60 |
| 93.07.14 | 330 | 0.08 | 0.01 | 0.11 | 0.04 | 0.33 | 0.11 | 4.47 | 0.10 | 2.30 | 0.08 | 3.89 | 0.19 | 1.78 | 0.09 |
| 93.10.14 | 422 | 0.04 | 0.02 | 0.05 | 0.01 | 0.11 | 0.05 | 3.68 | 0.21 | 2.09 | 0.31 | 3.22 | 0.11 | 1.71 | 0.28 |
| 92.08.18* | 0 | 0.06 | 0.04 | 0.04 | 0.00 | 0.16 | 0.03 | 16.2 | 0.31 | 12.1 | 0.21 | 13.5 | 0.31 | 10.3 | 0.60 |
| 92.08.25 | 7 | 0.04 | 0.02 | 0.04 | 0.02 | 0.11 | 0.06 | 16.1 | 0.31 | 11.4 | 1.11 | 13.0 | 0.32 | 9.91 | 1.01 |
| 92.09.27 | 30 | 0.02 | 0.01 | 0.03 | 0.01 | 0.04 | 0.01 | 15.8 | 1.48 | 10.8 | 0.09 | 12.7 | 1.28 | 9.61 | 0.12 |
| 92.10.15 | 58 | 0.08 | 0.02 | 0.09 | 0.02 | 0.19 | 0.10 | 14.6 | 0.79 | 10.1 | 0.69 | 11.8 | 0.59 | 8.89 | 0.51 |
| 93.05.06 | 261 | 0.03 | 0.01 | 0.05 | 0.02 | 0.13 | 0.12 | 12.6 | 0.28 | 6.91 | 0.41 | 10.4 | 0.38 | 5.56 | 0.28 |
| 93.07.14 | 330 | 0.11 | 0.10 | 0.15 | 0.15 | 0.41 | 0.40 | 10.8 | 0.31 | 5.89 | 1.01 | 9.01 | 0.21 | 4.71 | 0.79 |
| 93.10.14 | 422 | 0.06 | 0.02 | 0.04 | 0.01 | 0.05 | 0.02 | 6.61 | 0.71 | 3.19 | 0.48 | 5.58 | 0.61 | 2.62 | 0.40 |

*: The time when the samples were inserted into soil in the field.

Table 22. The $DT_{50}$ and $DT_{90}$ of chlorpyrifos, and cypermethrin isomers in artificially fortified soil

| Analytes | Fortification Concentrations | $DT_{50}$ (Days) | $DT_{90}$ (Days) |
|---|---|---|---|
| Chlorpyrifos | Low | 120 | 370 |
| | High | 92 | 390 |
| *Cis*-A | Low | 340 | NA* |
| | High | 390 | NA |
| *Trans*-C | Low | 290 | NA |
| | High | 320 | NA |
| *Cis*-B | Low | 360 | NA |
| | High | 390 | NA |
| *Trans*-D | Low | 280 | NA |
| | High | 290 | NA |

*: $DT_{90}$ can not be calculated due to the high residue concentration at the end of the experiment.

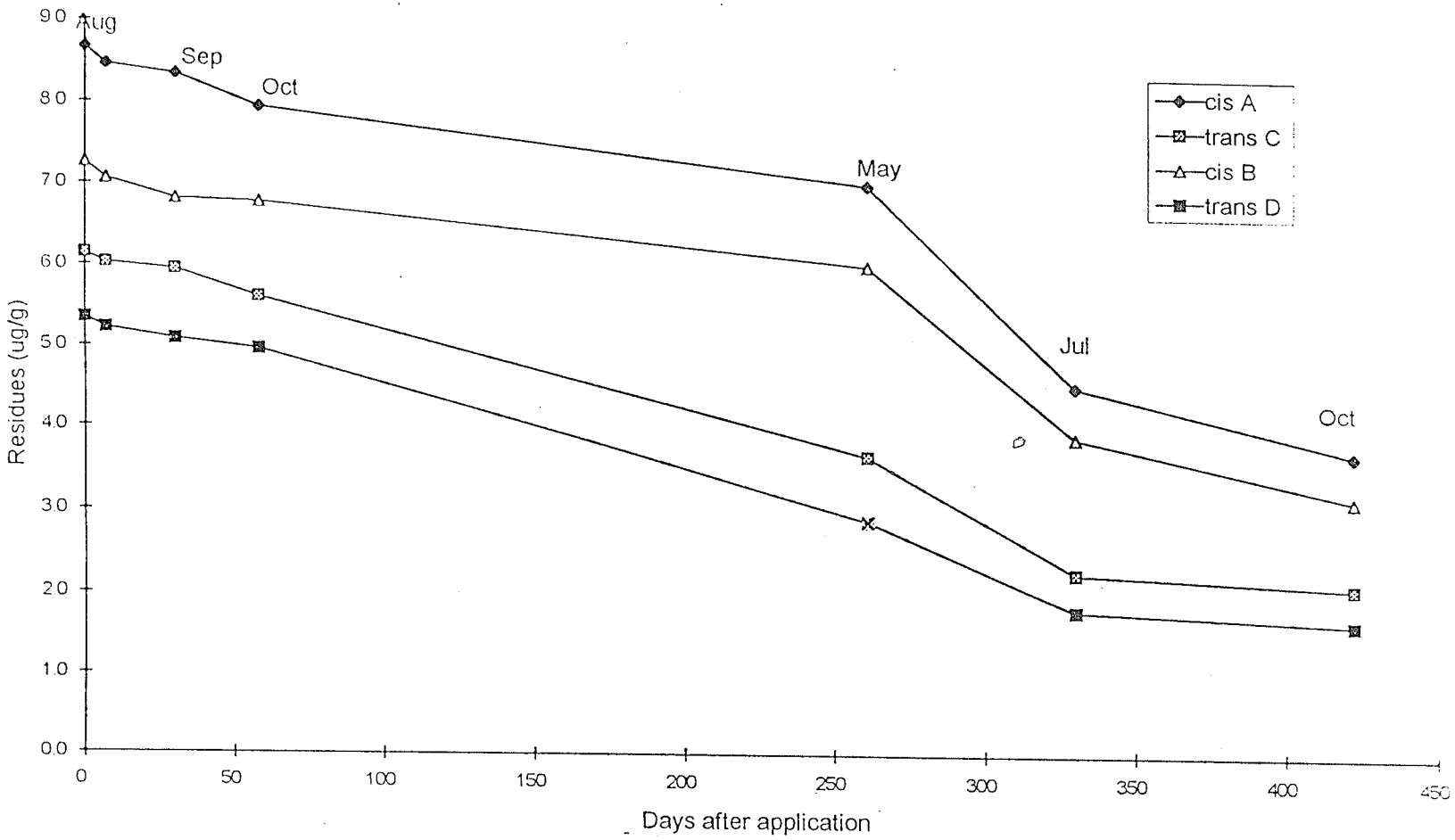



Figure 30. The degradation of four pairs of cypermethrin isomers at lower concentration in the artificially fortified soil

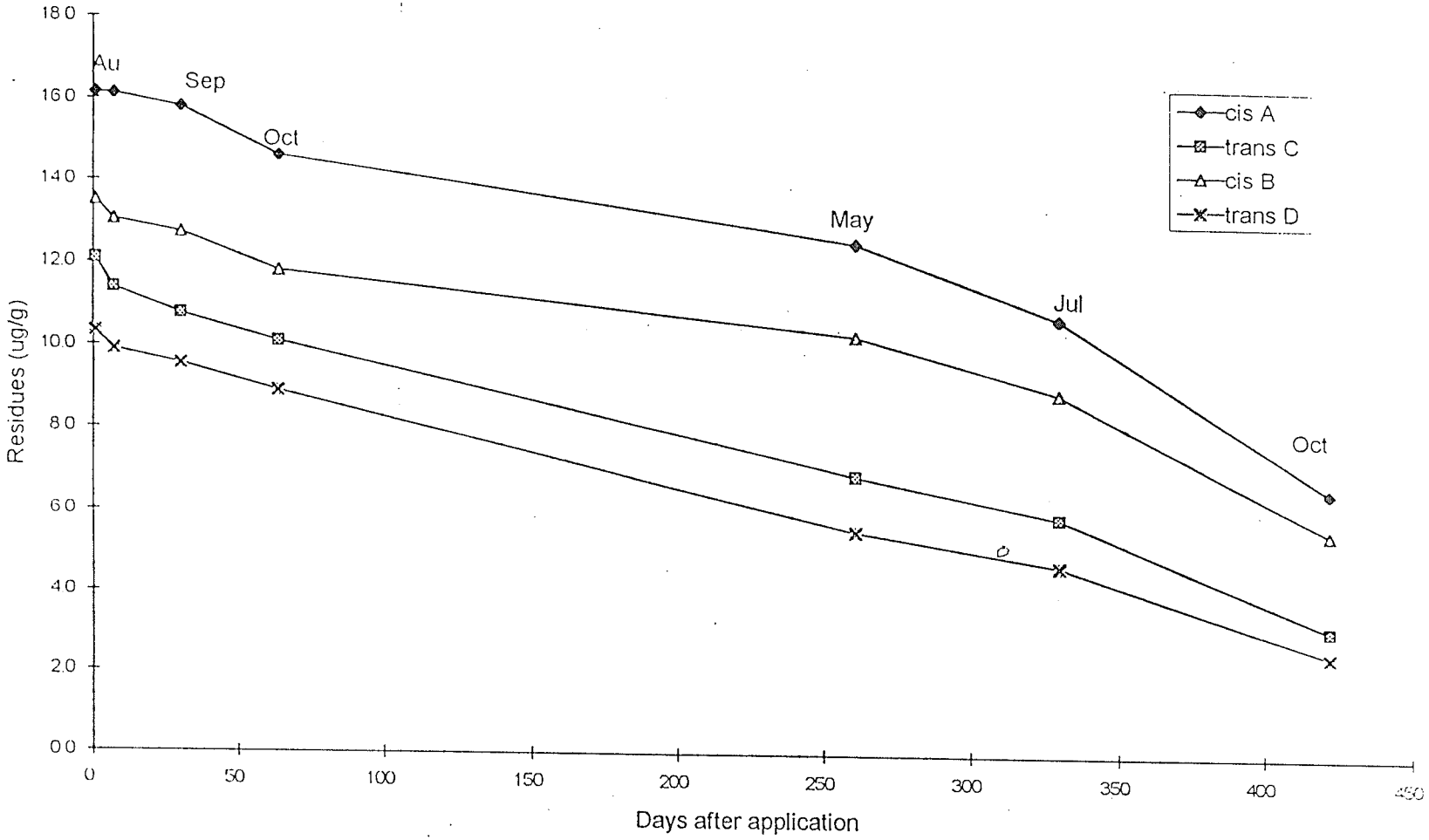



Figure 31. The degradation of four pairs of cypermethrin isomers at higher concentration in the artificially fortified soil

*trans*- D cypermethrin isomer pairs in the artificially fortified soil are 340, 290, 360, and 280 days for the lower level, and 390, 320, 390, and 290 days for the higher level. The data indicate that *trans*- cypermethrin isomers dissipated approximately 1.3 times more quickly than the *cis*- isomers; and the initial residue concentrations of cypermethrin isomers affect the dissipation rates with approximately 1.1 times longer $DT_{50}$ found in the higher level than in the lower level.

### *4.10 Availability of the surface residues of insecticides*

Table 23 displays the availability of chlorpyrifos and four pairs of cypermethrin isomers on the surface elm bark to glass fibre paper pressed against the treated elm bark surface for 1 minute at different sampling times. The insecticide residues transferred from the treated bark were 1.86 mg/m$^2$ for chlorpyrifos and 2.34 mg/m$^2$ (total of four pairs of isomers) for cypermethrin for samples taken when the treated bark surface had dried for about 1 hour after application. The availability of the insecticides dropped very quickly to 0.23 mg/m$^2$ for chlorpyrifos and 0.63 mg/m$^2$ for cypermethrin 4 days after the treatment.

