COMPARATIVE UPTAKE AND METABOLISM OF

FENITROTHION BY THREE SPECIES OF FOREST TREE

SEEDLINGS AND ONE SPECIES OF A RODENT

by

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Résumé

Mises en présence d'une solution aqueuse contenant 10 ppm de fénitrothion, thiophosphate de 0,0-diméthyle de 0-(méthyl-3-nitro-4phényle), des graines de pin blanc (*Pinus strobus L.*), d'épinette blanche (*Picea glauca L.*) et de bouleau jaune (*Betula alleghaniensis L.*) ont absorbé des quantités de ce pesticide au cours de leur germination. Dans le pin blanc, le fénitrothion détoxifié par une alkyl-transférase dépendant e la glutathione, a été transformé en fénitrothion déméthylé et en glutathione-S-méthyl. On a également activé ce pesticide en le transformant en fénitro-oxon, un composé plus toxique que le pesticide même. Il a été aussi démontré que le fénitrothion déméthylé peut être réactivé grâce à l'alkylation provoquée par le fénitrothion et donner alors le fénitrothion S-méthyl, un inhibiteur cholinestérasique encore plus puissant que le produit parent.

Le traitement des graines par des solutions aqueuses contenant 1,000 ppm de fénitrothion empêche la germination des graines de bouleau jaune mais non pas celle des graines de pin blanc ou d'épinette blanche. On a constaté que les fortes diminutions des concentrations de glutathione dans les tissus ont un rapport avec la toxicité observée dans les graines de bouleau jaune, qui ont absorbé beaucoup plus de pesticide que ne l'ont fait les graines des deux conifères.

Des expériences visant à rechercher les propriétés mutagènes du fénitrothion et de ses sept dérivés ont été effectuées; pour ce faire on a procédé à l'alkylation de l'ADN a pouvoir de reproduction dans une souche indicatrice de *Salmonella typhimurium*, sensible aux substitutions doubles alcalines causées par des agents alkylants. Ces expériences n'ont révélé aucune propriété mutagène.

(ii)

De jeunes rats ont suivi, pendant 7 jours, un régime
alimentaire contenant une concentration de fénitrothion égale à 10 ppm.
La comparaison due comportement de ces rats avec celui des rats pris comme
témoins a montré qu'il n'y avait aucune différence tant du point de vue
de leur poids que de celui de leur façon de manger et de boire. Le
fénitrothion présent dans le rein a été détoxifié, grâce à une désalkylation
en une position ortho, en fénitrothion déméthylé que l'on a décelé dans
l'urine. On n'a trouvé aucune trace de métabolite dans le cerveau, le foie,
le rein, la graisse omentale, le sang ou les testicules. On y a par
contre décelé la présence de fénitrothion intact. Les concentrations
de fénitrothion dans le foie et le rein sont remarquablement fortes.
Ce pesticide n'a apparemment provoqué aucun effet physiologique chez les
animaux traités.

INTRODUCTION

Fenitrothion, (0,0-dimethyl-0-(4-nitro-m-tolyl) phosphorothioate) is an organophosphorous insecticide. It has been used annually on increasingly larger areas of Canadian forests. Yule (1974) has shown that up to one part per million of fenitrothion was found to persist in foliage after 5 years of repeated annual applications at a dosage rate of 3 to 5 oz/acre. Initially a large part of the spray is lost; 50% within 2 days, but from 15 to 30% persists for up to 2 weeks (Yule and Duffy, 1972).

Metabolic and phytotoxic effects of the pesticide could be examined during the rapid growth of young seedlings under controlled laboratory conditions minimizing the extraneous variables found in the field. Therefore studies were initiated on white pine seeds with fenitrothion during stratification and germination.

Preliminary results showed that fenitrothion was absorbed from an aqueous solution containing 10 ppm fenitrothion by stratified and non-stratified germinating white pine seeds (Hallett et al 1974). A 10 ppm aqueous solution approximates the field concentration. Two metabolites, fenitro-oxon, and S-methyl fenitrothion were detected in late stages of germination. The mode of toxicity of fenitrothion to mammals and insects is via cholinesterase inhibition. Fenitro-oxon has been shown to be 10 to 100 times and S-methyl fenitrothion 100 to 1000 times more active as anticholinesterase agents than the parent compound (Hollingworth et al, 1967, and Kovacicova et al, 1973).

Germination studies were carried out therefore on treated stratified white pine seeds to quantitate the formation of fenitro-oxon, S-methyl fenitrothion, and desmethyl fenitrothion. The pathway of conversion of fenitrothion to the more toxic S-methyl isomer not

previously detected as a biological metabolite was followed with $OC^{14}H_3$ labelled fenitrothion.

Our previous studies showed that fenitrothion treatment did not produce any long term gross morphological defects in white pine seedlings after 6 months growth (Hallett et al 1974). White spruce, the target tree protected by the sprayings was also shown to resist toxic effects (Pomber and Weinberger, 1974). Both of these species are coniferous gymnosperms. Yellow birch, a deciduous angiosperm, prevalent in sprayed forests, showed slight effects on treatment with 10 ppm fenitrothion, and gross stunting after 10 days of germination treated at 100 ppm fenitrothion (Pomber and Weinberger, 1974). This effect was not evident in pine or spruce. Similar residue and metabolic studies were therefore carried out in whole seeds of birch and spruce to determine whether or not there were differences in absorption, metabolism, and detoxification of the pesticide.

Small rodents in the forest represent the second level productivity and are the basis of the food web upon which larger animals and birds prey. At times, up to 95% of forest seeds are eaten by rodents which depend on vegetation for their primary food supply (Ahbot, 1969). Young rats were fed a diet containing 10 ppm fenitrothion for 1 week [considerably higher than the amounts of fenitrothion that would be encountered to persist in seeds under normal field conditions (Hallett et al, 1974)]. Feeding habits and gross physiological effects were examined. The absorption of the pesticide from the diet was measured and the subsequent persistence and metabolism was followed in various organs.

A. MATERIALS

1. Reagents and Solvents:

Silica gel, 60 to 100 mesh (grade 950), was obtained from Grace Co. Ltd. It was heated overnight at 200°C to remove water. It was then cooled in a vacuum dessicator and stored in a reagent jar to prevent extraneous water uptake. Water content was adjusted by adding the appropriate amount by weight of distilled water to the absorbent in a reagent jar and equilibrating it overnight. All solvents used were nanograde and glass distilled. Methyl iodide was obtained from BDH Chemicals, Poole, England. Diazold (diazomethane) was obtained from Aldrich Chemical Co., U.S.A. Aquasolve Scintillation Fluid was obtained from New England Nuclear Limited, U.S.A.

2. Preparation of Fenitrothion and Metabolites:

(i) Fenitrothion, (0,0-dimethyl-0-(4-nitro-m-tolyl) phoshorothioate) technical grade, was obtained from Sumitomo Chemical Co., Japan. It was purified by a modification of the method of Kovacicova et al (1971). Ten grams of fenitrothion were added dropwise to the head of a stripping column at the bottom of which boiling toluene, 110°C entered counter-current wise. Fenitrothion was collected free from some volatile impurities at the bottom of the stripping column. It was then taken up in 100 ml of benzene and washed with four 100 ml aliquots of 1% ammonium hydroxide to hydrolyze fenitro-oxon and S-methyl fenitrothion contaminants. This was followed by four washings with distilled H_2O (pH 6.8) to remove any hydrolysis products. Residual water was removed from the organic phase with anhydrous sodium sulphate. The solvent was removed by rotary vacuum evaporation at 35°C. Traces of solvent were removed under a stream of nitrogen.

(ii) <u>Fenitro-oxon</u> (0,0-dimethyl-0-(4-nitro-m-tolyl-)phosphate was also obtained from Sumitomo Chemical Co., Japan. It was also synthesized by slow addition of 0,0-dimethyl phosphorochlorothioate in acetone to the sodium salt of 3-methyl-4-nitro phenol which had been prepared in benzene using sodium hydride. The yield was 92%. The product was further purified by column separation from residual fenitrothion.

(iii) <u>S-methyl fenitrothion</u> (S-methyl,0-methylo-0-(4-nitro-m-tolyl) phosphate was prepared by three methods: a. An excess of methyl iodide was added to fenitrothion dissolved in benzene. This mixture was refluxed for 0.5 h at 65°C to give a yield of 25% of the S-methyl product.

b. Pure fenitrothion was heated at 165°C for 0.5 h producing a 20% yield of the S-methyl isomer.

c. Pure S-methyl fenitrothion was prepared by J. Kovacicova (Research Institute of Agrochemical Tech., Bratislava, Czechoslovakia) via dealkylation of fenitrothion with potassium hydrosulphide and subsequent remethylation with dimethyl sulphate. The yield was 72%.

(iv) <u>Desmethyl fenitrothion</u> (0-methyl-0-(4-nitro-m-tolyl) phoshorothioicacid), was prepared by refluxing fenitrothion with potassium hydrosulphide for 5 h in methanol. The methanol was removed and the resulting oil was washed four times with benzene to remove residual fenitrothion. The yield was 50%.

(v) The hydrolysis product <u>3-methyl-4-nitrophenol</u> was obtained from Aldrich Chemical Co.

(vi) <u>Amino fenitrothion</u> was prepared by the reduction of

fenitrothion with chromous chloride in acetone giving a 93% yield (Forbes et al, 1973). Bis-fenitrothion and bis-S-methyl fenitrothion were obtained from V. Batora (Research Institute of Agrochemical Tech., Bratislava, Czechoslovakia). Trimethyl phosphate and triethyl phosphate were obtained from Aldrich Chemical Co. Ltd. Captan (N-trichloro methyl thio - tetrahydrophtalimide) was obtained from City Chemical Corp. New York. Benomyl (1-butylcarbamoyl) - benzimidazole - 2 - carbamic acid) was obtained from DuPont Chem. Co. Radioactive fenitrothion C14 labelled in the OCH3 position (specific activity .004 μC/uM) was obtained from Dr J.R. Duffy, University of Prince Edward Island.

3.   Biological Compounds

Glutathione and its reduced form were obtained from Fisher Scientific Co. Ltd. while cysteine, glycine, and L-glutamine were obtained as salts of the amino acids from BDH Chemical Co. S-CH3 glutathione was prepared by methylation of reduced glutathione in the following way: Glutathione (1.33 mMole) and 0.5 mMole NaOH were dissolved together in 25 ml of water. Methyl iodide (2 μMole) was added to the solution. Methanol was added to bring the mixture into one phase. The solution was allowed to stand for one hour and the methanol and methyl iodide were evaporated. The pH was adjusted to 3.0 by addition of 2 N HCl. The product yielded one spot on TLC with an Rf characteristic of S-methyl glutathione and its structure was confirmed by GC/MS.

4.   Forest Seeds:

White pine seeds (*Pinus strobus* L.), white spruce seeds (*Picea glauca*, Voss/Meunch), and yellow birch seeds (*Betula alleghaniensis*, Britt), were simply collected during the fall of 1972 in unsprayed regions of the Petawawa Forest Experiment Station (PFES) Ontario. Seeds were sterilized by washing

them for 15 minutes in a 1% solution of sodium hypochlorite. They
were then rinsed for another 15 minutes in continuously running
distilled water. Viability of birch seeds was ascertained by floating
the seeds on 80% ethanol. Seeds which floated contained embryos
insufficiently developed for germination and were discarded. All seeds
were stored dry at $5^{o}C$ until use to maintain high viability.

5. Animals (Rats):

Twelve disease free young male Sprague Dawley rats were ob-
tained from Ottawa Animal Supply Ltd. They weighed between 160 and
180 grams and were housed individually in stainless metabolism cages.
Rats were fed "Master Rat Chow" (Master Feeds Co.) in a powdered form.
The powder was supplemented with 3% Mazola corn oil (General Foods Co.).
Fenitrothion was added to the diet of 6 rats by first dissolving the
pesticide in the corn oil before it was added to the powdered feed.
The pesticide remained at stable concentration in the feed (10 ppm) when
kept at $20^{o}C$ for 1 day and $5^{o}C$ for 10 days.

6. Source of Indicating Bacteria:

A mutant culture His G-46; TA 1535 of *Salmonella typhimurium*
Li-2 was obtained from Dr. B. Ames, Dept. of Biochemistry, University of
California, Berkely, California.

B. METHODS

1. Treatment of Seeds with Fenitrothion:

Sterilized seeds were placed in 10 groups of 30. Population
homogenity was optimized by selecting seed groups with air dried weights
to within ± 5 mg. Groups of white pine seeds were first stratified or
pre-chilled in a 1.5×10^{-4} M solution of pure fenitrothion in distilled
water (pH 6.8) at $10^{o}C$ for 21 days in the dark. White spruce and yellow

birch seeds were similarly stratified at $5^{\circ}C$ for 28 days. A second set of seeds were treated in a 1.5×10^{-2} M emulsion of fenitrothion in distilled water. The seeds were then germinated on moist filter paper in sterile 9 cm plastic petri plates. Germination was also carried out in darkness with a diurnal temperature variation of $20^{\circ}C$ nights (8h) and $30^{\circ}C$ days (16h).

Following germination, pine seeds were dissected into three parts; the embryo, fleshy endosperm, and seed coat. Samples of replicate groups were taken during germination at 4 days (when the radicle has just emerged from the seed coat), 8 days (when the hypocotyl has emerged), 12 days (when the hypocotyl has developed), and 14 days (when the cotyledons have emerged from the seed coat). Birch and spruce seeds were not dissected due to their minute size. They were sampled in duplicate groups at 4, 10, 16, and 21 days of germination which corresponded to similar stages in morphological development as the sampling times for pine. Samples were frozen in liquid nitrogen and stored at $-70^{\circ}C$ until the pesticide was extracted.

2. Treatment of White Pine Seedlings with Radioactive Fenitrothion:

Sterile white pine seeds were stratified and germinated for 12 days as before except that no fenitrothion was added to the growth medium. The germinating seeds were then immersed in a 1.5×10^{-2} M emulsion of $OC^{14}H_3$ labelled fenitrothion in distilled water for 24 hours. The seeds were then germinated for an additional 48 hours on filter paper.

3. Treatment of Animals with Fenitrothion:

Twelve young Sprague-Dawley male rats were weighed and placed in metabolism cages so that their food and water intake and the urine and feces output could be determined. They were placed on a diet of

powdered "Master Rat Chow" containing 3% corn oil for 2 days prior to the experiment to allow them to become familiar with the cages and powdered diet. Six rats were fed chow fortified with 10 ppm fenitrothion and six without. Weight, food intake, water intake were measured daily at about 2 pm. and feces and urine were also collected. Rats were given fresh food and water daily. After seven days of feeding the rats were killed with ether. Five ml of blood was collected from the renal artery with a heparinized syringe. The liver, kidneys, brain, testes, and omental fat, were removed and weighed. Samples were frozen in liquid nitrogen and stored at $-70^{\circ}C$ until extracted. Treated rat feed was examined before and after the experiment for breakdown of fenitrothion during storage.

4. Extraction of Pesticide Residues:

a. Plant Material: Thirty seeds or seed parts were homogenized for 1 minute in 50 ml of acetonitrile with a Polytron sonicator. The homogenate was filtered under suction through Celite to remove seed debris. Another 50 ml of acetonitrile was sonicated in the flask to remove any residual pesticide on the sonicator and on the walls of the flask. This was also filtered through the same Celite. Oils and fats were removed by partitioning the acetonitrile with 50 ml of hexane 3 times (Getz, 1962). The extract was then flash evaporated almost to dryness and redissolved in 50 ml of benzene.

b. Plant Growth Medium: Water samples were concentrated to 2 ml and then extracted by partitioning with 2 ml of benzene 3 times.

c. Animal Material: (i) Liver, kidney, brain, testes, omental fat, and feces. One to two grams of material was homogenized in 50 ml of hexane in Polytron sonicator for 1 minute (Miyamoto et al., 1969). Then 50

ml of acetonitrile was added and the homogenate sonicated for a further minute. The acetonitrile was separated from the hexane layer and filtered through Celite under suction. Another 50 ml aliquot of acetonitrile was added to the hexane and the procedure repeated giving a final volume of 100 ml acetonitrile. The acetonitrile was then partitioned with another 50 ml of hexane. The extract was then flash evaporated almost to dryness and redissolved in hexane for GC analysis.

(ii) Blood. Two ml of blood was partitioned 3 times with 4 ml of chloroform. The organic and aqueous phases were separated by centrifugation at 250 x g for 1 minute. The chloroform was evaporated under a stream of nitrogen almost to dryness and redissolved in hexane for GC analysis.

(iii) Urine. Two grams of NaCl, 4 ml of benzene and 1 ml of 6 N HCl were added in that order to two ml of urine (Shafik and Enos, 1973). The mixture was stoppered and placed on a vortex for 1 minute. The benzene was separated by centrifuging for 1 minute at 250 x g.

5. Derivatization of Water Soluble Residue and Urine Extracts:

Water soluble metabolites were separated by washing the benzene once with an equal volume of water. The water was then removed by flash evaporation and the residues redissolved in 0.5 ml of methanol. A solution of pure diazomethane in methanol and ether was added until the solution was bright yellow. The mixture was stoppered and allowed to stand at 20°C for 30 minutes. It was then evaporated under a stream of nitrogen to the original 0.5 m. volume removing residual diazomethane. This procedure allowed detection and quantitation of desmethyl fenitrothion by gas chromatography. Derivatization of desmethyl fenitrothion

produced a 44% yield of fenitrothion and 56% of the S-methyl isomer. The overall recovery of extraction and derivatization was 78%.

6. Column Separation of Benzene Soluble Residues:

A slurry of silica gel and hexane was made. This was poured into a 25 x 450 mm glass column to a depth of 65 mm. Anhydrous sodium sulphate was added on top of the adsorbent to a depth of 10 mm. The sample was adsorbed to the column in 50 ml of benzene and elutions of 200 ml benzene and 100 ml acetone:benzene, 1:3, were made. The recoveries at the 0.5 ppm level were 90 to 95% for fenitrothion (found in the benzene fraction), and for fenitro-oxon and S-methyl fenitrothion (found in the acetone-benzene fraction).

7. Gas Chromatography:

A Pye (Model 104) gas chromatograph fitted with an alkali flame ionization detector (AFID), having a cesium bromide annulus was used. A glass column, 0.9 m x 4 mm ID, was packed with 100/120 mesh Gas Chrom Q coated with 3% OV-17 (Applied Science Labs State College, Pa.). With a column flow of 40 ml/min nitrogen, and a column temperature of 202°C, the retention time of fenitrothion was 3.4 min, and S-methyl fenitrothion 6.6 min. Better separation was necessary to quantitate fenitro-oxon residues. A glass column 1.8 m x 4 mm ID was packed with 4% SE30/6 % QF-1 on Chromsorb W. With a column flow of 40 ml/min and column temperature of 215°C the retention time of fenitro-oxon was 6.4 min and 3-methyl-4-nitro-phenol was 1.3 min. Duplicate determinations on all samples were carried out, bracketed by injections of sample standards. It was necessary to thoroughly condition the columns with S-methyl fenitrothion or fenitro-oxon throughout the experiment in order to get reproducible results. On-column injection was used and the

injector block was unheated. No on-column isomerization of fenitrothion was detected under these conditions.

8. Thin Layer Chromatography:

Baker-flex silica gel precoated sheets 250 microns thick were used to detect toxic residues. The plates were developed with a solvent system of ethyl acetate/cyclohexane 1:3. Spots were visualized using the enzyme inhibition technique described by Mendoza (1972) using extract of steer liver homogenate as the spray reagent. Fenitrothion has an Rf of 0.58, S-methyl fenitrothion 0.15, and fenitro-oxon 0.09. Desmethyl fenitrothion was chromatographed on cellulose plates due to its acidic nature. The plates were developed with a solvent system of 2,propanol: water:ammonium hydroxide, 75:24:1. Plates were developed with 2,6 diazobromoquionamine as spray reagent in glacial acetic acid. This is specific for P-S bonds of phosphorothionates (Menn et al 1957).

9. Extraction of Glutathione Conjugates:

Glutathion conjugates were extracted from seedlings treated with radioactive fenitrothion by sonication in 50 ml of 80% ice cold methanol for 1 minute. This was similar to the method used by Lamoureux (1970) for extracting glutathione-atrazine conjugates. The homogenate was filtered through Celite under suction and the flask and sonicator rinsed for 1 minute in cold methanol. The filtrate was evaporated almost to dryness and taken up in 1.0 ml of distilled water. The water was partitioned 3 times with chloroform to remove non-conjugated pesticide residues. Both water and chloroform extracts were then concentrated and examined by thin layer chromatography. Water soluble glutathione conjugates were chromatographed on cellulose plates. Two solvent systems were used, namely, butanol : acetic acid : water; (11:4:5)

(Morello et al, 1968), and phenol:water: (4:1), Johnson et al, 1966).
Plates were developed with ninhydrin dissolved in acetone. Portions of
plates were left undeveloped and areas with Rf's similar to S-methyl
glutathione were scraped off the plates and dissolved in methanol.
Aliquots were then hydrolysed in 6 N HCl for 20 h at $110^{\circ}C$ and re-
chromatographed. Other aliquots were concentrated to dryness and submit-
ted for analysis by direct probe mass spectroscopy. All ninhydrin
positive spots were scraped from the plate, dissolved in Aquasolve
scintillation fluid and counted on a Picker Nuclear Ansitron liquid
scintillation counter.

10. Quantitation of Free SH Groups in Seeds:

Seeds which had been stratified and germinated with and without
fenitrothion treatment for 12 days were examined for free SH groups re-
flecting levels of reduced glutathione present. Seeds were placed in
50 ml of 80% ice cold methanol (Lamoureux et al, 1970) containing 5% sul-
phosalicylic acid (Spragg and Yemm, 1958) and were sonicated with a Poly-
tron sonicator for 1 minute. The homogenate was then filtered and the
residue re-extracted as before. This was also filtered and the combined
filtrates were flash evaporated to 2 ml.

Free SH groups were measured colorimetrically using the nitro-
presside reaction of Grunert and Phillips (1951). Five ml of saturated
NaCl was added to the extract. After mixing, a 0.4 ml aliquot was placed
in a cuvette along with 0.2 ml of 0.067 M sodium nitroprusside and 0.2 ml
of a cyanide carbonate solution (1.5 M $NaCo_3$ and 0.067 M KCN). The red
colour was measured at minimum transmission of 520 nm using a Unicam SP-
800 double beam UV spectrophotometer. The amount of free SH groups, which
reflects approximately the amount of reduced glutathione present, was

calculated from a standard curve. Response is linear through a range of 1 to 100 μg of glutathione.