### *4.11 The penetration of the insecticides from the treated bark into cambium and wood tissue*

Table 24 shows the residue distribution of chlorpyrifos and cypermethrin isomers in elm bark, cambium, and wood tissues taken at different time intervals after application. The experimental results reveal that neither chlorpyrifos nor cypermethrin could be detected in cambium and wood tissue indicating that these

Table 23. Availability (n=3) of chlorpyrifos, and cypermethrin isomers from the treated elm bark surface

| Sampling Time | Days after application | *Cis*-A Residues (µg/ $m^2$) | SD (µg/ $m^2$) | *Trans*-C Residues (µg/ $m^2$) | SD (µg/ $m^2$) | *Cis*-B Residues (µg/ $m^2$) | SD (µg/ $m^2$) | *Trans*-D Residues (µg/ $m^2$) | SD (µg/ $m^2$) | Chlorpyrifos Residues (µg/ $m^2$) | SD (µg/ $m^2$) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 92.05.07 | 0 | 674 | 275 | 641 | 272 | 567 | 237 | 458 | 193 | 1860 | 1030 |
| 92.05.11 | 4 | 153 | 98.0 | 184 | 112 | 130 | 87.0 | 167 | 97.0 | 228 | 75.0 |
| 92.05.21 | 14 | 115 | 58.0 | 138 | 74.0 | 99.0 | 52.0 | 117 | 62.0 | 92.0 | 21.0 |
| 92.06.15 | 30 | 93.0 | 9.01 | 86.0 | 9.01 | 77.0 | 6.81 | 79.0 | 9.01 | 54.0 | 14.0 |
| 92.07.20 | 74 | 12.0 | 6.01 | 15.0 | 6.41 | 10.0 | 4.91 | 13.0 | 6.71 | 24.0 | 7.01 |
| 92.09.25 | 141 | 7.61 | 2.09 | 9.21 | 1.01 | 6.71 | 1.38 | 8.30 | 0.72 | 15.0 | 6.02 |
| 93.05.06 | 364 | 2.89 | 1.81 | 3.78 | 2.09 | 2.31 | 1.41 | 3.41 | 1.91 | 7.81 | 1.61 |
| 93.07.14 | 433 | 0.89 | 0.49 | 0.81 | 0.49 | 0.29 | 0.09 | 0.89 | 0.19 | 2.01 | 0.59 |
| 93.10.21 | 532 | 0.11 | 0.01 | 0.09 | 0.01 | 0.11 | 0.01 | 0.09 | 0.01 | 0.11 | 0.01 |

Table 24. The residues of chlorpyrifos, and cypermethrin isomers in bark, cambium, and wood tissue at different time intervals after application

| Sampling Time | Days after Application | Residues (μg/g) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *Cis*-A | | | *Trans*-C | | | *Cis*-B | | | *Trans*-D | | | Chlorpyrifos | | |
| | | Wood | Cambium | Bark | Wood | Cambium | Bark | Wood | Cambium | Bark | Wood | Cambium | Bark | Wood | Cambium | Bark |
| 91.08.15* | 0 | ND | ND | 123 | ND | ND | 107 | ND | ND | 75.1 | ND | ND | 50.1 | ND | ND | 1020 |
| 91.08.26 | 11 | ND | ND | 109 | ND | ND | 95.9 | ND | ND | 72.2 | ND | ND | 52.9 | ND | ND | 633 |
| 91.10.16 | 62 | ND | ND | 54.0 | ND | ND | 48.1 | ND | ND | 34.8 | ND | ND | 27.2 | ND | ND | 569 |
| 92.07.21 | 341 | ND | ND | 34.2 | ND | ND | 31.8 | ND | ND | 20.9 | ND | ND | 21.1 | ND | ND | 159 |
| 92.10.15 | 427 | ND | ND | 33.0 | ND | ND | 25.0 | ND | ND | 22.0 | ND | ND | 12.9 | ND | ND | 135 |
| 93.10.14 | 791 | ND | ND | 13.1 | ND | ND | 10.1 | ND | ND | 9.01 | ND | ND | 5.01 | ND | ND | 97.1 |

*: Application time of the insecticides.

two insecticides lack the ability to penetrate the bark. The residues of chlorpyrifos in the elm bark layer were still 97.1 μg/g after 791 days from the initial application of the insecticide.

## *4.12 Persistence of control effectiveness of chlorpyrifos and cypermethrin against elm bark beetles*

### 4.12.1 Behavioural observations of the elm bark beetle on treated elm bark

After the elm bark beetles were placed onto untreated, chlorpyrifos, and cypermethrin treated elm bark discs, the behaviour of the beetles in each treatment was observed.

#### *4.12.1.1 Untreated bark*

Approximately 50 minutes after the beetles were introduced onto the bark, dust produced by tunnelling through the bark was apparent. At that time, 50 of 60 test beetles had begun to make tunnels or at least initiate bark feeding or boring. The rest of the beetles moved over the bark. At 100 minutes after introduction, some beetles reached wood tissue, the normal overwintering site. At that time, ten beetles of the 60 beetles were still moving around on the bark surface. No dead beetles were found on the untreated bark during the experimental period of 130 minutes.

*4.12.1.2 In the chlorpyrifos treated bark*

At 50 minutes after beetles were placed on the chlorpyrifos treated elm bark, all the beetles were moving on the bark surface and did not make any tunnels. At 60 minutes, three of 60 test beetles appeared to be dying. The others were moving very slowly on the bark surface. At 90 minutes, approximately half of the 60 test beetles were dead. At 130 minutes, all 60 test beetles were dead. When the beetles were dead, the wings and legs of the dead beetles were extended. At the end of the experiment, trace dust was found at one location. A very shallow tunnel was produced by a beetle which died soon after.

*4.12.1.3 In the cypermethrin treated bark*

At 15 minutes after beetles were added on the cypermethrin treated bark, all the beetles moved very slowly with about 12 beetles lying on their backs. At 45 minutes, the beetles began to die. At 50 minutes, approximately 50% of the beetles were dead. At 65 minutes, all 60 test beetles were dead. No dust was found on the elm bark. Unlike the beetles killed by chlorpyrifos, the beetles killed by cypermethrin did not extend their wings and legs.

### 4.12.2 Insecticidal persistence of chlorpyrifos and cypermethrin

Tables 25 and 26 show the residues of chlorpyrifos and cypermethrin on the elm bark and the mortalities of beetles after 24 hours exposure to the treated elm bark and after 24 hours recovery at different sampling times in the 1991 and

1992 applications at the Beaudry Provincial Park. The experimental results indicated that chlorpyrifos was still 100% effective in killing the elm bark beetles after 791 days from the 1991 application and 532 days after 1992 application. The residue levels of chlorpyrifos in the elm bark after 791 days in 1991 and 532 days in 1992 application was 38 and 42 μg/g, respectively. Although some dust had been formed in the elm bark collected at 791 days after application, the tunnels produced by the beetles were only in the surface bark, and were much shorter than the normal overwintering tunnels.

The bioassay results of elm bark beetles exposed to the cypermethrin treated elm bark indicate that the effectiveness of cypermethrin in killing the elm bark beetles remained at 100% after 62 days from the 1991 application and 74 days from the 1992 application. In excess of 80 % mortality was recorded in both 1991 at 341 and 364 days, respectively (Table 26).

Table 25. The mortality (n=3) of elm bark beetles in the chlorpyrifos treated elm bark

| Sampling Date | Days after Application | Control Mortality(%) | | Residues (μg/g) | Chlorpyrifos Mortality (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | | 24 hr test | Post-recovery | | 24 hr Test | SD | Post-recovery | SD |
| 91.08.15* | 0 | 10 | 10 | 444 | 100 | 0 | 100 | 0 |
| 91.08.19 | 4 | 5 | 10 | 294 | 100 | 0 | 100 | 0 |
| 91.08.26 | 11 | 10 | 10 | 273 | 100 | 0 | 100 | 0 |
| 91.09.19 | 32 | 5 | 5 | 247 | 100 | 0 | 100 | 0 |
| 91.10.16 | 62 | 10 | 10 | 177 | 100 | 0 | 100 | 0 |
| 92.05.20 | 279 | 0 | 5 | 119 | 100 | 0 | 100 | 0 |
| 92.07.21 | 341 | 5 | 5 | 59.4 | 100 | 0 | 100 | 0 |
| 92.10.15 | 427 | 0 | 0 | 56.3 | 100 | 0 | 100 | 0 |
| 93.05.03 | 627 | 0 | 0 | 44.3 | 100 | 0 | 100 | 0 |
| 93.10.14 | 791 | 0 | 0 | 39.7 | 100 | 0 | 100 | 0 |
| 92.05.07* | 0 | 5 | 5 | 388 | 100 | 0 | 100 | 0 |
| 92.05.11 | 4 | 0 | 5 | 237 | 100 | 0 | 100 | 0 |
| 92.05.21 | 14 | 5 | 5 | 173 | 100 | 0 | 100 | 0 |
| 92.06.15 | 30 | 10 | 10 | 155 | 100 | 0 | 100 | 0 |
| 92.07.20 | 74 | 0 | 0 | 111 | 100 | 0 | 100 | 0 |
| 92.09.25 | 141 | 0 | 0 | 67.7 | 100 | 0 | 100 | 0 |
| 93.05.06 | 364 | 5 | 5 | 54.6 | 100 | 0 | 100 | 0 |
| 93.07.14 | 433 | 5 | 5 | 54.3 | 100 | 0 | 100 | 0 |
| 93.10.21 | 532 | 0 | 0 | 42.4 | 100 | 0 | 100 | 0 |

*: Application time of chlorpyrifos.

Table 26. The mortality (n=3) of elm bark beetles in the cypermethrin treated elm bark

| Sampling Date | Days after Application | Control Mortality (%) | | Residues (μg/g) | Cypermethrin Mortality (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | | 24 hr Test | Post-Recovery | | 24 hr test | SD | Post-recovery | SD |
| 91.08.15* | 0 | 10 | 10 | 171 | 100 | 0 | 100 | 0 |
| 91.08.19 | 4 | 5 | 10 | 119 | 100 | 0 | 100 | 0 |
| 91.08.26 | 11 | 10 | 10 | 112 | 100 | 0 | 100 | 0 |
| 91.09.19 | 32 | 5 | 5 | 96.6 | 100 | 0 | 100 | 0 |
| 91.10.16 | 62 | 10 | 10 | 69.6 | 100 | 0 | 100 | 0 |
| 92.05.20 | 279 | 0 | 5 | 53.9 | 92 | 2.9 | 97 | 2.9 |
| 92.07.21 | 341 | 5 | 5 | 49.2 | 82 | 7.6 | 83 | 10 |
| 92.10.15 | 427 | 0 | 0 | 43.9 | 83 | 7.6 | 85 | 2.9 |
| 93.05.03 | 627 | 0 | 0 | 24.6 | 68 | 7.6 | 70 | 5 |
| 93.10.14 | 791 | 0 | 0 | 15.7 | 37 | 2.9 | 38 | 5 |
| | | | | 0 | | | | |
| 92.05.07* | 0 | 5 | 5 | 95.6 | 100 | 0 | 100 | 0 |
| 92.05.11 | 4 | 0 | 5 | 68.7 | 100 | 0 | 100 | 0 |
| 92.05.21 | 14 | 5 | 5 | 60.6 | 100 | 0 | 100 | 0 |
| 92.06.15 | 30 | 10 | 10 | 56.3 | 100 | 0 | 100 | 0 |
| 92.07.20 | 74 | 0 | 0 | 45.9 | 100 | 0 | 100 | 0 |
| 92.09.25 | 141 | 0 | 0 | 39.2 | 92 | 2.9 | 93 | 2.9 |
| 93.05.06 | 364 | 5 | 5 | 28.2 | 82 | 7.6 | 83 | 5.8 |
| 93.07.14 | 433 | 5 | 5 | 15.3 | 63 | 10 | 63 | 10 |
| 93.10.21 | 532 | 0 | 0 | 11.4 | 22 | 7.6 | 23 | 7.6 |

*: Application time of cypermethrin.

# 5. Discussion

## *5.1 Analytical method development*

The high accuracy indicated in the recovery data and good precision reflected in the SDs in the previous tables indicate that the analytical methods developed to determine the residues of chlorpyrifos, the four cypermethrin isomer pairs, and their metabolites in elm bark, litter, soil, and glass fibre filter paper at actual residue levels are of high quality.

Chlorpyrifos, oxon, and pyridinol could be quantitatively extracted from elm bark, litter, and soil matrices under acidic conditions (Tables 3, 4, 5). These three analytes could be chromatograghed together after simple liquid-liquid partition, and methylation. The attainment of excellent resolution between chlorpyrifos and oxon and the high reproducibility and sensitivity of the gas chromatograph analysis to the oxon in make separation of chlorpyrifos and oxon prior to gas chromatographic analysis unnecessary. In the previous analytical method (Braun, 1974) to determine the residues of chlorpyrifos, oxon, and pyridinol in a sample, normally pyridinol was first separated from the chlorpyrifos and oxon through liquid-liquid partition by adjusting the pH of the solution. The oxon analogue was separated from its parent by passing the extract through a silica gel column which decomposed the oxon into pyridinol. The pyridinol was then derivatized prior to gas chromatographic analysis. Thus, chlorpyrifos, oxon, and pyridinol in one sample had to be analyzed on the GC separately. That chlorpyrifos, oxon, and

pyridinol can now be extracted and then chromatographed together is much simpler and more productive. The detection limits for chlorpyrifos, oxon, and pyridinol in elm bark, litter, and soil achieved in these analytical methods were adequate in these situations because of the high residue concentration in the samples. These analytical methods could be directly utilized or slightly modified to determine the residues of chlorpyrifos, oxon, and pyridinol in matrices which contained the target analytes above the detection limits. These analytical methods could not have been used to analyze chlorpyrifos, oxon, and pyridinol together in matrices which contained lower concentrations than the detection limits and more interfering materials, because the oxon can not resist decomposition in the commonly used cleanup adsorbents, such as silica gel (Braun, 1974).