11. Detection of Mutagenicity of Fenitrothion and Metabolites Using Enteric Bacteria:

The method of Ames (1971) was used to test the ability of fenitrothion and its derivatives to cause mutations in bacterial DNA. A mutant culture of *Salmonella typhimurium* Li-2 was used; $HisG_46$; a histidine requiring mutant susceptable to base-pair substitutions caused by alkylating agents. Bacteria were plated on sterile plastic petri plates containing minimal agar medium (Vogel-Bonner 1956; 1.5% agar, 2.0% glucose). A mixture of 0.1 ml of a nutrient broth culture of bacteria (3 x 10^8 bacteria) and 2 ml of melted (45°C) 0.6 agar containing 0.5% NaCl, 0.1 μMole histidine, and 0.1 μMole biotin was prepared for each plate, poured onto the agar, and spread by rotating the plate slowly. Pesticides and derivatives were first dissolved in spectrophotometric grade dimethyl sulphoxide which is sterile. This solvent dissolved all of the compounds (as many were not water soluble) and was not toxic to the bacteria. Dilutions were made in DMSO and 1.0 ml aliquots were spread over the surface of the plate. Duplicate plates were made in each case. In a second experiment the pesticide was added to the plate along with the "S-9" liver homogenate of Garner et al (1972). The S-9 mixture contained: 0.3 ml of S-9 liver homogenate, 8mM $MgCl_2$, 33 mM KCl, 5mM glucose-6-phosphate, 4mM TPN, and 100mM sodium phosphate (pH7.4). The homogenate allows biological activation of potential alkylators.

12. Nuclear Magnetic Resonance Spectroscopy:

NMR spectra were obtained on a Varian T-60 Spectrometer. Optimal spectra were obtained from 10% solutions in deuterated DMSO.

13. Mass Spectrometry:

Mass Spectra were obtained on a Finnigan 300 Mass spectrometer. Spectra of fenitrothion and metabolites were obtained by GC/MS whereas spectra of S-methyl glutathione and amino acids were obtained by direct probe analysis.

RESULTS

1. Metabolism of Fenitrothion by White Pine:

Preliminary results showed that fenitrothion was absorbed from a 10 ppm aqueous solution, approximating field concentration, by stratified and non-stratified germinating white pine seeds. Two metabolites, fenitro-oxon, and S-methyl fenitrothion were detected in late stages of germination (Hallett et al 1975). Germination studies were carried out to quantitate the formation of fenitro-oxon, S-methyl fenitrothion, and desmethyl fenitrothion, a non-toxic intermediate proposed in the formation of the S-methyl isomer.

The amount of fenitrothion absorbed during germination of stratified white pine seeds is shown in Figure 1. Initially at 4 days germination a high (160 ppm) concentration of fenitrothion is found in the seed coat. Lower concentrations are found in the endosperm (70 ppm) and embryo (24 ppm). During the course of germination the concentration falls in the seed coat to 60 ppm at 14 days of germination. There is however a concomitant increase in the concentration of fenitrothion in the endosperm to 180 ppm at 14 days. This is paralleled by an increase in the concentration in the embryo to 120 ppm at 14 days germination.
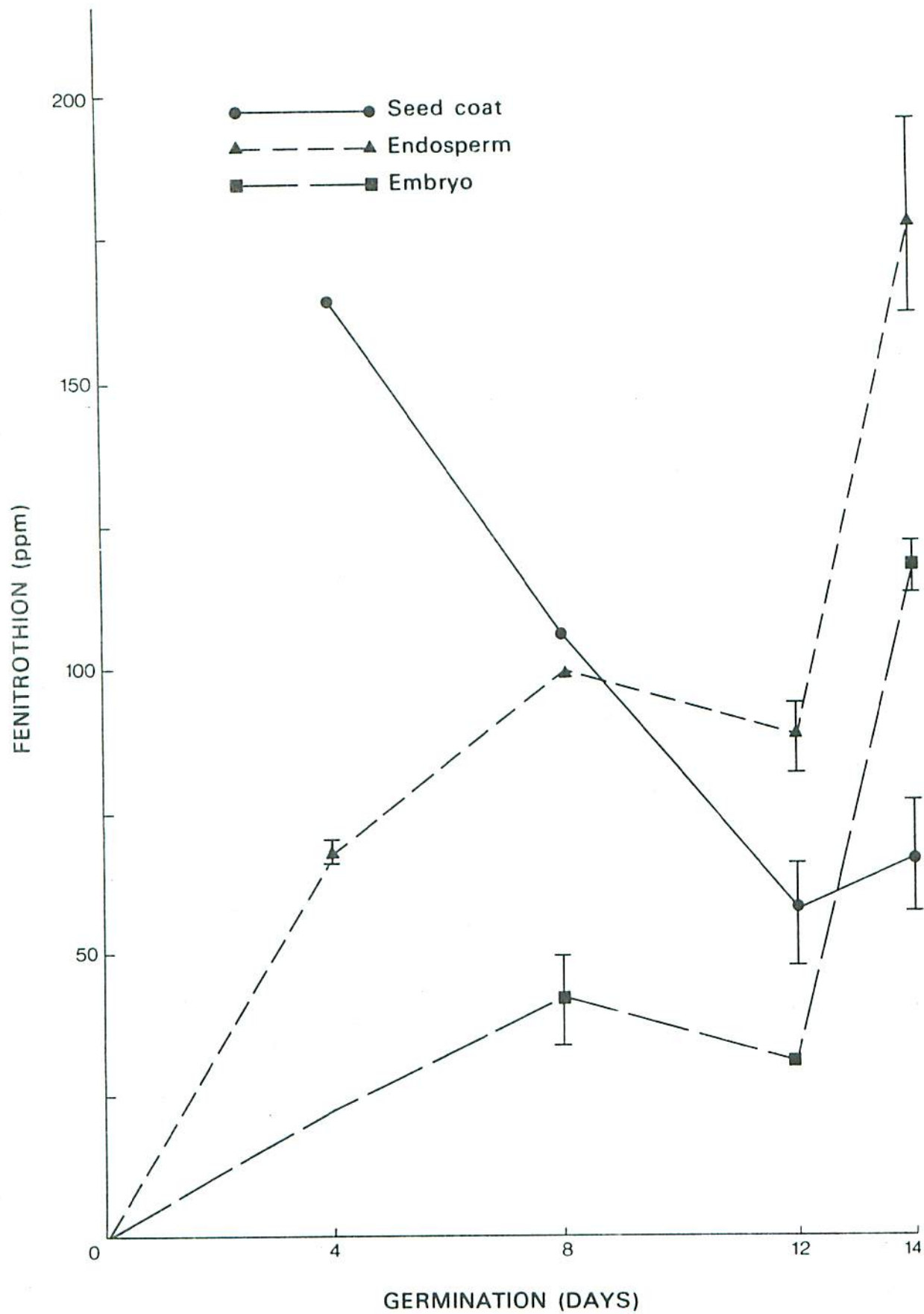
Figure 1: Uptake of Fenitrothion by White Pine Seeds
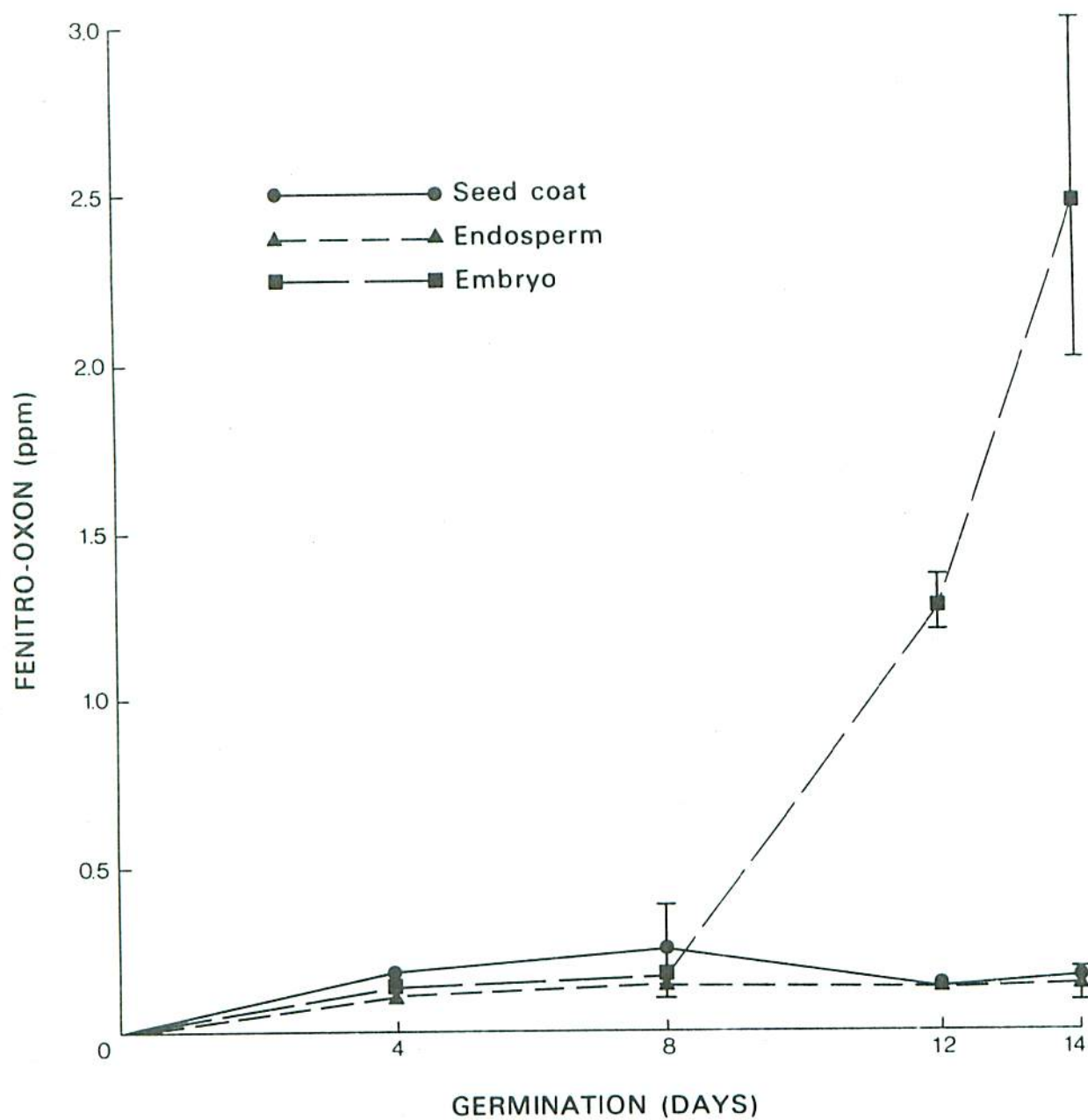
Figure 2: Formation of Fenitro-oxon in White Pine Seeds During Germination.
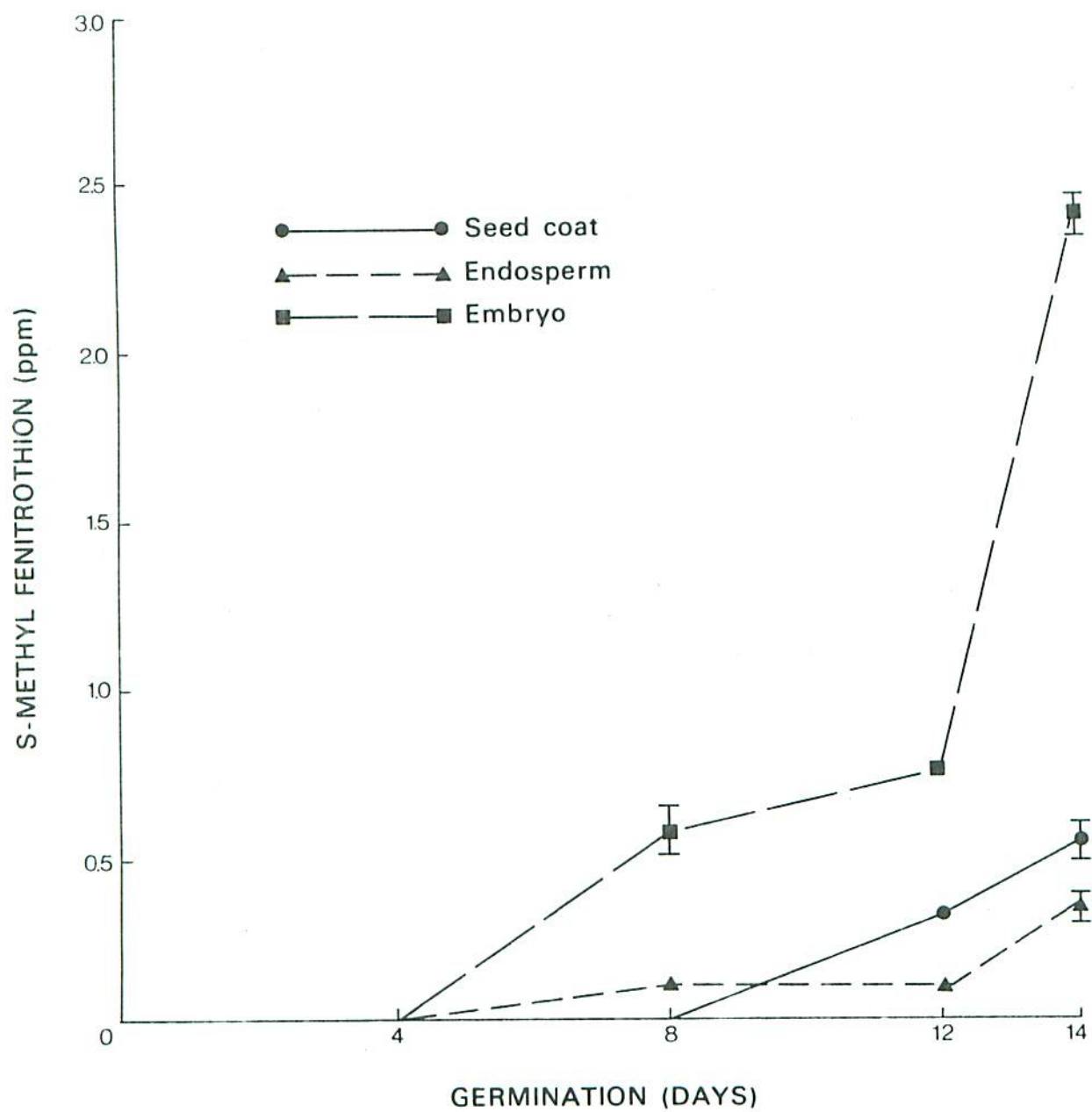
Figure 3:   Formation of S-methyl Fenitrothion in White Pine Seeds
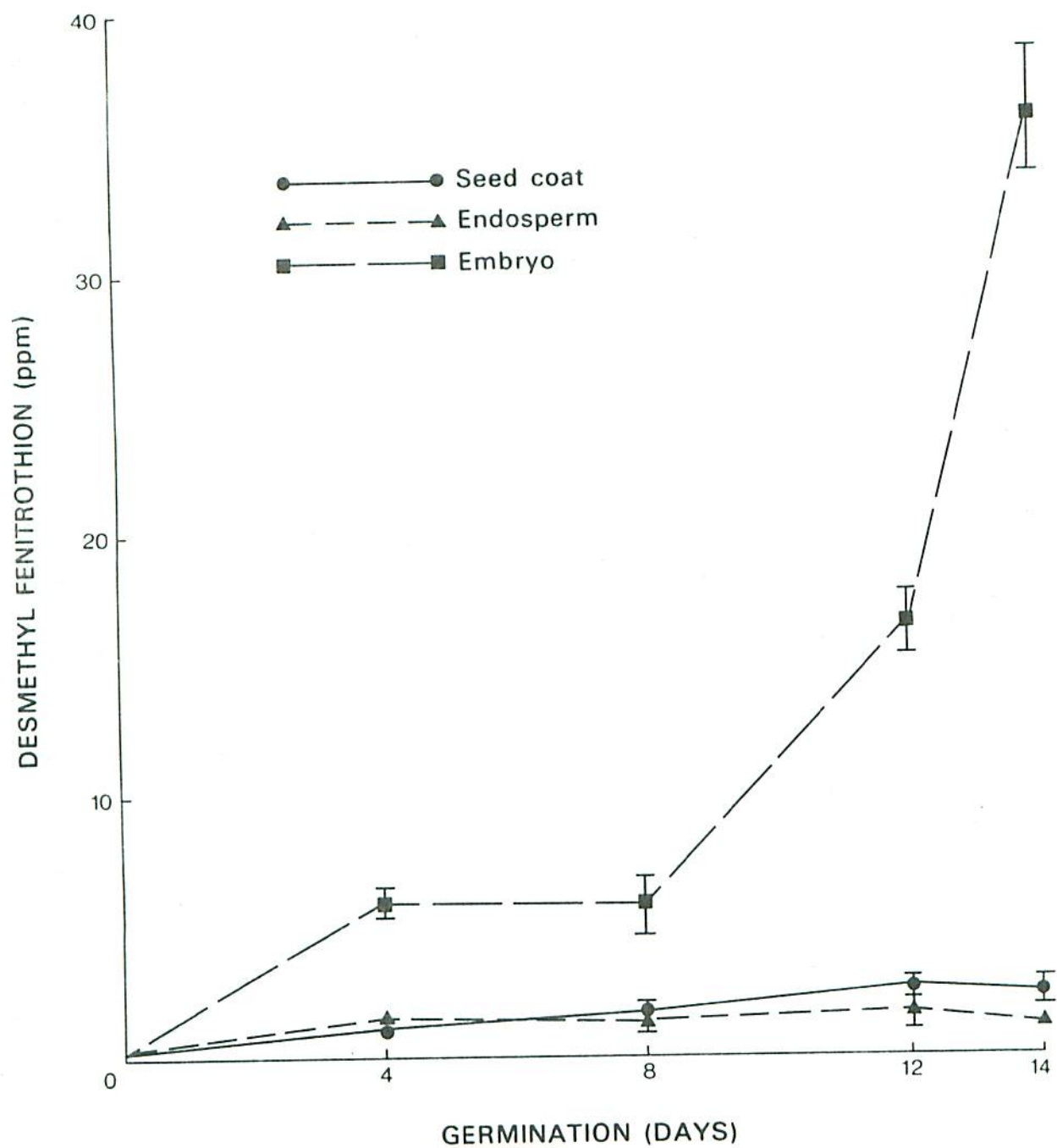   During Germination.