Some analytical methods for the determination of chlorpyrifos, oxon, and pyridinol in various matrices already exist. Struble and McDonald (1973) developed methods to analyze chlorpyrifos and oxon in wheat plants and kernels and average recoveries ranged from 85 to 95% for chlorpyrifos and from 58 to 93% for oxon. The recoveries of chlorpyrifos and oxon in corn and grass determined by gas chromatography with flame photometric detection ranged from 96 to 100% and 85 to 90%, respectively (Bowman and Beroza, 1967). The recoveries of the published analytical methods for chlorpyrifos and pyridinol range from 86 to 110%, and from 79 to 99%, respectively, in dates (Mourer *et al.*, 1990), and averaged 88% in peppermint hay and peppermint oil (Inman *et al.*, 1981). Braun (1974) developed a method to analyze chlorpyrifos, oxon, and pyridinol in

vegetable tissue and obtained average recoveries of 95% for chlorpyrifos and 85% for oxon and pyridinol.

The gas chromatograms of cypermethrin isomer pairs and their derivatized metabolites in different matrices indicate that there were no significant interfering peaks at retention times of the seven target peaks for the elm bark and soil matrices, but there was an interfering peak at the retention time of 21.5 minutes in the litter control sample which coincided with the retention time of the *cis*-B cypermethrin pair. The identity of the interfering peak in the litter was not determined. The probability that the peak is a cypermethrin pair is quite low because other cypermethrin isomer pairs did not exist in the chromatograms of the control litter sample and the four cypermethrin isomer pairs appear proportionately in the chromatograms of litter samples taken around the treated elm trees. The quantification of *cis*-B cypermethrin isomers in the litter was made by comparing the difference of the peak area obtained from the treated litter sample minus the peak area of the peak with the same retention time in the control litter to the standard curve made from the standard solution with the known amount.

The analytical methods developed to determine the residues of cypermethrin isomers and their metabolites in elm bark, litter, and soil matrices allow the target analytes to be quantitatively extracted from elm bark, litter, soil, and glass fibre filter paper and to be analysed altogether on the gas chromatograph after simple liquid-liquid partition cleanup and proper derivatization of the three metabolites. Similar analytical methods were developed by Class

(1992) in which the residues of cypermethrin isomers and major metabolites in spruce bark, and soil were extracted with dichloromethane by Soxhlet, and cleaned up on a Florisil column, and analysed by GC-ECD, GC-MS or HPLC. In GC analysis, all eight possible cypermethrin isomers appeared in four peaks with each peak representing a pair of *cis*- or *trans*- isomers. Three of the four pairs of cypermethrin isomers were further slightly separated in normal phase HPLC. Chapman and Harris (1978) developed analytical methods for cypermethrin parent compounds in vegetable crops. The recoveries for the methods ranged from 72% to 99% at the 0.01 μg/g level. Average recovery of 105% in celery for cypermethrin was also reported (Braun and Stanek, 1982).

The analytical methods developed here were simple in operation and productive. The parent compounds and their metabolites could be separated readily through liquid-liquid partition by adjusting the pH value in the solution. But the separation between the parent cypermethrin and its metabolites during the course of sample preparation and the consequent separate GC analyses consume more reagents and labour. This separation is only necessary when the simultaneous analyses of cypermethrin and its metabolites cannot be carried out due to the presence of interfering material or to significant differences in the amount of cypermethrin and its metabolites in the same sample. The detection limits of cypermethrin isomers and their metabolites in elm bark, litter, and soil were sufficient to deal with the analytical task at hand. These analytical methods appear suitable to be directly used or slightly modified to determine the residues of

cypermethrin isomers and their metabolites in other matrices. If lower detection limits are required, additional cleanup procedures, such as column chromatography on Florisil or silica gel, could be applied to reduce the interfering material. The cypermethrin isomers and the derivatization products of CCA, and PBA with α-bromo-2,3,4,5,6-pentafluoro toluene do not degrade when they pass through these adsorbents.

### *5.2 Dissipation of the insecticides in elm bark*

The difference of the initial residues of chlorpyrifos at different application times and experimental sites was attributed to experimental error of application and to variation in bark texture of the test elm trees used. The rougher bark normally found on the larger trees could intercept larger amount of insecticides at application and would then have higher initial residue values when the residual concentration of insecticides in bark is expressed as insecticide weight per unit weight of bark. The initial residues of approximately 400 μg/g for chlorpyrifos in elm bark are very high compared to the initial residues of 1-10 μg/g found in common crops such as corn (Abdel-All *et al.*, 1990; Leuck *et al.*, 1968). High initial chlorpyrifos residues of 1449 - 4000 μg/g in pine bark were reported after the pine trunks were treated with 1 or 2% chlorpyrifos (EC) solutions (Brady *et al.*, 1980; Berisford *et al.*, 1981,1991). The authors found that the residues dissipated very quickly initially, dropping 50% within the first month, and then dissipation slowed down. This is a similar to the dissipation pattern seen in my research. First order

dissipation half-life of 5.0 - 6.9 months were calculated in their experiments. The DT50 of chlorpyrifos in the elm bark ranged from 11 to 68 days in my study. The initial residues of total cypermethrin in elm bark are much lower than those of chlorpyrifos mainly due to the lower application dosage of cypermethrin (4.8 times lower than chlorpyrifos). This experiment did not determine the effect of bark position on the trees on the dissipation of the insecticide on the elm bark, but Page (1983) indicated that there was no significant difference (P = 0.01) between the residues of gamma-HCH and chlorpyrifos-methyl in Ponderosa pine bark (*Pinus ponderosa* Laws.) on samples from the north or south sides of the trees.

According to the $DT_{50}$ and $DT_{90}$ of the insecticides (Tables 8, 11), the insecticides dissipated more quickly during the earlier stage than during the later period after application. The dissipation data of the insecticides during summer and winter period in elm bark presented in Figures 13 - 20 also reveal that the insecticides dissipate more quickly during the summer time than during the winter time, presumably due to higher temperature, and rainfall which are favourable for the dissipation of insecticides from the target matrix through the such processes as the molecular decomposition and relocation of the insecticides from the target area to non-target area. Some dissipation of insecticides occurred during the first winter in the 1991 application. The dissipation of the insecticides in elm bark became very slow after 341 days from the application in 1991, dissipating approximately 33 - 39 % within the 450 days; and after 364 days in the 1992 application, dissipating approximately 22 - 32 % within 168 days for chlorpyrifos. The possible tight

association between the insecticide molecules and the bark components or probable penetration of the insecticide into inner bark in the later stage might slow down the dissipation processes. The dissipation trend observed during the later sampling indicates that the effective residues of chlorpyrifos in elm bark may last for a number of years.

The dissipation of insecticides in the environmental compartments is caused by the translocation and transformation of the target analytes and bonded residues. In the translocation process, target analytes move from the target matrices to non-target ones through such processes as leaching, runoff, evaporation, and partitioning into other environmental compartment without decomposition. In the transformation, the target molecules are decomposed into other substances by means of hydrolysis, photolysis and biological activity. The dissipation of chlorpyrifos and cypermethrin in the elm bark reflected in Tables 7, 9, and 10 is caused by the sum of the above mentioned processes.

The ester bond structures of chlorpyrifos and cypermethrin molecules determine that these two insecticides are susceptible to the hydrolytic degradation. The reported hydrolysis half-life of chlorpyrifos at near neutral pH (~7) and ambient temperature (~ 25 °C) were from 29 (Chapman and Cole, 1982) to 74 days (Batzer *et al.*, 1990). The reported photolytic half-life of chlorpyrifos applied on various surfaces ranged from 2.2 days (Chen *et al.*, 1984) on glass plates to 52.6 days (Walia *et al.*, 1988) on a leaf surface (*Polystichum setiferum*) depending on the light source, surface characteristics, and modifiers on the surface. The half-

life of cypermethrin is one or more years at acidic pH (Hall *et al.*, 1981). The photodegradation half-life of cypermethrin on soil thinly coated on plates in natural sunlight ranged from 8 to 16 days (Takahashi *et al.*, 1985a). Considering the dryness of elm bark and amount of direct sunlight reaching the basal portion of elm trunks and the hydrolytic and photolytic half-life of chlorpyrifos and cypermethrin on other matrices, the relative importance of photolytic and hydrolytic reactions in the overall dissipation of chlorpyrifos and cypermethrin in the elm bark may be fairly low.

Physical transportation such as leaching and volatility may play a major role in the dissipation of insecticides in the elm bark, especially soon after the application. The adjuvants used in the insecticide formulation help the non-polar active ingredients of the insecticides to be evenly distributed in the aqueous solution during application. After the mixture of the active ingredients and adjuvant reach the target surface, it can be expected that the adjuvant in the mixture will make the active ingredient more susceptible to leaching of rainfall or dew. It is also expected that leaching plays a smaller role in the overall dissipation of the insecticides with more adjuvant leached due to its relatively large water solubility and adsorption of the insecticide molecules into the dead organic components of the elm bark due to their low water solubility. No data about the leaching of chlorpyrifos or cypermethrin on tree bark or plant surfaces are available, but leaching of these two insecticides in soil is very slow (Thiegs, 1964; McCall, 1985; Kaufman *et al.*, 1981).

Volatility may be another important route for the dissipation of the insecticide in the elm bark. McCall *et al.* (1985) estimated the relative importance of volatility. Corn plants were treated with chlorpyrifos at a rate of 1.12 kg/ha and placed in a growth chamber (30 °C) with constant air speed of 0.22 m/sec (0.8 km/h). Within 48 h, 79.3 % had volatilized from the corn leaf surfaces. The authors concluded that volatility loss was the major mechanism of chlorpyrifos dissipation from the plant leaf surfaces. Due to the higher vapour pressure of chlorpyrifos (1.8 - 2.0 X $10^{-5}$ mm Hg at 25 °C) (Chakrabarti and Gennrich, 1987) than cypermethrin (1.4 X $10^{-9}$ Hg at 20 °C)(Travis, 1990; Ferraro, 1990), evaporation may play a smaller role in the dissipation of cypermethrin from elm bark than is true for chlorpyrifos. Veierov *et al.* (1988) conducted a study to demonstrate how leaching and volatility affected the dissipation of chlorpyrifos on plant leaves. From the experimental results, the authors found that surface residues dissipated 1.4-4.4 times more rapidly than internal leaf residues demonstrating the important role of evaporation on the dissipation rate. Any factors which facilitate chlorpyrifos to penetrate into leaf tissue would reduce the dissipation rate from plant leaves. This could be demonstrated by the half-life of 11.8, 68.7-72.7, and >470 hours for EC, mineral oil, and cottonseed oil formulations, respectively. The oil formulations slowed the volatile loss of chlorpyrifos from the treated plant leaves by increasing the ability of oil-formulated chlorpyrifos to penetrate into leaf tissue (26-56%) *vs.* the EC (13%) and reducing leaching. The insecticides dissipated from the target matrices through volatility find their fate in the atmosphere. However, extensive air

monitoring for volatilized chlorpyrifos residues following indoor or outdoor applications has demonstrated that toxicologically significant levels are not reached from a human exposure standpoint (Naffziger *et al.,* 1985; Currie *et al.,* 1990; Glotfelty *et al.*, 1990; Brady *et al.*, 1991).

Considering that the insecticides failed to penetrate into the cambium layer, as shown in Table 24, and that the bark sample used in the experiment contained cambium layer, the residue concentrations of the insecticides in the thin surface bark layer would have been several times higher than the values expressed in Tables 7, 9, and 10. Results in the test of contact residue availability indicate that the chlorpyrifos or cypermethrin residues transferred from the treated bark surface to glass fibre filter paper pressed against on it for 1 minute are below the acceptable daily intake for human subjects even just after application. But the 100 % mortality of elm bark beetles exposed on the chlorpyrifos treated elm bark indicated in Table 25 without producing any dust indicates that the residues are still on the surface and are still available to the contact insects. Residues of the insecticides are therefore probably adsorbed on the thin surface layer of elm bark.

### *5.3 Dissipation of the insecticides in litter*

As a result of the large contact area/mass of litter, the high concentration of insecticide solution and the relatively low application efficiency, the initial residues of the two kinds of insecticides in litter are extremely high (187 - 916 μg/g for chlorpyrifos, and 176 - 417 μg/g for total cypermethrin) (Tables 12, 14, 15)

compared to the initial residues encountered in common agricultural crops. Howell and George (1984) studied chlorpyrifos persistence in apple orchards in Washington. Chlorpyrifos (EC) was applied one to three times at 1.2 and 2.4 g/L, either as a full cover or an under tree debris spray. The residues of chlorpyrifos in litter 1 month after the last application (September) were 297 - 501 μg/g for the low and 1011 - 1736 μg/g for the high rate of application, and by the next spring (April) had dissipated to 1.9 - 14.6 % of the level in September. The residue levels of chlorpyrifos in my experiment were much lower than the ones in the above experiment. The sampling error and variation in the amount of litter on the ground when the insecticides were applied all contribute to the difference of initial insecticide residues between the application times and between the experimental sites. As far as the application times are concerned, the initial residues of the insecticides in litter were lower in the 1992 application than in the 1991 application. When the insecticides were applied in May, 1992, the larger amount of litter from the previous fall probably had the effect of diluting the residue concentration in litter.

Insecticide residues in litter dissipated much more quickly than in bark. The insecticide residues in litter decreased very quickly, presumably through evaporation of the residues into air, transfer into soil through leaching, and decomposition of litter originally contaminated by the insecticides, replacement of the contaminated litter in the treatment area by fresh litter from the untreated areas by wind and other agents, and dilution of the contaminated litter by freshly fallen

leaves every fall. The dissipation mechanism of chlorpyrifos in bark discussed above is also applicable to litter. The mobility of litter on the ground and high humidity in litter greatly increase the dissipation rate of chlorpyrifos in litter.

The data in Table 12, 14, and 15 indicate that the dissipation rates of the insecticides in the litter are much slower during winter period than during the summer period. During the winter time, the litter on the ground is also covered with a layer of snow. The dissipation agents active during the summer time such as high temperature and rainfall and micro-organism can play much smaller role in the dissipation of the insecticides in litter during the winter time.

The larger standard deviations indicate that homogeneous samples of litter are more difficult to obtain and accurately measure than bark samples, because of the uneven distribution of litter components and of residues in litter. Elm bark was evenly sprayed with chlorpyrifos solution, while litter was contaminated unintentionally resulting in uneven distribution of the insecticides.

The impact of such high insecticide residues in litter on non-target organisms is not clear. They may have had a negative impact on the animals which reside in or walk over the litter layer. Litter can act as a medium to contaminate the non-target area when the contaminated litter is translocated from the treated area to other places by wind or other agents.

## *5.4 The residues and dissipation of the insecticides in soil*

### 5.4.1 The residues of the insecticides in soil after overspray of elm bark

Although soil is not the treated medium, spray drift can directly reach soil during application. Furthermore, the residues originally in elm bark and litter might be transferred to soil through stem flow and flooding and decomposition of contaminated litter into soil. The layer of litter on the ground effectively reduces the opportunity of spray drift reaching the soil. The initial insecticide residues in soil (averaging 13.4 μg/g for chlorpyrifos and 0.91 μg/g for total cypermethrin isomers) were no higher than the initial insecticide residues found in soil in typical crop fields [ranging from 8.0 μg/g in a corn field (McKellar *et al.,* 1972) to 28.2 μg/g in an onion field (Harris *et al.,* 1973)], although much higher concentrations of insecticide solutions were applied in this study. After application, the insecticide residue concentrations in soil fluctuated. Oliver *et al.* (1987) applied chlorpyrifos (EC) aqueous solutions (1.12 a.i. kg/ha) over the centre of each tree row with spray boom in a swath. The initial residues of chlorpyrifos in the upper inch of soil beneath and between plant canopies were 0.4 - 0.8 and 0.2 - 2.3 μg/g, and average soil dissipation half-life of 1.3 - 4.4 days was observed. Bush *et al.* (1987) and Neary *et al.* (1988) studied the behavior of chlorpyrifos in pine forest stands in Florida. Chlorpyrifos (EC) was applied as a 2% drench to each tree trunk from the ground to the lower crown. The initial residues of chlorpyrifos in the upper 5 cm

soil were 1.25 μg/g. The residues of chlorpyrifos in the soil increased to 3.20 μg/g in the later stages of the experiment. The major routes of pesticide movement from the treated trees to the soil were hypothesized to be stem washoff, crown leaching, and litterfall. Compared to the results of the above research, the residues of chlorpyrifos in the soil in my experiment are much higher.

The data in Tables 17, 18, and 19 indicate that higher initial insecticide residues were found in soil following the August application (averaging 24.1 μg/g for chlorpyrifos, and 1.08 μg/g for total cypermethrin isomers) than following the May application (averaging 2.42 μg/g for chlorpyrifos, and 0.65 μg/g for total cypermethrin isomers). The layer of litter accumulated in the previous fall appears to have decreased the amount of the insecticide spray drift which reached the soil when the insecticide was applied in May. In August, a much thinner layer of litter remained on the ground and more insecticide spray drift reached the soil resulting in higher initial residues in soil.

The residues of chlorpyrifos in the soil are relatively high and persistent compared to agricultural situations (0.72 - 1.4 μg/g at the application rate of 3.36 kg/ha and no detection after one year (Fontaine *et al.,* 1987)). There are few studies on the effect of chlorpyrifos on non-target organisms in soil. Funderburk *et al.* (1990) investigated the effects of granular chlorpyrifos on nontarget arthropods in peanut field. The authors applied granular chlorpyrifos (Lorsban 15G) at the rate of 2.24 kg/ha in a 30 to 35 cm band over the row and found that densities of

spiders were significantly decreased by the chlorpyrifos application. But the red imported fire ant (*Solenopsis invicta* Buren), earwigs (Dermaptera: Labiduridae), damsel bugs (Hemiptera: Nabidae), and bigeyed bugs (Hemipera: Lygaeidae) were not significantly affected. The initial residues of chlorpyrifos in the soil were not given. But according to the similar condition of another research, the application rate of 2.4 kg/ha in soil produced the initial residues of 7.7 μg/g (Chapman and Harris, 1980).

The residues of cypermethrin in the soil in the current study are not especially high compared to the typical agricultural situation. It is expected that the soil residues of cypermethrin will not exert a large effect on the non-target organisms. The toxicity of cypermethrin for the earthworm (*Eisenia foetida*) has been assessed in an artificial soil test system. Worms were exposed to dosages of 0 - 100 μg/g soil for 14 days. No mortality was found (Inglesfield and Sherwood, 1983; Inglesfield, 1984). When cypermethrin was applied to a sandy loam soil at a rate of 2.5 or 250 μg/g, no effect was found on the rate of carbon dioxide evolution (Cook, 1978). The highest residue concentration of cypermethrin found in soil during my study was 10.1 μg/g, lower amount than the highest dosage used in the above mentioned studies.

### 5.4.2 The dissipation of the insecticides in fortified soil

The $DT_{50}$ of chlorpyrifos in fortified soil under forest conditions were 120 days for the lower initial concentration (20 μg/g), and 92 days for the higher initial

concentration (40 μg/g). The reported half-life of chlorpyrifos in soil ranges from 3.74 (Kuhr and Tashiro, 1978) to 146 days (Van De Steene *et al.*, 1989; 1990). In most cases, the reported half-life of chlorpyrifos in soil was less than 50 days. The fact that the number of moles of pyridinol detected was much less than that of chlorpyrifos lost suggests that produced pyridinol degraded further or was leached or washed away by water due to its high water solubility; also chlorpyrifos may have degraded through pathways that do not give rise to pyridinol.

The $DT_{50}$ values of cypermethrin isomer pairs in fortified soil under forest conditions ranged from 280 to 360 days for the lower initial concentration (30 μg/g), and from 290 to 390 days for the higher initial concentration (60 μg/g) with *cis*-isomers having longer half-life than *trans*-isomers. Roberts and Standen (1982) reported that typical half-life for the parent racemic cypermethrin was 2 to 4 weeks in the growing season and *cis*-isomers often had a longer half-life than the *trans*-isomers. Harris *et al.* (1981) reported that the concentration of initial residues affected the degradation rates of cypermethrin in soil which were 2 to 3 times lower in the soil with an initial concentration of 10 μg/g than that with an initial concentration of 0.5 μg/g.

Due to the high $K_d$ values of these two insecticides (1862.0 mL/g) (Sharom *et al.*, 1980) for chlorpyrifos and 16000 (Travis 1990; Ferraro, 1990) for cypermethrin in soil, laboratory and field studies on the leaching and runoff of these two insecticides indicate that they do not move vertically and horizontally in soil and normally remain in the top soil (0 - 10 cm) (Thiegs, 1964; McCall *et al*,

1984; Jackson, 1977; Kaufman *et al.*, 1981; Stevens & Hill, 1980). The dissipation of these two insecticides through volatility from soil is also low. When chlorpyrifos was applied at 67.8 $\mu g/cm^2$ to a sand and 11.2 $\mu g/cm^2$ to a silt loam soil and then incubated at 25 °C with a simulated wind speed of 0.002 km/hour, 2.6 and 9.3 % of the applied chlorpyrifos had volatilized (Racke *et al.*, 1991). It seems that abiotic and biological transformations plays a major role in the dissipation of chlorpyrifos and cypermethrin in soil. It can be expected that any factors which affect abiotic chemical reactions and microbial activity, such as temperature, moisture content, and application rate, will accelerate or retard the dissipation rate of pesticides in soil.

The much longer $DT_{50}$ of the pesticides in Manitoba forest soil than in studies in the crop field soil may results from a number of factors including a lack of micro-organisms which degrade the insecticides, lower temperature, and soil texture. Clay soils strongly adsorb chlorpyrifos or cypermethrin as indicated by the very high $K_d$ values and consequently reduce the availability of insecticides for dissipation.

### *5.5 Availability of surface residues of insecticides*

The highest availability of the insecticide residues on the treated elm bark surface to the glass fibre paper held to its surface was found just after the application. If it is assumed that the area of a child's hand is approximately 0.01 $m^2$ (or 10 cm x 10 cm), the dosage available to a child from one such contact with

a treated tree would be approximately 0.02 mg just after treatment, and should be much less thereafter in each case. The acceptable daily intake (ADI) for human is 0.01 mg/kg (Tomlin, 1994) for chlorpyrifos and 0.05 mg/kg (World Health Organization, 1989) for cypermethrin. A child would weigh approximately 15 to 40 kg; therefore, the ADI for such a child would be approximately 0.3 to 0.4 mg/day for chlorpyrifos and 1.5 to 2.0 mg/day for cypermethrin. Clearly, the dosage available from the treated elm trees by one touch is well below the level of human toxicological concern even on the first day after application, and would diminish after that. The transfer of the insecticides to the bare hand from chlorpyrifos- or cypermethrin- treated elm bark at the application dosage used in this experiment appears to be of low concern. Even if the human skin has 15 times higher affinity for the chlorpyrifos and 75 times higher affinity for cypermethrin than the glass fibre filter paper, and the obtained insecticides are all absorbed by skin, the insecticide obtained by such touch is still below the level of human toxicological concern.

The absorption rates of chlorpyrifos and cypermethrin through skin into the human seem to be very low. Nolan *et al.* (1984) examined the dermal absorption of chlorpyrifos in human volunteers and reported that only 1.35 % of the applied dose (5.0 mg/kg) penetrated the skin during a period of 180 hours. Cheng *et al.* (1989) reported similarly minimal dermal absorption of chlorpyrifos in goats, in which a maximum of 0.3% of the applied dose was present in the circulatory system after 12 hours. Higher absorption rates of cypermethrin were reported. A man was given a single dermal application of 50 mg cypermethrin in hexylene

glycol/shellsol AB on the underside of the forearm. The majority of the application (35 mg) was removed from the skin after 4 hours. No metabolites of CCA and its glucuronide were detected during 96 hours after dosing (Coveney & Eadsforth, 1982). In the another experiment by Van Sittert *et al.* (1985), two male volunteers were given a single dermal application of 25 mg cypermethrin in hexylene glycol/Shellsol A on the underside of the forearm. An average of 53% of the originally applied cypermethrin was removed from the skin 4 hours after application. Approximately 0.1 % was excreted as the urinary metabolite, CCA, during 72 hours after application.

### *5.6 Test of insecticidal persistence of the insecticides against elm bark beetles*

The method to determine the insecticidal persistence of the insecticides on the treated elm bark taken at different intervals from field proved simple and effective. Covering the untreated area of bark samples produced during sampling with paraffin wax ensured that all the test beetles were exposed to the insecticide treated area increasing the experimental accuracy and precision. After having been introduced to the untreated control bark disks, the elm bark beetles began to make tunnels and would have reached the wood tissue under field conditions. The temperature and humidity used in the experiment may have been higher than those found in the field at time when the applied insecticides are expected to be

active, i.e. in the late fall when the beetle begins moving to the basal trunk of elm trees to overwinter and spring when overwintering beetles emerge.

Chlorpyrifos was 100% effective in killing the test beetles for the whole experimental period. The fact that the detected amount of chlorpyrifos in the elm bark after 791 days from application was still approximately three times higher than the initial amount of chlorpyrifos found in common agricultural crops which effectively control insects on crops, and that the residues dissipated very slowly after 791 days of application, implies that chlorpyrifos might be effective for some further time. A longer sampling time is needed to establish the relation between the mortality of the beetles and the chlorpyrifos residue concentration in the elm bark.

Although the mortality values reflected in the bioassay test according to the mortality determining criteria declined gradually with time, elm beetles which were not included in the mortality value were disabled severely by cypermethrin in that they could move only very short distances and appeared to lose the ability to make overwintering tunnels. The fate of the beetles affected by cypermethrin under field conditions is not known. Their failure to move long distances or to make overwintering tunnels could mean that these beetles would not survive under winter conditions. If this is the case, the period of 100% control of the elm bark beetle would be extended to 627 days after application in August or 433 days after application in May. A few beetles were found to make tunnels to wood tissue in

the bark samples indicating that cypermethrin failed to control the elm bark beetle effectively beyond the above stated times.