Figure 4: Formation of Desmethyl Fenitrothion in White Pine Seeds During Germination.

Fenitrothion was metabolized into fenitro-oxon by the germinating seedlings as shown in Figure 2. Low concentrations are found in the seed coat and endosperm (0.2 ppm) over the 14 day period. However the concentration rises in the growing embryo to 1.4 ppm at 12 days and 2.5 ppm at 14 days of germination.

S-methyl fenitrothion was also formed in the maturing seedlings as shown in Figure 3. The seed coat and endosperm contained low concentrations of this metabolite; at maximum, only 0.5 ppm in the seed coat and 0.3 ppm in the endosperm. S-methyl fenitrothion was found at 0.5 ppm in the embryo by 8 days of germination, and the concentration rose to 2.4 ppm after 14 days of germination.

Figure 4. shows the concentration of desmethyl fenitrothion found in the germinating seeds. This metabolite was found in low concentrations (1.0 ppm) in the seed coat and endosperm throughout germination. The embryo contained 0.5 ppm of the desmethyl derivative after 4 days germination and this concentration rose rapidly between 8 and 14 days germination to 36 ppm.

Although the extraction and GC method was sensitive for detection of aminofenitrothion, no residues of this metabolite were found. The main hydrolysis product, 3-methyl, 4-nitrophenol was not evident in detectable amounts, however, methods of detection are 100 times less sensitive to this metabolite than fenitrothion. The water moistening the seeds was monitored for metabolites throughout the experiment. None were detected.

2. Metabolism of Fenitrothion by White Spruce and Yellow Birch:

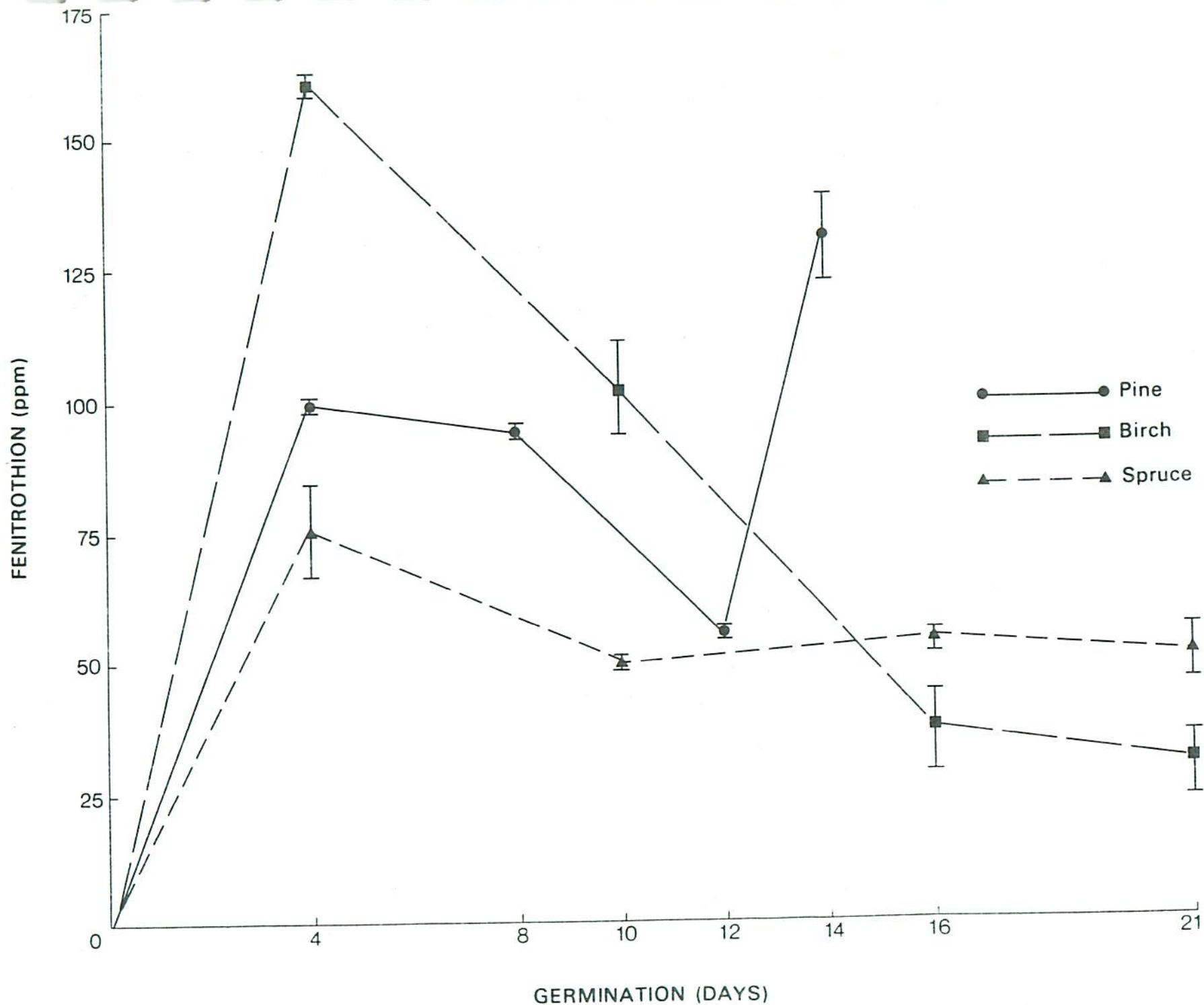Previous studies showed that fenitrothion treatment did not pro-

Figure 5: Uptake of Fenitrothion by Seeds of Three Forest Species During Germination.
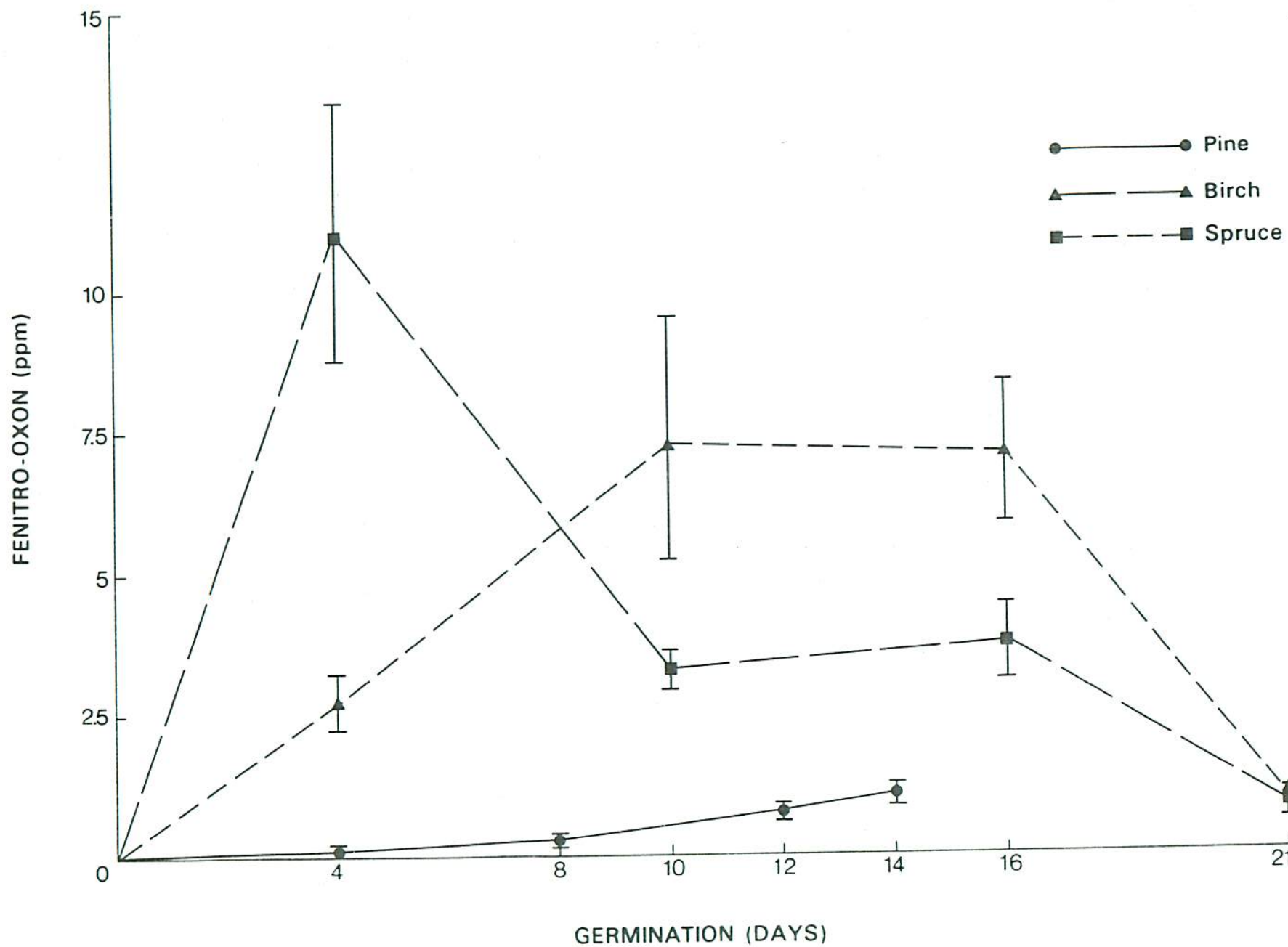
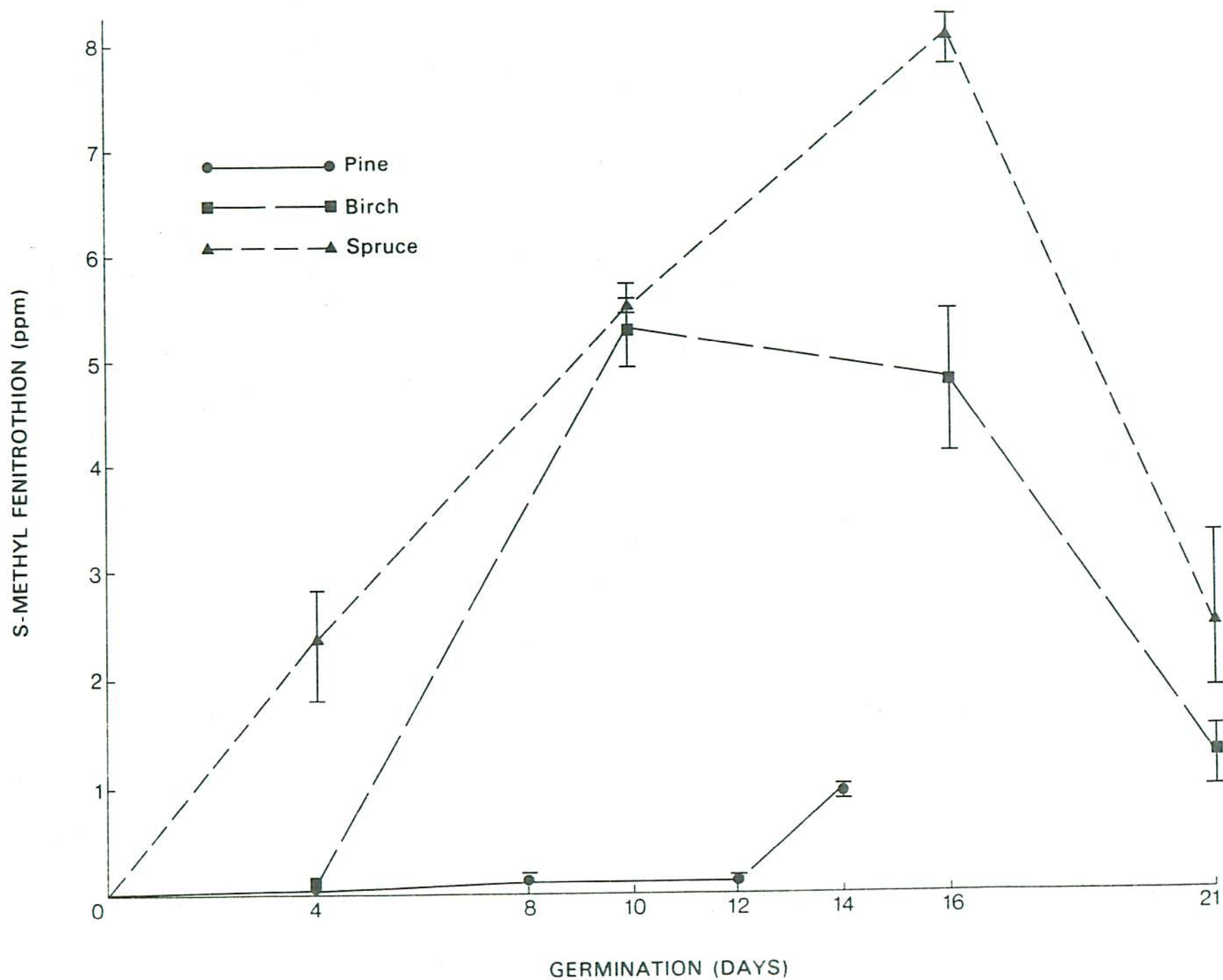Figure 6: Contents of Fenitro-oxon in Seeds of Three Species During Germination.

Figure 7: Concentration of S-methyl Fenitrothion in Seeds of Three Species.
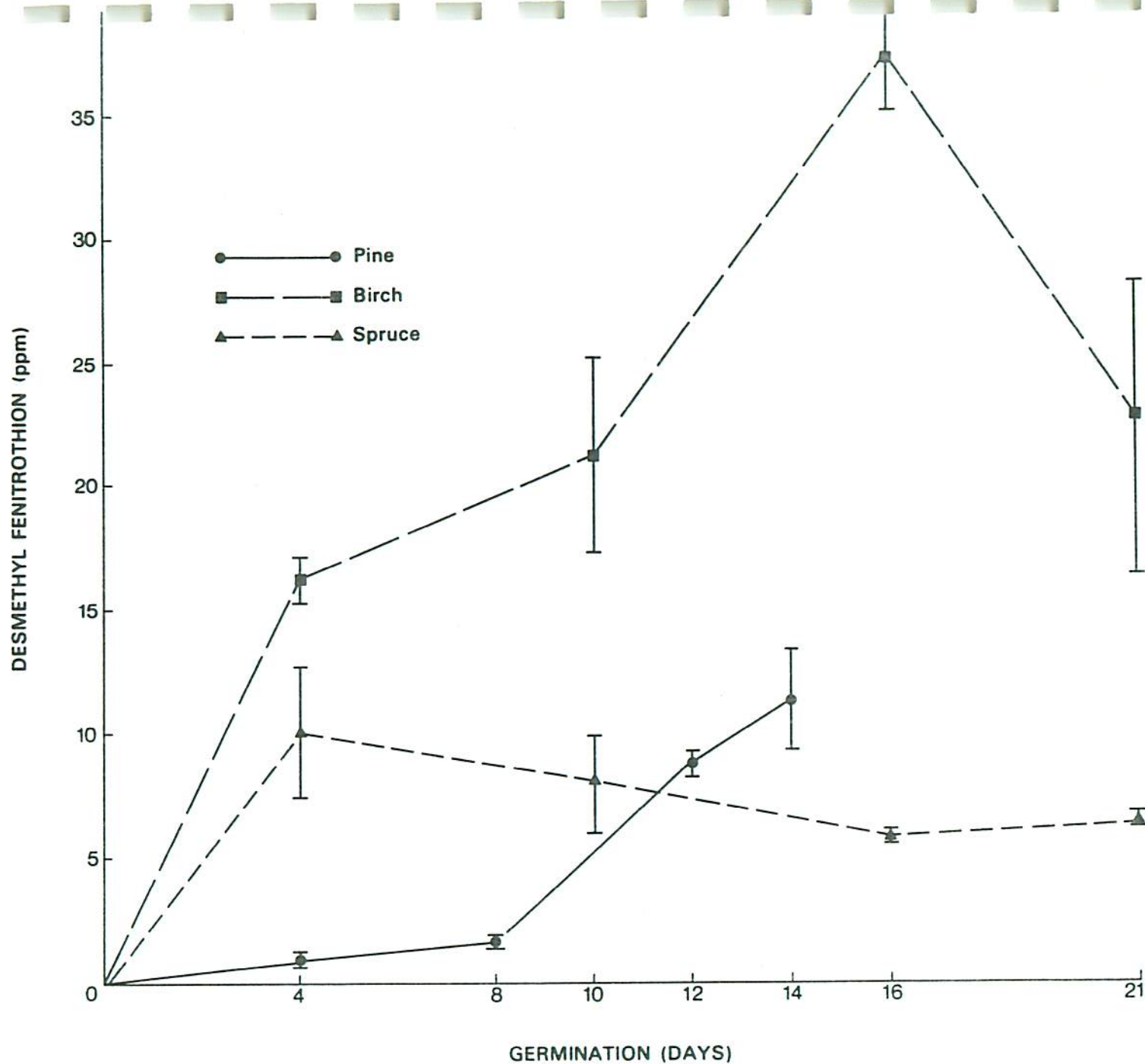
Figure 8: Course of formation of Desmethyl Fenitrothion in Seeds of Pine, Birch & Spruce.