### *5.7 The suitability of cypermethrin to replace or use in conjunction with chlorpyrifos to control of elm bark beetle*

The suitability of cypermethrin to substitute for chlorpyrifos partly or completely for control of elm bark beetles should be further assessed based on my experimental results. The efficacy of both insecticides at selected application dosages, the persistence, the degradation rates in different non-target environmental matrices, and the physicochemical properties of these two chemicals should be examined further. Whether or not cypermethrin is able to replace chlorpyrifos to control elm bark beetles is a matter of judgment, but the results of the foregoing research provide assistance.

After the basal 1 m of elm trunks were treated with the 100 x aqueous dilution (4.8 g a.i./L) of 48 EC Dursban® (chlorpyrifos) in August, 1991, chlorpyrifos was 100% effective in killing elm bark beetle for the entire experimental period of up to 791 days (entire experimental period). A longer periods of 100% control might be expected considering the residue level of chlorpyrifos in elm bark after 791 days (41.3 μg/g) and the slow dissipation rate expected. After the basal areas of elm trunks were treated with the 400 X aqueous dilution (1 g a.i./L) of 40 EC Ripcord® (cypermethrin), a rate 4.8 X lower than that for chlorpyrifos, the mortality of beetles on treated elm bark was 100%

between 62 and 279 days. Although the mortality in the bioassay test declined gradually with time, a further number of elm bark beetles which were not included in the mortality value were severely disabled by cypermethrin, and could move only very short distances and appeared to have lost the ability to make overwintering tunnels. They would probably die under winter conditions if they could not overwinter in the normal place. If this were the case, the 100% control effectiveness period of cypermethrin against the elm bark beetle would be extended to 627 days after which some overwintering tunnels were successfully established in the cypermethrin treated elm bark. Considering that cypermethrin applied to the elm trunks can only interact to control the elm bark beetle in the fall when the beetles begin to move to the basal trunk areas to overwinter and in the spring when the overwintering beetles emerge from the overwintering tunnels in the same basal trunk areas, two calendar years of 100% control could be expected if Ripcord® is applied just before the beetles begin to overwinter, i.e., in early September.

After the elm trunks had been treated with chlorpyrifos, high initial residues (916 μg/g) were detected in litter. Even after 791 days from application, 7.5 μg/g chlorpyrifos still remained in litter. The chlorpyrifos residues in soil ranged from 0.8 to 28 μg/g during the whole experimental period; 2 μg/g chlorpyrifos could still be detected after 791 days from application. After the elm bark had been treated with cypermethrin, the highest initial residues of total

cypermethrin isomers in litter were 416 μg/g. No cypermethrin residues could be detected in litter 341 days after application. The total residues of cypermethrin isomers found in soil ranged from below detection to 10 μg/g. No cypermethrin could be detected in soil after 627 to 791 days from the fall application and 433 to 532 days from the spring application. Cypermethrin has relatively lower residue levels and shorter residue persistence in non-target environmental matrices, such as litter and soil.

Chlorpyrifos has higher acute toxicity ($LD_{50}$ (rats): 135 - 163 *vs* 251 - 4123 mg/kg for cypermethrin), much higher water solubility (2 *vs* 0.04 mg/L), much higher vapor pressure ($2 \times 10^{-5}$ *vs* $1.4 \times 10^{-9}$ mm Hg), and a much lower soil sorption coefficient (995 *vs* 2000 mL/g) than cypermethrin. The higher acute toxicity of chlorpyrifos is potentially more harmful to non-target organisms, such as human beings and small animals which come in contact with the treated area, especially when the initial residue levels are so high. The higher water solubility and lower soil sorption coefficient of chlorpyrifos mean that the chemical has a higher potential to be translocated and to contaminate non-target environmental matrices. The higher vapor pressure of chlorpyrifos indicates that the insecticide has higher potential to escape in the vapor phase from the contaminated matrices, such as bark, litter, and soil to pollute air, a potential negative effect when chlorpyrifos is applied to elm trees in urban areas where human population is relatively dense.

Considering the factors discussed, it is suggested that cypermethrin applied in the fall could replace chlorpyrifos in the control of overwintering elm bark beetles on elm trees in urban areas if two years of control effectiveness are satisfactory. My study shows that chlorpyrifos seems to provide a longer period of effectiveness. The longer effectiveness persistence is helpful to save labor costs, especially when the number of wild elm trees is large and scattered so widely, and also when access may be a problem. The effect of higher chlorpyrifos residues in the non-target matrices, such as litter, soil on the non-target organisms should be assessed while considering the saving of labor. Any measures to reduce the amount of chlorpyrifos distributed to non-target matrices, such as shielding the non-target area, increasing the application efficiency to the elm bark, or decreasing the application dosage, could be considered.

The mortality of native elm bark beetles exposed on chlorpyrifos treated elm bark collected on the last sampling in the experiment was still 100%. If correlation between beetle mortality and chlorpyrifos residues on the elm bark need to be established, sampling and analyses of residues and bioassay should be continued. If possible, the mortality of the elm bark beetle should be investigated under field conditions to confirm the bioassay results obtained under laboratory conditions.

# 6. Summary and Conclusions

Applying insecticides on the base of elm trunks to suppress elm bark beetles is a very important component of the integrated control program of Dutch elm disease in Manitoba. Experiments were designed and implemented in the native elm stands at the Glenlea Research Station along the Red River south of the City of Winnipeg and the Beaudry Provincial Park along the Assiniboine River west of the city beginning in August, 1991, and May, 1992.

Analytical methods using GC-ECD were developed to determine the residues of chlorpyrifos, four pairs of cypermethrin isomers, and their major metabolites in elm bark, litter, and soil with ball mill extraction, and in glass fibre filter paper with Soxhlet extraction. The average recoveries in three replicates of all tested matrixes ranged from 90 to 100% with standard deviations ranging from 0.4 to 8.2% for chlorpyrifos, oxon, and pyridinol, from 82 to 110%, above 90% in most cases, with the standard deviations from 2.0 to 11% for cypermethrin and metabolites. The accuracy, precision, and the sensitivity of the analytical methods were considered acceptable for this study.

The initial residue concentration of chlorpyrifos in elm bark ranged from 388 to 444 μg/g at both experimental sites and at both application times at each site, about 40 times the initial residue concentration of chlorpyrifos normally found in the common crops such as wheat or corn. The initial residues of chlorpyrifos dissipated quickly with $DT_{50}$ ranging from 11 to 68 days. The dissipation rate of

chlorpyrifos during the later period became much slower with the dissipation $DT_{90}$ ranging from 560 to 730 days. Approximately 90% of applied chlorpyrifos dissipated from the treated elm surface after 791 days from the treatments at the two sites. Bioassay results on the effectiveness of chlorpyrifos on the elm bark discs collected from treated trees after pre-determined intervals indicated that it was still 100% effective in killing bark beetles after 791 days (1991 application) and 532 days (1992 application). It is hypothesized that chlorpyrifos would still be effective against the elm bark beetles for some period of time after 791 days, considering the high residues of chlorpyrifos on the bark at that time and subsequent slow dissipation rates.

The initial residues of *cis*-A, *trans*-C, *cis*-B, and *trans*-D cypermethrin in the elm bark ranged from 28.8 to 58.9, from 24.2 to 53.1, from 24.9 to 34.9, and from 17.7 to 25.2 μg/g, respectively, in the elm bark. The residues of four pairs of cypermethrin isomers dissipated more quickly shortly after application and then slowed down thereafter. The $DT_{50}$ and $DT_{90}$ values of *cis*-A, *trans*-C, *cis*-B, and *trans*-D in elm bark ranged from 25 to 140 days, and from 209 to 365 days, respectively. The residues of *cis*-isomers dissipated approximately 89% after 791 days while *trans*-isomers dissipated approximately 92% during the same period. The bioassay results on the effectiveness of cypermethrin to control the elm bark beetle indicated that cypermethrin was 100% effective in killing elm bark beetles on treated elm bark for approximately two months with in excess of 80 % mortality measured up to 364 days after treatment. However, the surviving beetles were

affected by cypermethrin in that they could only move a very short distance and lost the ability to make overwintering tunnels. If it is assumed that these beetles would die in field, the 100% effective control period of cypermethrin could be extended to 627 days for the August application and 437 days for the May application. This is a projected mortality based on laboratory data only.

The initial residues of chlorpyrifos in litter ranged from 187 to 916 μg/g depending on the amount of litter on the ground at time of application. The $DT_{50}$ and $DT_{90}$ values of chlorpyrifos in litter ranged from 3.9 to 59 days, and from 55 to 310 days, respectively. The residues of chlorpyrifos in litter dissipated about 99% by 791 days from application. The residue decline was probably correlated to the dilution by freshly fallen leaves. The residues of chlorpyrifos in soil after the basal elm bark was treated with 48 EC Dursban® aqueous solution fluctuated from 0.78 to 28.2 μg/g during the entire experimental period. The residues of chlorpyrifos in soil remained at 1.04 -2.09 μg/g after 791 days from the application. Chlorpyrifos in soil wrapped in glass fibre filter paper and embedded in soil under the litter layer in the field had a $DT_{50}$ of from 92 to 120 days depending on the initial residues.

The initial residues of *cis*-A, *trans*-C, *cis*-B, and *trans*-D in litter ranged from 54.2 to 141, from 41.0 to 125, from 47.7 to 88.3, and from 32.7 to 62.9 μg/g, respectively, depending on the application time, and application sites. The residues of four pairs of cypermethrin isomers initially dissipate more quickly and slow down thereafter. The $DT_{50}$ and $DT_{90}$ values of *cis*-A, *trans*-C, *cis*-B, and *trans*-

D in litter range from 8.8 to 29 days and from 95 to 300 days, respectively. No cypermethrin could be detected 341 days after application. Cypermethrin residues of the total isomers found in soil after treatment to bark ranged from below detection limits to 10.1 μg/g. No cypermethrin was detected in soil 791 days after the fall application and 532 days after spring application. The residues of *cis*-A, *trans*-C, *cis*-B, and *trans*-D in soil wrapped in glass fibre filter paper and embedded in soil under the litter layer in the field degraded with $DT_{50}$ ranging from 340 to 390, from 290 to 320, from 360 to 390, and from 280 to 290 days, respectively, depending on the initial residue concentration.

The results of tests to determine the availability of the applied insecticides on the elm bark to non-target organisms indicated that the chlorpyrifos or cypermethrin residues transferred from the treated bark to the contact objects is well below the acceptable daily intake for humans. No chlorpyrifos or cypermethrin was detected in the cambium layer and wood tissue, indicating that these insecticides do not penetrate the bark layer.

Based on the experimental results and the physicochemical properties of chlorpyrifos and cypermethrin, it is suggested that cypermethrin could be used as an alternative, or along with, chlorpyrifos as part of an elm bark beetle management program. The use of cypermethrin near water bodies needs to be further investigated.

After the entire experimental period of 791 days for the application of chlorpyrifos in August, the mortality of the elm bark beetle was still 100 %. If the

relationship between the elm bark beetle mortality and the residue concentration of chlorpyrifos in the elm bark is to be established, further samples should be taken for residue analyses and bioassay. The fate of those elm bark beetle which are poisoned by cypermethrin but not killed and lose the ability to make normal overwintering tunnels under field conditions should be further investigated so that the control persistence of cypermethrin can be determined. The effect of the high residues of chlorpyrifos and cypermethrin in litter and soil on non-target organisms should be further studied.

# 7. References

Abdel-All, A., Khamis, A.E., Edrisha, M.S., Antonious, G.F., Efficiency and persistence of certain insecticides on soybean and broadbean plants, *Alexandria Sci. Exch.*, 11, 1, 1990.

Afifi, F.A., Kansouh, A.S.H., Dissipation and fate of chlorpyrifos and aldicarb in clay soil under laboratory conditions. *Ain. Shams. Univ. Fac. Agric. Res. Bull.,* 1401, 1, 1980.

Baker, P.G., Bottomley, P., Determination of residues of synthetic pyrethroids in fruit and vegetables by gas-liquid and high-performance liquid chromatography. Analyst, 107, 206, 1982.

Banger, M.H., Cuthbert R.A., Seegrist D.G., Statistical correlations between GLC assay and smaller European elm bark beetle bioassay, *J. Econ. Entomol.*, 66, 79, 1973.

Barger, J.H., Dutch elm disease and methoxychlor. *U.S. For. Serv. Res. Pap. NE-353.* 5pp., 1976.

Batzer, F.R., Fontaine, D.D., White, F.H., Aqueous photolysis of chlorpyrifos, DowElanco, Indianapolis, IN (unpublished report), 1990.

Beattie, R.K., How the Dutch elm disease reached America, *Proc. Natl. Shade Tree Conf.,* 9, 101, 1933.

Berisford, C.W., Brady, U.E., Ragenovich, I.R., Residue studies. In: Hastings, F.L.,Coster, J.E.(eds), Field and laboratory evaluations of insecticides for southern pine beetle control. U.S. Dept Agriculture, Southern Forest Experiment Station, Forest Service, SE-21, pp 11-12, 27, 32, 33, 1981.

Berisford, C.W., Dalusky, M.J., Bush, P.B., Taylor, J.W., Berisford, Y.C., Efficacy persistence, ground deposition, and human exposure of polymer-encapsulated lindane and chlorpyrifos used for control of the southern pine beetle. *Phytoprotection*, 72, 15, 1991.

Betrem, J. C., De iepenziekte en de iepenspintkever, *Tijdschr. Plantenziekten,* 35, 273,1929.

Bidlack, H.D., Degradation of chlorpyrifos in soil under aerobic, aerobic/anaerobic, and anaerobic conditions, DowElanco, Indianapolis, IN (unpublished report), 1979.

Bonsen, K.J.M., Scheffer, R.J., and Elgersma, D.M., Barrier zone formation as a resistance mechanism of elms to Dutch elm disease, *IAWA Bull. New Ser.,* 6,71, 1985.

Bowman M.C., Beroza M., Determination of Dursban and its oxygen analog in corn and grass by gas chromatography with flame photometric detection, *J. Agric. Food Chem.*, 15, 651, 1967.

Bowman, B.T., Sans, W.W., Effect of temperature on the water solubility of insecticides, *J. Environ. Sci. Hlth. B*, 20, 625, 1985.

Brady, U.E., Berisford, C.W., Hall, T.L., Hamilton, J.S., Efficacy and persistence of chlorpyrifos, chlorpyrifos-methyl, and lindane for preventive and remedial control of the southern pine beetle. *J. Econ. Entomol.*, 73, 639, 1980.

Brady, U.E., Tippins, R., Perry, J., Young, J.R., Wauchope, R.D., Chlorpyrifos exposure of workers entering sweet corn after chemigation, *Bull. Environ. Contam. Toxicol.*, 46, 343, 1991.

Brasier, C. M., Genetics of pathogenicity in *Ceratocystis ulmi* and its significance for elm breeding, *in Resistance to Diseases and Pests in Forest Trees,* Heygroek, H. M., Stephan, B. R., and von Weissenberg, K., Eds., Pudoc, Wageningen, the Netherlands, 224, 1982.

Brasier, C.M., The future of Dutch elm disease in Europe, in *Research on Dutch Elm Disease in Europe*, Burdekin, D, A., Ed., Forestry Commission Bulletin No. 60, Her Majesty's Stationery Office, London, 96, 1983.

Braun, H.E., Gas-liquid chromatographic determination of residues of chlorpyrifos and leptophos, including their major metabolites, in vegetable tissue, *J. Assoc. Off. Anal. Chem.*, 57, 182, 1974.

Braun, H.E., Ritcey, G.M., Ripley, B.D., McEwen, F.L., Frank, R., Studies of the disappearance of nine pesticides on celery and lettuce grown on Muck soils in Ontario, 1977-1980, *Pestic. Sci.*, 13, 119, 1987.

Braun, H.E., Stanek, H., Application of the AOAC multiresidue method to determination of synthetic pyrethroid residues in celery and animal products, *J. Assoc. Off. Anal. Chem.,* 65, 685, 1982.

Bromley, S.W., Tests on elm Scolytus 1949, *J. Econ. Entomol.*, 43, 397, 1950.

Brust, H.F., A summary of chemical and physical properties of O,O - diethyl - O - (3,5,6, - trichloro - 2 - pyridyl) phosphorothioate, DowElanco, Indianapolis, IN, (unpublished report), 1964.

Brust, H.F., A summary of chemical and physical properties of Dursban, *Down to Earth*, 22, 21,1966.

Buisman, C.J., The Dutch elm disease, *Phytopathology,* 20, 111, 1930.

Buisman, C. J., *Ceratostomella ulmi*, de geslachtelijke vorm van *Graphium ulmi* Schwarz, *Tijdschr. Plantenziekten, 38, 1, 1932.*

Burt, P.E., Elliot, M., Farnham, A.W., Janes, N.F., Needham, P.H., Pulman, D.A., The pyrethrins and related compounds, XIX, Geometrical and optical isomers of 2,2-dimethyl-3-(2,2-dichlorovinyl)-cyclopropane carboxylic acid and insecticidal esters with 5-benzyl-3-furylmethyl and 3-phenoxybenzyl alcohols, *Pest. Sci.*, 5,791, 1974.

Bush, P.B., Taylor, J.W., McMahon, C.K., Neary, D.G., Residues of lindane and chlorpyrifos in firewood and woodsmoke. *J. Entomol. Sci.,* 22, 131, 1987.

Buth, J.L., and Ellis, R.A., Bark beetle research and control in Winnipeg, 1975 to 1981, *Proceedings of the Dutch elm disease symposium and workship*, October 5-9, Winnipeg, Manitoba, 451, 1981.

Camilleri, P., Alkaline hydrolysis of some pyrethroid insecticides, *J. Agric. Food Chem.*, 32, 1122, 1984.

Casida, J.E., Pyrethrum flowers and pyrethroid insecticides, *Environmental Health Perspectives*, 34, 189, 1980.

Chakrabarti, A., Gennrich, S.M., Vapor pressure of chlorpyrifos., DowElanco, Indianapolis, IN(unpublished report), 1987.

Chapman, J.W., The introduction of a European scolytid, the smaller elm bark beetle, *Scolytus multistriatus* (Marsh.) into Massachusetts, *Psyche.*,17, 63, 1910.

Chapman, R.A., Chapman, P.C., Persistence of granular and EC formulations of chlorpyrifos in a mineral and an organic soil incubated in open and closed containers. *J. Environ. Sci. Hlth. B*, 21, 447, 1986.

Chapman, R.A., Cole, C.M., Observations on the influence of water and soil pH on the persistence of insecticides, *J. Environ. Sci. Hlth. B,* 17, 487, 1982.

Chapman, R.A., Harris, C.R., Extraction and liquid-solid chromatography cleanup procedures for the direct analysis of four pyrethroid insecticides in crops by gas-liquid chromatography, *J. Chromatogr.*, 166, 513, 1978.

Chapman, R.A., Harris, C.R., Persistence of chlorpyrifos in a mineral and an organic soil, *J. Environ. Sci. Health*, B, 15, 39, 1980.

Chapman, R.A., Harris, C.R., Persistence of four pyrethroid insecticides in a mineral and an organic soil, *J. Environ. Sci. Health*, B, 16, 5, 605, 1981.

Chapman, R.A., Tu, C.M., Harris, C.R., Cole, C., Persistence of five pyrethroid insecticides in sterile and natural, mineral and organic soil, *Bull. Environ. Contam. Toxicol.*, 26, 513, 1981.

Chen, Z.M., Zabik, M.J., Leavitt, R.A., Comparative study of thin film photodegradative rates for 36 pesticides, *Ind. Eng. Chem. Prod. Res. Dev.,* 23, 5, 1984.

Cheng, T., Bodden, R.M., Puhl, R.J., Bauriedel, W.R., Absorption, distribution, and metabolism of [$^{14}$C]chlorpyrifos applied dermally to goats. *J. Agric. Food Chem.*, 37,1018, 1989.

Class, T.J., Environmental analysis of cypermethrin and its degradation products after forestry application, *Intern. J. Environ. Anal. Chem.*, 49, 189, 1992.

Coats, J.R., O'Donnel-Jeffery, N.L., Toxicity of four synthetic pyrethroid insecticides to rainbow trout, *Bull. Environ. Contam. Toxicol.*, 23, 250, 1979.

Collius, C.W., Buchanan, W.D., Whitten, R.R., and Hoffmann, C.H., Bark beetles and other possible insect vectors of the Dutch elm disease *Ceratostomella ulmi* (Schwarz) Buisman, *J. Econ. Entomol.,* 29, 169,1936.

Cook, K.A., Determination of the effects of WL 43467 on microbial activity in soil. I. Effects on carbon dioxide evolution, Sittingbourne, Shell Research (BLGR.0003.78), 1978.

Coveney, P.C., and Eadsforth, C.V., The metabolism of cypermethrin in man (3). Urinary excretion following a single dermal dose of cypermethrin, Sittingbourne, Shell Research (SBGR.82.290), 1982.

Crummett, W.B., O,O-diethyl O-(3,5,6-trichloro-2-pyridyl) phosphorothioate hydrolysis rate, DowElanco, Indianapolis, IN (unpublished report), 1963.

Currie, K.L., McDonald, E.C., Chung, L.T.K., Higgs, A.R., Concentrations of diazinon, chlorpyrifos, and bendiocarb after application in offices, *Am. Ind. Hyg. Assoc. J.*, 51, 23, 1990.

Dawson, J., The determination of the solubility of chlorpyrifos in water according to EEC/OECD guidelines (flask method). DowElanco, Indianapolis, IN (unpublished report), 1989.

Day, S.R., Leahey, J.P., $^{14}$C-cypermethrin: aqueous photodegradation in sunlight, Fernhurst, Imperial Chemical Industries, (Unpublished ICI Report No. RJ0154B), 1980.

De Bruihn, J., Busser, F., Seinen, W., Hermens, H., Determinations of octanol/water partition coefficients for hydrophobic organic chemicals with the slow-stirring method, *Environ. Toxicol. Chem.*, 8, 499, 1989.

Dimond, A.E., The origin of symptoms of vascular wilt diseases, in *Phytotoxins in Plant Diseases,* Wood, R.K.S.,Ballio, A., and Graniti, A., Eds., Academic Press, London, 289, 1972.

Dutta, D.N., Goswami, M.M., Dissipation of Dursban residues from mustard (*Brassica sp.*) crop. *J. Res. Assam. Agric. Univ.*, 3, 186, 1982.

Elgersma, D.M., Resistance mechanisms of elms to *Ceratocystis ulmi*, *Meded. Phytopathol. Lab.* "Willie Commelin Scholten", 77, 1, 1969.

Elgersma, D.M., Resistance mechanisms of elms to Dutch elm disease, in *Resistance to Diseases and Pests in Forest Trees,* Heybroek, H.M., Stephan, B.R., and von Weissenberg, K., Eds., Pudoc, Wageningen, the Netherlands, 143, 1982.

Elgersma, D.M., Host-parasite interactions in Dutch elm disease, in *Research on Dutch elm Disease in Europe.* Proc. European Economic Community Research Seminar, Guernsey, Channel Islands, Burdekin, D.A., Ed., Forestry Commission Bulletin No. 60., Her Majesty's Stationery Office, London, 78, 1983.

Elliott, M., Farnham, A.W., Janes, N.F., Needham, P.H., Pulman, D.A., Insecticidal activity of the pyrethrins and related compounds, VII, Insecticidal dihalovinyl analogues of cis and trans chrysanthemates, *Pest. Sci.*, 6, 537, 1975.

Environmental Protection Agency, Pesticide fact handbook, V2, Noyes Data Corporation, New Jersey, U.S.A., p148, 1990.

Felsot, A., Dahm, P.A., Sorption of organophosphorus and carbamate insecticides by soil, *J. Agric. Food Chem.*, 27, 557, 1979.

Ferraro, C.F., Personal communication, FMC Corporation, Chemical Group, Princeton, NJ, U.S.A., 1990.

Fontaine, D.D., Teeter, D., Photodegradation of chlorpyrifos in the vapor phase, DowElanco, Indianapolis, IN (unpublished report), 1987.

Fontaine, D.D., Wetters J.H., Weseloh, J.W., Stockdale, G.D., Young, J.R., Swanson, M.E., Field dissipation and leaching of chlorpyrifos, DowElanco, Indianapolis, IN (unpublished report), 1987.

Frank, R., Braun, H.E., Ritcey, G., McEwen, F.L., Sirons, G.J., Pesticide residues in onions and carrots grown on organic soils, Ontario, 1975-80, J. Econ. Entomol., 75, 560, 1982.

Fransen, J.J., Enkele gegevens omtrent de verspreiding van de door *Graphium ulmi* Schwarz veroorzaakte eiepenziekt door de iepenspintkever, *Eccoptogaster (Scolytus) scolytus* F. en *Eccoptogaster (Scolytus) multistriatus* Marsh. in verband met de bestrijding dezer ziekte, *Tijdschr. Plantenziekten*, 37, 49, 1931.

Fransen, J.J., De kleine iepenspintkever *Scolytus* (*Eccoptogaster) multistriatus* Marsh. als verbreider der iepenziekte, *Tijdschr. Plantenziekten*, 38, 197, 1932.

Fransen, J.J., and Buisman, C.J., Indectieproeven op verschillende iepensoorten met behulp van iepenspintkevers, *Tijdschr. Plantenziekten*, 41, 221, 1935.

Funderburk, J.E., Braxton, L.B., Lynch, R.E., Nontarget effects of soil-applied chlorpyrifos on defoliating pests and Arthropod predators in peanut, *Peanut Sci.*, 17, 113, 1990.