duce any long term gross morphological defects in white pine seedlings after 6 months growth. White spruce, the target tree protected by the sprayings was also shown to resist toxic effects (Pomber and Weinberger, 1974). Both of these species are coniferous gymnosperms. Yellow birch, a deciduous angiosperm, prevalent in sprayed forests, showed slight effects on treatment with 10 ppm fenitrothion, and gross stunting after 10 days of germination when treated with 1000 ppm fenitrothion. This effect was not evident in pine or spruce (Pomber and Weinberger, 1974). Similar residue and metabolic studies were therefore carried out in whole seeds of birch and spruce to examine any difference in absorption, metabolism, and detoxification of the pesticide.

The amount of fenitrothion absorbed by whole pine, spruce, and birch seeds is shown in Figure 5. By 4 days of germination, yellow birch had absorbed 160 ppm of fenitrothion, 1.5 times more than pine and 2 times more than spruce. This level fell rapidly in birch but only slightly in spruce and increased in pine during later stages of germination.

Fenitro-oxon (Figure 6) reached a high of 11 ppm by 4 days of germination in birch. The maximum in spruce (7.5 ppm) was not reached until 10 days of germination or until 14 days in pine (14 ppm).

The concentration of S-methyl fenitrothion (Figure 7) rose rapidly and reached a high of 5 ppm by 10 days germination in birch. Higher levels were found in spruce (8.0 ppm) at 16 days of germination. The concentrations found in pine were far lower.

Desmethyl fenitrothion (Figure 8) was found in high concentration (15 ppm) by 4 days of germination in birch. The concentration in-
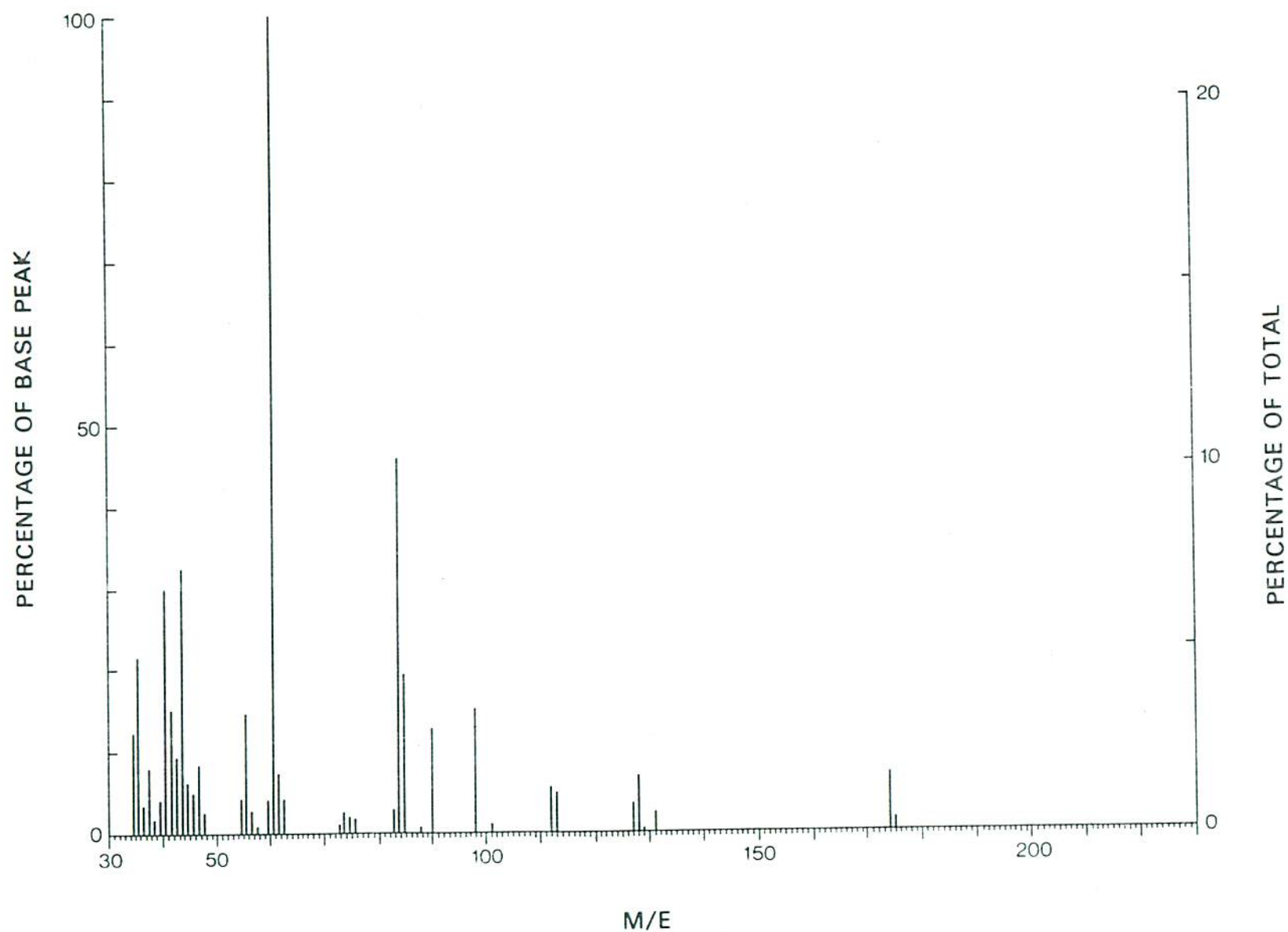
Figure 9: Mass Spectrum of Glutathione After Methylation.

creased to 37 ppm by 16 days germination. Lower levels were found in spruce (9.3 ppm) which showed a gradual decline to 5 ppm by 21 days germination. Levels in pine increased during germination to 11.2 ppm in the young seedlings.

3. Dealkylation of Fenitrothion by Germinating Seedlings:

Fenitrothion has been shown to be dealkylated by an alkyl transferase using glutathione, a common tripeptide, as substrate to form desmethyl fenitrothion, and S-methyl glutathion in mammalian liver and kidney (Hollingworth et al, 1969). Desmethyl fenitrothion which is non-toxic was found in white pine embryos, and in white spruce and yellow birch during germination. It was suspected to be an intermediate in forming the toxic S-methyl isomer. Therefore $OC^{14}H_3$ labelled fenitro-thion was used to treat seeds between 12 and 15 days of germination. Extraction of glutathione conjugates from the seeds yielded a series of ninhydrin positive compounds (Table 1). The major compound was radio-active (1500 DPM) and had an Rf similar to that of S-methyl glutathione. When this molecule was hydrolysed and re-chromatographed, ninhydrin posi-tive molecules with Rf's similar to glutamine, cysteine, S-methyl cys-teine, and glycine were evident in approximately equal amounts. The structure of S-methyl glutathione was confirmed by mass spectroscopy (Figure 9). The base peak m/e 61 is indicative of the CH_2-S-CH_3 fragment characteristic of S-methyl cysteine and S-methyl glutathione. Peaks at m/e 84 and 34 are characteristic of glutamic acid and glycine respectively. They are the other two constituent amino acids of glutathione. A compound with an Rf similar to S-methyl cysteine containing 500 DPM was also re-covered from the seedlings.

Thin layer chromatography of the chloroform extract containing pesticide residues showed three spots sensitive to the enzyme inhibition technique (Table 2). A spot identified as fenitrothion contained 300,000 DPM, S-methyl fenitrothion 1500 DPM, and fenitro-oxon 1000 DPM.

4. Metabolism of a High Concentration of Fenitrothion by Yellow Birch:

Fenitrothion treatment at 1,000 ppm was shown to inhibit the germination of yellow birch seeds (Pomber and Weinberger, 1974). The seeds were found to produce a short radicle and hypocotyl up to 10 days of germination whereupon they stopped growing and underwent rapid senesence. Figure 10, shows that birch seeds treated with 1,000 ppm of the pesticide absorbed a maximum of 5,000 ppm fenitrothion after 4 days of germination. This was a 32 times increase in concentration absorbed over the 10 ppm treatment. Levels of metabolites increased only 3 times in the case of fenitro-oxon (32 ppm) and S-methyl fenitrothion (16.7 ppm), and 5 times for desmethyl fenitrothion (155 ppm).