Furuzawa, K., Mikami, N., Yamada, H., Miyamoto, J., Metabolism of the pyrethroid insecticide cypermethrin in cabbages, *J. Pestic. Sci.*, 11, 253, 1981.

Gardiner, L.M. and Webb, D.P, Test of chlorpyrifos for control of the North American elm bark beetle, *Hylurgopinus rufipes* Eichh., *Canadian Forestry Service Report*, 0-X-311, 1980.

Getzin, L.W., Dissipation of chlorpyrifos from dry soil surfaces, *J. Econ. Entomol.,* 74, 707, 1981a.

Getzin, L.W., Degradation of chlorpyrifos in soil: Influence of autoclaving, soil moisture, and temperature, *J. Econ. Entomol.*, 74, 158, 1981b.

Getzin, L.W., Rosefield. I., Organophosphorus insecticide degradation by heat-labile substances in soil, *J. Agric. Food Chem.,* 16, 598, 1968.

Glotfelty, D.E., Majewski, J.S., Seiber, J.N., Distribution of several organophosphorus insecticides and their oxygen analogues in a foggy atmosphere, *Environ. Sci. Technol*, 24, 353, 1990.

Goh, K.S., Edmiston, S., Maddy, K.T., Meinders, D.D., Margetich, S., Dissipation of dislodgeable residue of chlorpyrifos and dichlorvos on turf, *Bull. Environ. Contam. Toxicol.,* 37, 27,1986.

Greig, B.J.W, Further experiment with thiabendazole (TBZ) for control of Dutch elm disease, *Arboricultural Journal*, 10, 191, 1986.

Hall, J.S., Leahey, J.P., Curl, E.A., Cypermethrin: photodegradation on a soil surface, Ferhurst, Imperial Chemical Industries, (Unpublished ICI Report No. RJ0192B), 1981.

Hansen, D.H., Goodman, L.R., Crip, G.M., Macauley, S.F., Early life-stage test methods for Gulf Toadfish (*Opasnus beta*) and results using chlorpyrifos, *Ecotoxicol. Environ. Safety,* 11, 15, 1986.

Harris, C.R., Chapman, R.A., Harris, C., Laboratory studies on the persistence and behavior in soil of 4 pyrethroid insecticides, *Can. Entomol.*, 113, 685, 1981.

Harris, C.R., Chapman, R.A., Tolman, J.H., Moy, P., Henning, K., Harris, C., A comparison of the persistence in a clay loam of single and repeated annual applications of seven granular insecticides used for corn rootworm control, *J. Environ. Sci. Hlth. B.*, 23, 1, 1988.

Harris, C.R., Svec, H.J., Toxicological studies on cutworms, I, Laboratory studies on the toxicity of insecticides to the dark-sided cutworm, *J. Econ. Entomol.*, 61, 788, 1968.

Harris, C.R., Svec, H.J., Sans, W.W., Toxicological studies on cutworms. X. Laboratory and field microplot studies on effectiveness and persistence of some experimental insecticides used to control the black cutworm in organic soil, *J. Econ. Entomol.*, 66, 203, 1973.

Heller, S.R., Herner, A.E., ARS Pesticide Properties Date Base, USDA-ARS Systems Research Laboratory, Beltsville, MD, USA,1990.

Helling, C.S., Turner, B.C., Pesticide mobility: determination in soil by thin-layer chromatography, *Science*, 162, 562, 1968.

Heybroek, H.M., Three aspects of breeding trees for disease resistance, in *Proc. 2nd World Consultation on Forest Tree Breeding,* Food and Agriculture Organization, Rome, 519, 1970.

Heybroek, H. M., Elgersma, D. M., and Scheffer, R. J., Dutch elm disease: anecological accident, *Outlook Agric.*, 11, 1, 1982

Hoog, G. S. de and Scheffer, R.J., *Ceratocystis* vs. *Ophiostoma*: a reappraisal, *Mycologia*, 76, 292, 1984.

Howell, J.F., George, D.A., Residues of chlorpyrifos and its pyridinol metabolite in apples, in twig bark, and in undertree debris, U.S. Dept Agriculture, *Agricultural Res Serv*, Beltsville, MD, ARS-9, pp1-12, 1984.

Hummel, R.A., Crummet, W.B., Solubility of ethel (O,O, - diethyl O - (3,5,6 trichloro - 2 -pyridyl) phosphorothioate in various solvents. DowElanco, Indianapolis, IN (unpublished report), 1964.

Hunt, J., Taxonomy of the genus *Ceratocystis, Lloydia,* 19, 1, 1956.

Inglesfield, C., Toxicity of the pyrethroid insecticides cypermethrin and WL 85871 to the earthworm *Eisenia foetida* Savigny, *Bull. Environ. Contam. Toxicol.*, 33, 568, 1984.

Inglesfield, C., and Sherwood, C.M., Toxicity of cypermethrin to the earthworm, *Eisenia foetida* L (Oligochaeta: Lumbriculidae) in laboratory tests, Dittingbourne, Shell Research (SBGR.83.070), 1983.

Inman, R.D., Kigemagi, U., Deinzer, M.L., Determination of chlorpyrifos and 3,5,6-trichloro-2-pyridinol residues in peppermint hay and peppermint oil, *J. Agric. Food Chem.*, 29, 321, 1981.

Iwata, Y., O'Neal, J.R., Barkley, J.H., Dinoff, T.M., Dusch, E., Chlorpyrifos applied to California citrus: Residue levels on foliage and on and in fruit, *J. Agric Food Chem.* 31, 603, 1983.

Jackson, C., The leaching of WL 43467 through laboratory soil columns, Sittingbourne, Shell Research, (Unpublished Report BLGR.0150.77), 1977.

Kanazawa, J., Relationship between the soil sorption constants for pesticides and their physicochemical properties, *Environ. Toxicol. Chem.*, 8, 477, 1989.

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

Kenaga, E.E., Whitney, W.K., Hardy J.L., Laboratory tests with Dursban insecticide, *J. Econ. Entomol.,* 58, 1043, 1965.

Klisenko, M.A., Pis'mennaya, M.V., Photochemical transformations of organophosphorus pesticides in air, *Gig. Rr. Prof. Zabol.*, 3, 56, 1979.

Kuhr, R.J., Tashiro, H., Distribution and persistence of chlorpyrifos and diazinon applied to turf, *Bull. Environ. Contam. Toxicol.,* 20, 652, 1978.

Lal, S., Lal, R., Bioaccumulation, metabolism, and effects of DDT, fenitrothion, and chlorpyrifos on *Saccharomyces cerevisiae, Arch. Environ. Contam. Toxicol.,* 16, 753, 1987.

Lanier, G.N., Fungicides for Dutch elm disease: comparative evaluation of commercial products, *Journal of Arboriculture*, 13, 189, 1987.

Lanier, G.N., Sherman, J.F., Rabaglia, R.J., and Jones, A.H., Insecticides for control of bark beetles that spread Dutch elm disease, *Journal of Arhoriculture*, 10, 265, 1984.

Leahey, L.P., The metabolism and environmental degradation of the pyrethroid insecticides, *Outlook Agric.*, 10,, 135, 1979.

Lester, D.I. and Smalley, E.B., Response of *Ulmus pumila and U. pumila x rubra* hybrids to inoculation with *Ceratocystis ulmi*, *Phytopathology*, 62, 848, 1972.

Leuck, D.B., Bowman, M.C., Beck, E.W. , Dursban insecticide persistence in grass and corn forage, *J. Econ. Entomol.*, 61, 689, 1968.

Macalady, D.L., Wolfe, N.L., New perspectives on the hydrolytic degradation of the organophosphorothioate insecticide chlorpyrifos, *J. Agric. Food Chem.,* 31, 1139, 1983.

Manitoba, The Dutch elm disease act, Being chapter D107 of the continuing consolidation of the statutes of Manitoba, March, 6 pp, 1984.

Marchetti, L., D'Aulerio, A.Z., Valle, E,D., Lodi, M., Dalla-Valle, E, Trial stem injections for control of Dutch elm disease*, Monti-e-bosche,* 41, 48, 1990.

May, C., The Dutch elm disease, *Proc. Nat. Shade Tree conf.,* 6, 91, 1930.

McCall, P.J., Column leaching and sorption studies with chlorpyrifos, DowElanco, Indianapolis, IN (unpublished report), 1985.

McCall, P.J., Swann, R.L., Bauriedel, W.R., Volatility characteristics of chlorpyrifos from soil and corn, DowElanco, Indianapolis, IN (unpublished report), 1985.

McCall, P.J., Soil adsorption properties of $^{14}$C-chlorpyrifos, DowElanco, Indianapolis, IN (unpublished report), 1987.

McCall, P.J., Oliver, G.R., McKellar, R.L., Modeling the runoff potential and behavior of chlorpyrifos in a terrestrial-aquatic watershed, DowElanco, Indianapolis, IN (unpublished report), 1984.

McDonald, I.A., Howes, D.A., Gillis, N.A., The determination of the physicochemical parameters of chlorpyrifos, DowElanco, Indianapolis, IN (unpublished report), 1985a.

McDonald, R.A., Karris, G.C., Chakrabarti, A., The melting behavior, heat of melting, specific heat capacity, thermal conductivity, and vapor pressure of a recrystallized Dursban insecticide, DowElanco, Indianapolis, IN, (unpublished report), 1985b.

McKellar, R.L., Wetters, J.H., Dishburger, H.J., Residues of chlorpyrifos and 3,5,6-trichloro-2-pyridinol in soil from corn fields treated with Dursban insecticides, DowElanco, Indianapolis, IN (unpublished report), 1972.

McLeese, D.W., Metcalfe, C.D., Zitko, V., Lethality of permethrin, cypermethrin and fenvalerate to salmon, lobster and shrimp, *Bull. Environ. Contam. Toxicol.*, 25, 950, 1980.

Meikle, R.W., Kurihara, N.H., DeVries, D.H., Chlorpyrifos: The photodecomposition rates in dilute aqueous solution and on a surface, and the volatilization rate from a surface, *Arch. Environ. Contam. Toxicol.*, 12, 189, 1983.

Melin, E. and Nannfeldt, J. A., Researches into the blueing of ground woodpulp, *Sven. Skogsvardforen. Tidskr.,* 32, 397, 1934.

Michalyna, W., Report of the detailed soil survey of the Glenlea Research Station, Canada - Manitoba soil survey, Canada Department of Agriculture / Department of Soil Science, University of Manitoba, 1963.

Michalyna, W., Gardiner, W., Podolsky, G., Soils of the Winnipeg region study area, Canada - Manitoba soil survey, Agriculture Canada / Department of Soil Science, University of Manitoba, 1975.

Miles, J.R.W., Tu, C.M., Harris, C.R., Persistence of eight organophosphorus insecticides in sterile and non-sterile mineral and organic soils, *Bull. Environ. Contam. Toxicol.*, 22, 312, 1979.

Miles, J.R.W., Harris, C.R., Tu, C.M., Influence of temperature on the persistence of chlorpyrifos and chlorfenvinphos in sterile and natural mineral and organic soils, *J. Environ. Sci. Hlth. B*, 18, 705, 1983.

Miles, J.R.W., Harris, C.R., Tu, C.M., Influence of moisture on the persistence of chlorpyrifos and chlorfenvinphos in sterile and natural mineral and organic soils, *J. Environ. Sci. Hlth. B*, 19, 237, 1984

Miller, H.J. and Elgersma, D.M., The growth of aggressive and nonaggressive strains of *Ophiostoma ulmi* in susceptible and resistant elms, a scanning electron microscopial study, *Neth. J. Plant Pathol.*, 82, 51, 1976.

Miyamoto, J., Mikami, N., Degradation of pyrethroid insecticide in the field, In: IUPAC Pesticide chemistry, human welfare and the environment, Oxford, Pergamin Press, pp.193-200, 1983.

Moreau, C., Coexistence des formes *Thielaviopsis* et *Graphium* chez une souche de *Ceratocystis major* (van Beyma) nov. comb. Remarques sur les variations des *Ceratocystis, Rev. Mycol.,* 17 (Suppl. Colonial 1), 17, 1952.

Mortland, M.M., Raman, K.V., Catalytic hydrolyses of some organic phosphate pesticides by copper (II), *J. Agric. Food Chem.,* 15, 163, 1967.

Mourer, C.R., Hall, G.L., Whitehead W.E., Shibamoto, T., Gas chromatographic method for determination of chlorpyrifos and Its metabolite 3,5,6-trichloro-2-pyridinol (TCP) in dates, *J. Assoc. Off. Anal. Chem.,* 73, 294,1990.

Naffziger, D.H., Sprenkel, R.J., Mattler, M.P., Indoor environmental monitoring of Dursban L.O. following broadcast application, *Down to Earth*, 41, 1, 1985.

Neary, D.G., Bush, P.B., McMahon, C.K., Cantrekk, R.L., Taylor, J.W., Persistence of nine forest pesticides in the surface horizon of a typic quartzipsamment soil of the Ocala National Forest, *Soil Crop Sci. Soc. Florida Proc.,* 47, 127, 1988.