5. Effect of Fenitrothion Treatment on Seedling Glutathione Levels:

Desmethyl fenitrothion was shown to be a major metabolite of fenitrothion formed in pine, spruce, and birch seeds during germination of treated seeds. Highest levels were found in birch. Glutathione was found to be the substrate acceptor of the methyl on O-dealkylation of fenitrothion in pine seedlings forming S-methyl glutathione. Table 3. shows that treatment with fenitrothion at the 10 ppm level only slightly depleted glutathione levels in all three seed species. Treatment at the 1000 ppm level depleted the level of glutathione slightly in spruce (25%), moderately in pine (43%), and drastically in birch (75%).
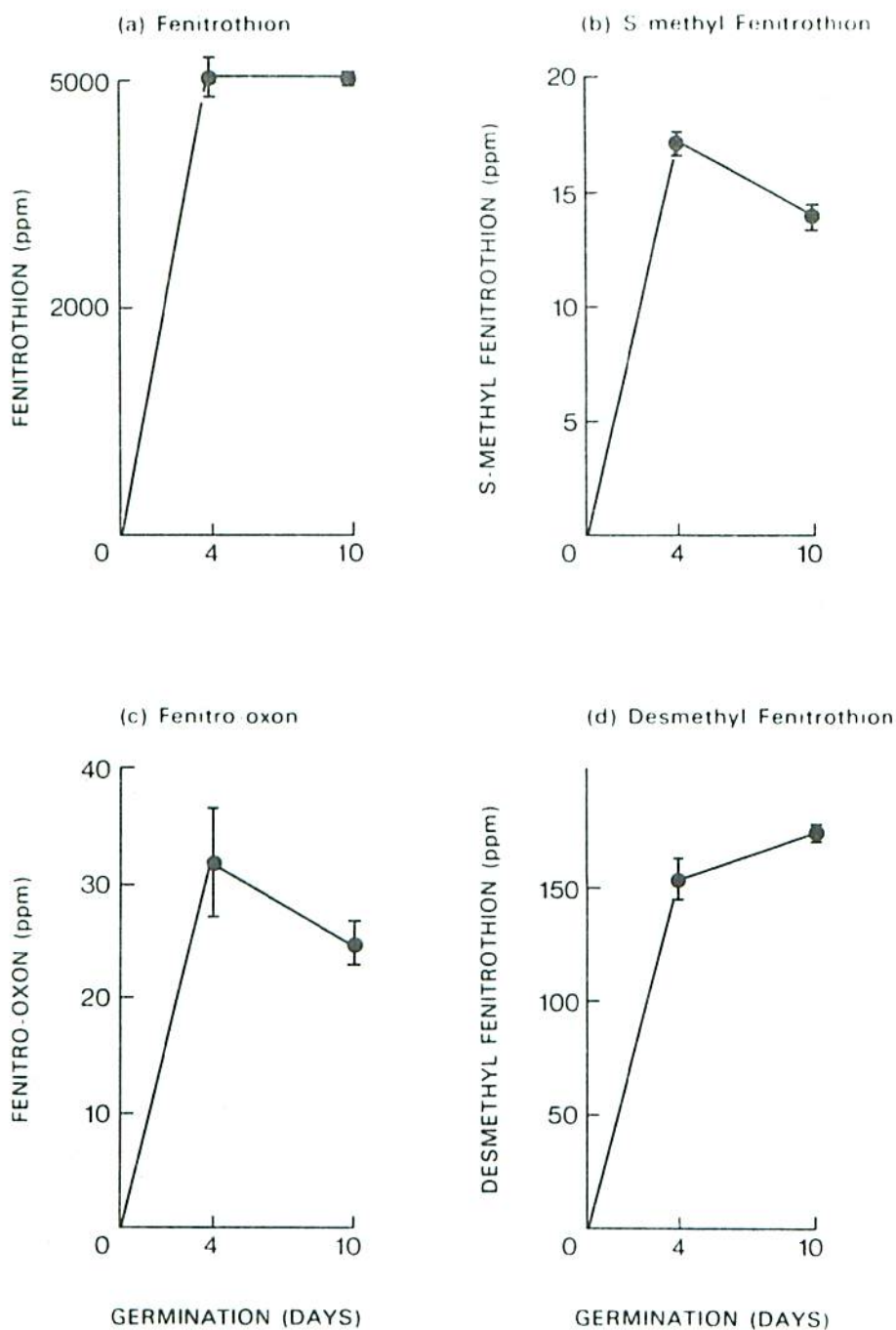
Figure 10: Metabolism of Fenitrothion and Formation of Metabolites in Yellow Birch Seeds treated with 1000 ppm of the insecticide.

6. Formation of S-methyl Fenitrothion:

Methyl phosphate esters have been shown to act as alkylating
agents. Eto (1968) showed that fenitrothion would alkylate desmethyl feni-
trothion in the S position when incubated in acetone for 14 hours at $60^{\circ}C$
to give a 16% yield of S-methyl fenitrothion. A similar reaction was
followed in DMSO by NMR. In a 0.3 M mixture of fenitrothion and desmethyl
fenitrothion, S-methyl fenitrothion represented 26% of the reaction mix-
ture after 21 hours at $60^{\circ}C$ and 10.3% after 21 hours at $35^{\circ}C$ (Table 4).
In a 0.3 M mixture of fenitro-oxon and desmethyl fenitrothion, S-methyl
fenitrothion represented 10.7% of the reaction mixture after 21 hours at
$60^{\circ}C$ but there was no reaction after 21 hours at $35^{\circ}C$. No breakdown of
fenitrothion, fenitro-oxon, desmethyl fenitrothion, or S-methyl feni-
trothion was evident after 21 hours at $35^{\circ}C$ or $60^{\circ}C$.

7. Detection of Mutagenicity Using Enteric Bacteria as Indicator:

In the previous section fenitrothion and fenitro-oxon were shown
to be chemical alkylating agents. Fenitrothion and metabolites: fenitro-
oxon, S-methyl fenitrothion, aminofenitrothion bis fenitrothion bis S-
methyl fenitrothion, 3-methyl-4-nitrophenol were tested for their potential
to be mutagenic via alkylation of nucleic acids causing base pair sub-
stitutions. All compounds were tested with and without liver homogenate
as a biological activator. All gave a negative response at concentrations
of 1,000, 100, 50, and 10 ppm. Trimethyl phosphate (TMP) and Triethyl
phosphate (TEP) gave a negative response in this system although they have
shown positive responses to mutagenicity in other systems (Vogel-Bonner,
1956). In contrast fungicides benomyl and captan gave a positive response.
The response to captan is concentration-dependant at low levels as shown
in Table 5.

TABLE I

Thin Layer Chromatogram of Extract from White Pine Seedlings

Treated with $OC^{14}H_3$ Fenitrothion

(Ninhydrin Positive Substances)

	Rf VALUES				
	SOLVENT SYSTEM A			SOLVENT SYSTEM B	
COMPOUND	STANDARD	EXTRACT	HYDROLYSIS PRODUCT	STANDARD	EXTRACT
S-Methyl Glutathion	.55	.55		.59	.59
Glutathion	.47	(1500 DPM)		.55	
Cysteine	.46		.49	.54	
S-methyl Cysteine	.59	.59	.59	.62	
Glutamic Acid	.375	(500 DPM)	.38	.53	
Glycine	.325		.32	.27	

```
                                 glu
         S-methyl Glutathion     cys- S - CH3
                                 gly
```

TABLE II

Thin Layer Chromatogram of Extract from White Pine

Seedlings Treated with $OC^{14}H_3$ Fenitrothion

(Enzyme Inhibition)

COMPOUND	Rf VALUES		RADIOACTIVITY
	Standard	Extract	DPM
Fenitrothion	.58	.58	300,000
S-methyl Fenitrothion	.15	.15	1,500
Fenitro-oxon	.09	.09	1,000

TABLE III

Concentration of Glutathione in 12 Day Old Tree Seedlings Treated
with Two Concentrations of Fenitrothion

Glutathione µg/gram seed

SPECIES	CONTROL	10 ppm	1000 ppm
White Spruce	901.1 ± 9.2	883.2 ± 6.1	662.5 ± 4.7
White Pine	828.3 ± 8.5	796.5 ± 32.1	459.0 ± 17.5
Yellow Birch	656.9 ± 6.3	625.6 ± 14.7	168.1 ± 25.0

TABLE IV

Alkylation of Desmethyl Fenitrothion

(Followed by NMR)

Compound	Value	Coupling Constant
Fenitrothion	OCH_3 = 6.10	J = 17.5 cps
	CH_3Aryl = 7.48	
Fenitro-oxon	OCH_3 = 6.11	J = 15 cps
	CH_3Aryl = 7.46	
S-methyl Fenitrothion	OCH_3 = 6.05	J = 16 cps
	CH_3Aryl = 7.46	
	SCH_3 = 7.40	J = 25 cps
Desmethyl Fenitrothion	OCH_3 = 6.15	J = 17.5 cps
	CH_3Aryl = 7.48	
Desmethyl + Fenitrothion: Time 21 hours	Temp = 60°C	$\frac{SCH_3}{OCH_3}$ X 100 = $\frac{6}{25}$ X 100 = 24%
	Temp = 35°C	$\frac{SCH_3}{OCH_3}$ X 100 = $\frac{3}{29}$ X 100 = 10.3%
Desmethyl + Fenitro-oxon:	Temp = 60°C	$\frac{SCH_3}{OCH_3}$ X 100 = $\frac{3}{28}$ X 100 = 10.7%
	Temp = 35°C	No Reaction

TABLE V

Mutagenic Potential of Captan Fungicide

Concentration in ppm	Number of Colonies	
	A	B
1000	+++	+++
750	+++	+++
500	+++	+++
250	+++	2,000
100	180	84
50	66	67
25	20	18
10	12	10
1	11	6
DMSO	4	5

8. Rat Feeding Study:

In sections 1,2,3 and 4 it was shown that germinating forest seeds absorbed and concentrated fenitrothion from aqueous solutions containing 10 ppm of the pesticide. This concentration approximated the field concentration aerial spray of 4 oz./acre. It was shown that the seeds metabolized the pesticide into two more toxic derivatives: fenitro-oxon, and S-methyl fenitrothion, or was dealkylated to form desmethyl fenitrothion. Rats were therefore fed a diet containing 10 ppm fenitrothion and were monitored accordingly.

The rats ate the contaminated diet quite willingly as shown in Figure 11. and consumed the same amount of food as the control animals (approximately 160 grams over 7 days). They drank the same amount of water (Figure 12), and gained weight at the same rate as the control animals (Figure 13). The rats appeared quite healthy after 7 days of eating the contaminated diet and having consumed 1.610 grams of fenitrothion (Figure 14).

Very little fenitrothion was eliminated in the feces (Figure 14). It was either absorbed or hydrolysed in the gut cavity. Considerably more fenitrothion was eliminated in the urine after being dealkylated to the non-toxic desmethyl form.

Figure 15. shows that fenitrothion was absorbed from the diet into various organs of the rat. Although the concentration in the blood was very low (0.2 ppm) there was accumulation of the pesticide in tissues of low metabolic turnover and blood supply. The omental fat contained 0.35 ppm, the testes 0.05 ppm and the brain 0.075 ppm. The liver and kidney have a rich blood supply and rapid metabolic turnover. The liver

contained the highest concentration of fenitrothion, 3.2 ppm. The kidney contained 2.64 ppm. Neither fenitro-oxon, nor S-methyl fenitrothion, nor desmethyl fenitrothion were found in detectable levels (0.05 ppm) in any of these organs.