Neely, D., Municipal control of Dutch elm disease in Illinois cities, *Plant Dis. Rep.,* 56, 460, 1972.

Nolan, R.J., Rick, D.L., Freshour, N.L., Saunders, J.H., Chlorpyrifos: Pharmacokinetics in human volunteers, *Toxicol. Appl. Pharmacol.,* 73, 8, 1984.

Novozhilov, K.V., Petrova, T.M., Andreev, Y.B., Degradation of Basidin and Dursban in Povolahya soils and their toxicity for click beetle larvae, *Agrokhimiya*, 10, 123, 1982.

Oliver, G.R., McKellar, R.L., Woodburn, K.B., Eger, J.E., McGee, G.G., Ordiway, T.R., Field dissipation and leaching study for chlorpyrifos in Florida citrus, DowElanco Indianapolis, IN (unpublished Report), 1987.

Page, M., Degradation and persistence of gamma-HCH and chlorpyrifos-methyl in Ponderosa pine bark, *Pestic. Sci.*, 14, 571, 1983.

Page, M., and Greene, L., *Scolytus multistriatus*, Laboratory Bioassay, *1974-75 Insecticide and Acaricide Tests*, 3, 143, 1978.

Pajares, J.A, and Lanier, G.N., Pyrethroid insecticides for control of European elm bark beetle (Coleoptera: Scolytidae), *J. Econ. Entomol.,* 82, 873, 1989.

Phillipsen, W.J., Ascerno, M.E., Landwehr, V.R., Colonizations, emergence, and survival of *Hylurgopinus rufipes* and *Scolytus multistriatus* (Coleoptera:

Scolytidae) in insecticide-treated elm wood, *J. Econ. Entomol.*, 79, 1347, 1986.

Quattlebaum, E.C., The use of sevin carbaryl insecticide in a Dutch elm disease prevention program, *Proceedings of the Dutch elm disease symposium and workship*, October 5-9, Winnipeg, Manitoba, 510, 1981.

Rabaglia, R.J., Twig crotch by *Scolytus multistriatus* (*Coleoptera:Scolytidae*). Sampling for feeding intensity and evaluation of carbaryl for prophylaxis. *Master of Science Thesis*, SUNY Coll. *Environ. Sci. and Fov.*, 81pp.,1980.

Racke, K.D. Environmental fate of chlorpyrifos, *Reviews of Environmental Contamination and Toxicology*, 131,44, 1993a.

Racke, K.D., Environmental fate of chlorpyrifos, *Reviews of Environmental Contamination and Toxicology*, 131, 60, 1993b.

Racke, K.D., Coats, J.R., Titus, K.R., Degradation of chlorpyrifos and its hydrolysis product, 3,5,6-trichloro-2-pyridinol, in soil. *J. Environ. Sci. Hlth. B*, 23, 527, 1988.

Racke, K.D., Coats, J.R., Enhanced biodegradation of insecticides in Midwestern corn soils, In: Rache, K.D., Coats, J.R.(eds) Enhanced biodegradation of pesticides in the environment. *ACS Symp. Series 426*, Washington, DC, 93, 1990.

Racke, K.D., Laskowski, D.A., Schultz, M.R., Resistance of chlorpyrifos to enhanced biodegradation in soil, *J. Agric. Food Chem.*, 38, 1430, 1990.

Robbie, A. Life cycle of the native elm bark beetle, *Hylurgopinus rufipes*, Personal communication, 1989.

Roberts, T.R., Standen, M.E., Degradation of the pyrethroid cypermethrin NRDC 149(±)-α-cyano-3-phenoxy-benzyl(±)-cis, trans-3-(2,2-dichlorovinyl-2,2-dimethylcyclopropanecarboxylate and the respective *cis*-(NRDC 160) and the *trans*-(NRDC 159) isomers in soils. *Pestic. Sci.*, 8, 305, 1977.

Roberts, T.R., Standen, M.E., Further studies of the degradation of the pyrethroid insecticide cypermethrin in soils, *Pestic. Sci.*, 12, 285, 1981.

Roberts, T.R., Standen, M.E., Further studies of the degradation of the pyrethroid insecticide cypermethrin in soils, *Pestic. Sci.*, 12, 285, 1982.

Roepke, W., Verdere gegevens omtrent de iepenziekte en de iepenspintkever, *Tijdschr. Plantenziekten,36, 231, 1930.*

Rouchaud, J., Metsue, M., Gustin, F., van de Steene, F., Pelerents, C., Benoit, F., Ceustermans, N., Gillet, J., Vanparys, L., Soil and plant biodegradation of chlorpyrifos in fields of cauliflower and Brussels sprouts crops, *Toxicol. Environ. Chem.*, 23, 215, 1989.

Royal Society of Chemistry, The Agrochemicals Handbook, Royal Society of Chemistry, Nottingham, England, 425 pp, 1983.

Sakata, S., Mikami, N., Matsuda, T., Miyamoto, J., Degradation and leaching behavior of the pyrethroid insecticide cypermethrin in soils, *J. Pestc. Sci.*, 11, 71, 1986.

Sapiets, A., Swine, H., Tandy, M.J., Cypermethrin, In: Zweig G, Sherme, J (eds.) Analytical methods for pesticides and plant growth regulators, Vol. XIII, Academic Press, New York, p33, 1984.

Scheffer, R.J., Mechanisms involved in biological control of Dutch elm disease, *Journal of Phytopathology*, 130, 265, 1990.

Schmimmel, S.C., Garnas, R.L., Patrick, J.M., Moore, J.C., Acute toxicity, bioconcentration, and persistence of AC 222, 705, benthiocarb, chlorpyrifos, fenvalerate, methyl parathion, and permethrin in the estuarine environment, *J. Agric. Food Chem.*, 31, 104, 1983

Schwarz, M. B., Das Zweigsterben Der Ulmen, Trauerweiden und Pfirsichbaume, *ede Md. Phytopathol. Lab.* "Willie Commelin Scholten", 5, 1, 1922.

Sears, M.K., Chapman, R.A., Persistence and movement of four insecticides applied to turfgrass, *J. Econ. Entomol.*, 72, 272, 1979.

Shaaban, A.M., Zidan, Z.H., Sobeiha, A.M.K., El-Zemaity, M.S., Some factors influencing the persistence of certain pesticides in soil and water, *Bull. Entomol. Soc. Egypt., Econ. Ser.*, 12, 137, 1981.

Sharom, M.S., Miles, J.R.W., Harris, C.R., McEwen, F.L., Persistence of 12 insecticides in water, *Water Res.,* 14, 1089, 1980.

Smith, G.N., Ultraviolet light decomposition studies with Dursban and 3,5,6-trichloro-2-pyridinol, *J. Econ. Entomol.,* 61, 793, 1966.

Smith, J.L., Rust, M.K., Vapor activity of insecticides used for subterranean termite (Isoptera: Rhinotermitidae) control, *J.. Econ. Entomol.*, 84, 181, 1991.

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

Spehar R.L., Tanner DT, Nordling BR, Toxicity of the synthetic pyrethroids, permethrin and AC 222, 705 and their accumulation in early life stages of fathead minnows and snails, *Aquatic. Toxicol.*, 3, 171, 1983.

Spierenburg, D., Een onbekende Ziekte in de iepen. I, *Versl. Meded. Plantenziektenkundige Dienst Wageningen*, 18, 3, 1921.

Spierenburg, D., Een onbedende Ziekte in de iepen. II, *Versl. Meded. Plantenziektenkundige Dienst Wageningen*, 24, 3, 1922.

Standen, M.E., The leaching and degradation of the insecticide WL 43467 when applied in sheep-dip solution to soil, Sittingbourne, Shell Research, (BLGR.0141.77)(Unpublished report), 1977.

Stephenson, R.R., Aquatic toxicity of cypermethrin, I, Acute toxicity to some fresh water fish and invertebrates in laboratory tests, *Aquat. Toxicol.,* 2, 175, 1982.

Stevens, J.E.B., Hill, I.R., Mobility of cypermethrin and its degradation products in soil columns, Fernhurst, Imperial Chemical Industries, (Report No. RJ/0166-B)(Unpublished ICI data), 1980.

Struble, D.L., McDonald S., Residue analysis of chlorpyrifos and Its oxygen analogue in field-treated wheat plants, *J.. Econ. Entomol.*, 66, 769, 1973.

Szeto, S.Y., Mackenzie, J.R., Vernon, R.S., Comparative persistence of chlorpyrifos in a mineral soil after granular and drench applications, *J. Environ. Sci. Hlth. B*, 23, 541, 1988.

Takahashi, N., Mikami, N., Matsuda, T., Miyamoto, J., Hydrolysis of the pyrethroid insecticide cypermethrin in aqueous media, *J. Pestic. Sci.*, 10, 643, 1985a.

Takahashi, N., Mikami, N., Matsuda, T., Miyamoto, J. Photodegradation of the pyrethroid insecticide cypermethrin in water and on soil surface, *J. Pestic. Sci.*, 10, 629, 1985b.

Tattar, T.A., Disease of shade trees (revised edition),191, Academic Press, Inc., San Diego, California, 92101, U.S.A., 1989.

Thiegs, B.J., Degradation and leaching of Ethel $^{36}Cl$ in soil, DowElanco, Indianapolis, IN (unpublished report), 1964.

Thiegs, B.J., Degradation of [$^{14}C$] Dursban in soil, DowElanco, Indianapolis, IN (unpublished report), 1966.

Tomlin, C., The pesticide manual (a world compendium), 10th edition, British Crop Protection Council and the Royal Society of Chemistry, UK, p200, 1994.

Travis, K.Z., Personal communication, ICI, Agrochemicals, Berkshire, England, 1990.

Upadhyay, H. P., *A Monograph of Ceratocystis and Ceratocystiopsis*, University of Georgia Press, Athens, Georgia, 1981.

Van de Steene, F., De Smet, G., Verstraeten, R., Doeltreffendheid tegen de koolvlieg, *Delia brassicae B.* en mikrobiuele afbraak van chloorfenvinfos, chloorpyrifos en trichloronaat, *Revue de l'Agric*, 42, 445, 1989.

Van de Steene, F., Melkebeke, T., Verstraeten, R., Breakdown of carbofuran, chlorpyrifos, fonofos, and bromofos-ethyl applied to soil from four regions of Belgium, *Med Fac Landbouww Rijksuniv Gent*, 55,107, 1990.

Van Sittert, N.J., Eadsforth, C.V., Bragt, P.C., Human dermal dose-excretion study with Ripcord, The Hague, Shell International Petroleum Maatschappy, (HSE.85.009), 1985.

Vanderplank, J.E., *Disease Resistance in Plants*, Academic Press, New York,1968.

Vanderplank, J.E., *Disease Resistance in Plants*, 2nd ed., Academic Press, New York, 1984.

Veierov D., Berlinger M.J., Fenigstein A., The residual behaviour of fenpropathrin and chlorpyrifos applied as aqueous emulsions or oil solutions to greenhouse tomato leaves, *Med. Fac. Landbouww Rijksuniv Gent.*, 53, 1535, 1988.

Walia, S., Dureja, P., Mukerjee, S.K., New photodegradation products of chlorpyrifos and their detection on glass, soil, and leaf surfaces, *Arch. Environ. Contam. Toxicol.*, 17, 183, 1988.

Wanchope, R.D., ARS/SCS pesticide properties database, version 1.9, preprint, 1989.

Ware, G.W., Buck, N.A., Estesen, B.J., Dislodgable insecticide residues on cotton foliage: comparison of ULV/cottonseed oil *v.* aqueous dilutions of 12 insecticides, *Bull. Environ. Contam. Toxicol.*, 31, 551, 1983.

Weijman, A. C. M. and de Hoog, G.S., On the subdivision of the genus *Ceratocystis*, *Antonie van Leeuwenhoek*, 41, 353, 1975.

Westcott, N.D., Reichle, R.A., Persistence of deltamethrin and cypermethrin on wheat and sweetclover, *J. Environ. Sci. Health, B*, 22, 91, 1987.

Westerdijk, J., Is de iepenziekte een infectieziekte?, *Tijdschr. Ned. Heidemaatsch.*, 40, 333, 1928.

Westwood, A.R., A cost benefit analysis of Manitoba's integrated Dutch elm disease management program, *Proc. Entomol. Soc. Manitoba*, 47, 44, 1991.

Wetters, J.H., McKellar, R.L., Ordiway, T.R., Miller, P.W., Dislodgeable residues of chlorpyrifos on turf grasses following application of Dursban 4E insecticide or Dursban 50W insecticide, DowElanco, Indianapolis, IN, (unpublished report), 1985.

Wilson, M. and Wilson, M.J.F., The occurrence of the Dutch elm disease in England, *Gard. Chron.*, 83, 31, 1928.

Wollenweber, H, W., Das Ulmensterben und sein Erreger. *Z. Pilzkd.* 8, 162, 1929.

World Health Organization, Cypermethrin, Environmental Health Criteria 82, Geneva, p115, 1989.

Worthing, C.R., Hance, R.J., The Pesticide Manual, 9 th edition, The British Crop Protection Council, 1991.