If the concentrations are extrapolated to the whole organ (half was sampled in most cases) the amount of fenitrothion contained in these could be determined (Figure 16). The liver contained 33 μg, kidney 4.8 μg, fat 1.1 μg, brain 0.6 μg, and testes 0.4 μg. The total amount of fenitrothion accumulated in these organs was 39.9 μg in a rat weighing 227 grams or 176 mg/kg.
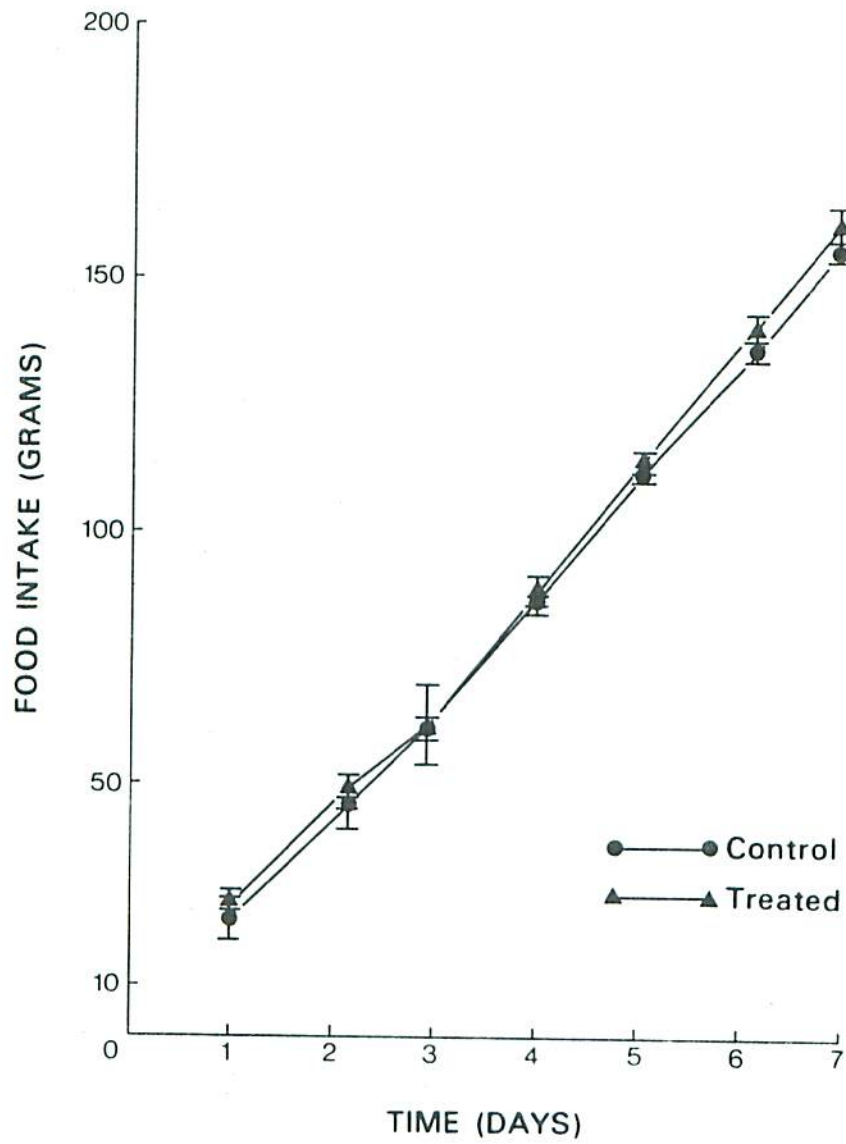
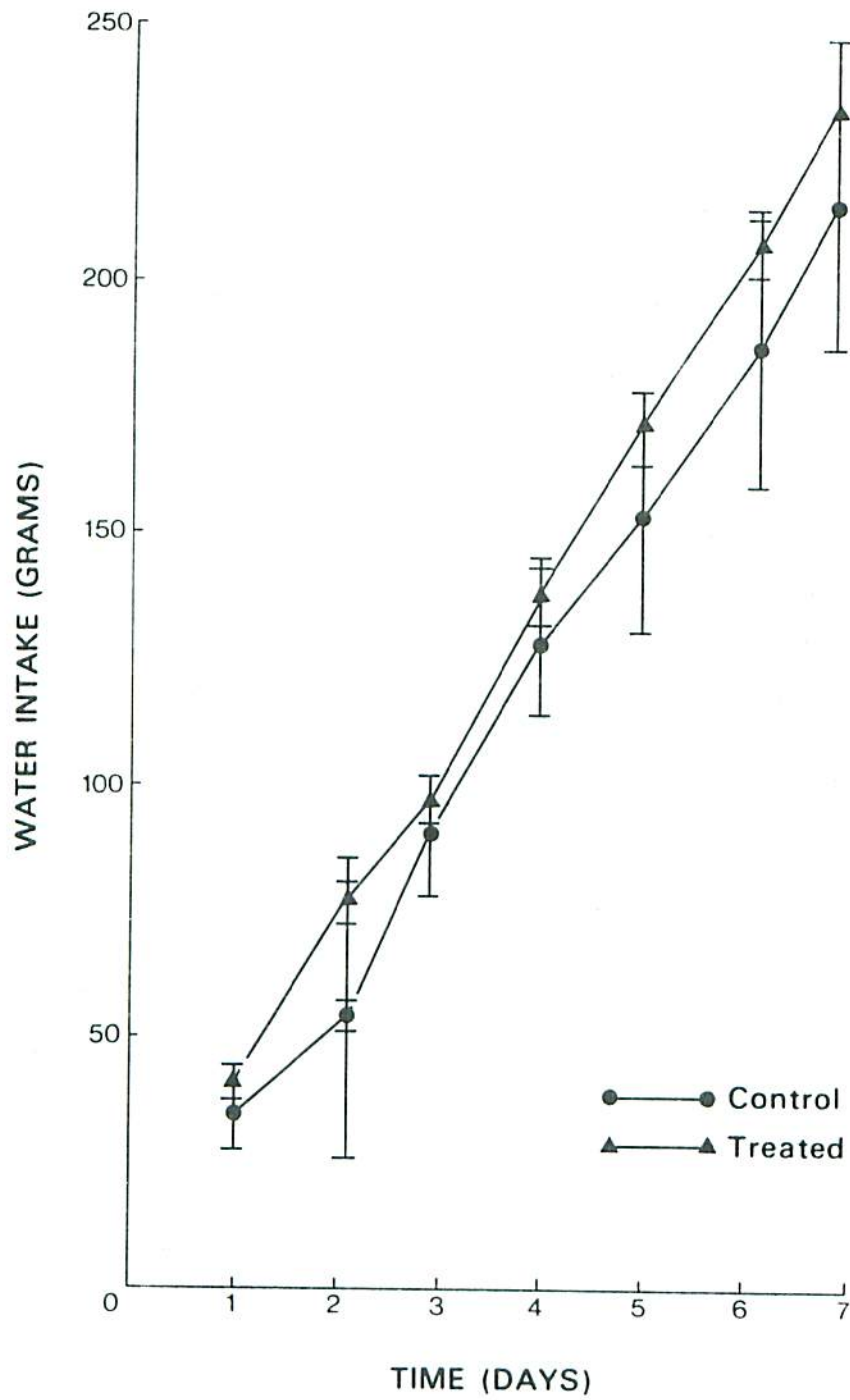Figure 11: Effect of Fenitrothion on Food Intake by Rats.

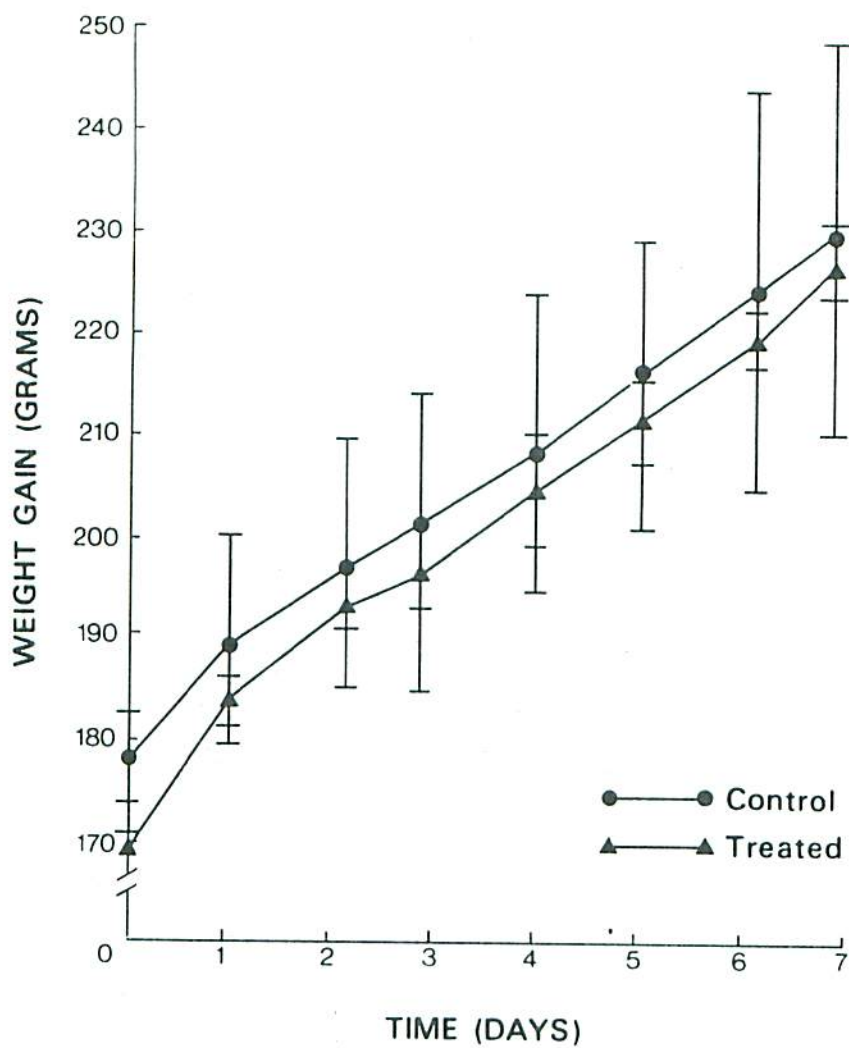Figure 12: Effects of Fenitrothion on Water Intake by Rats.

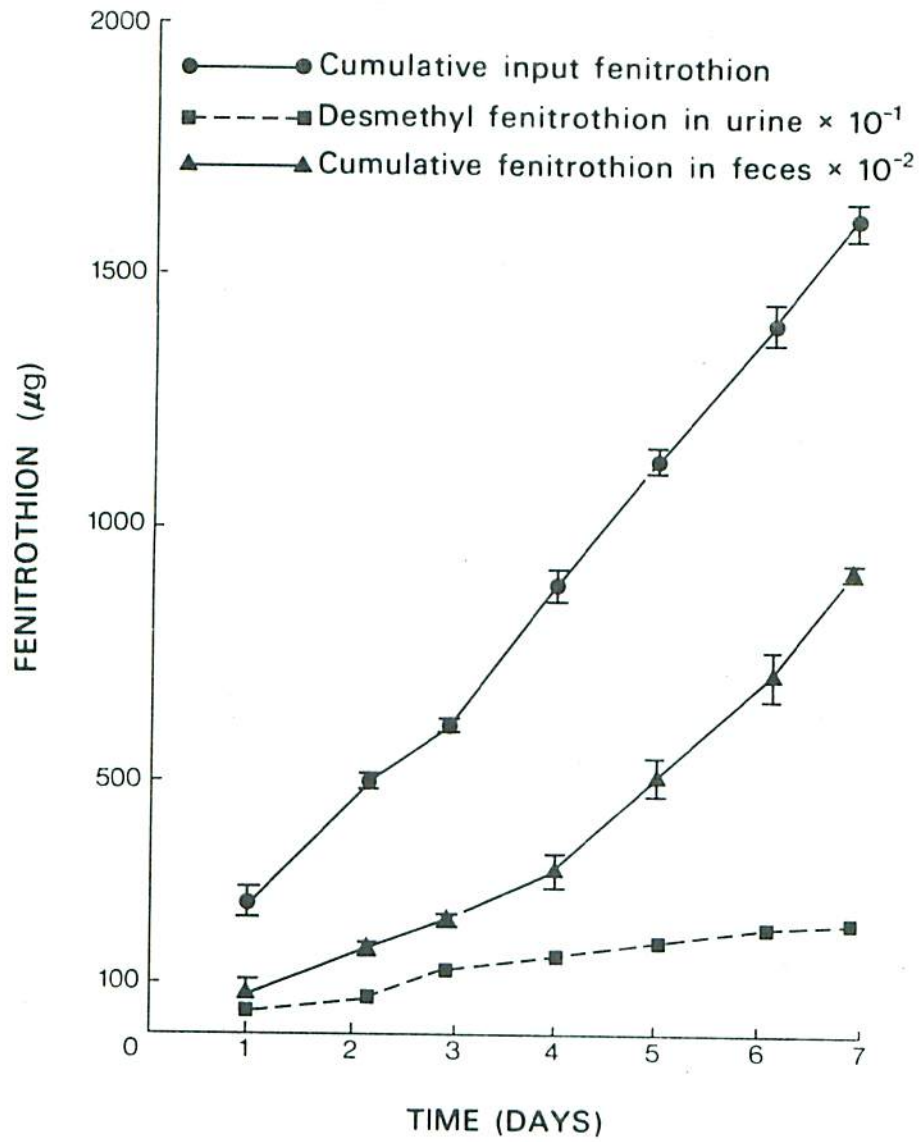Figure 13: Effects of Fenitrothion on Weight Gain of Rats.

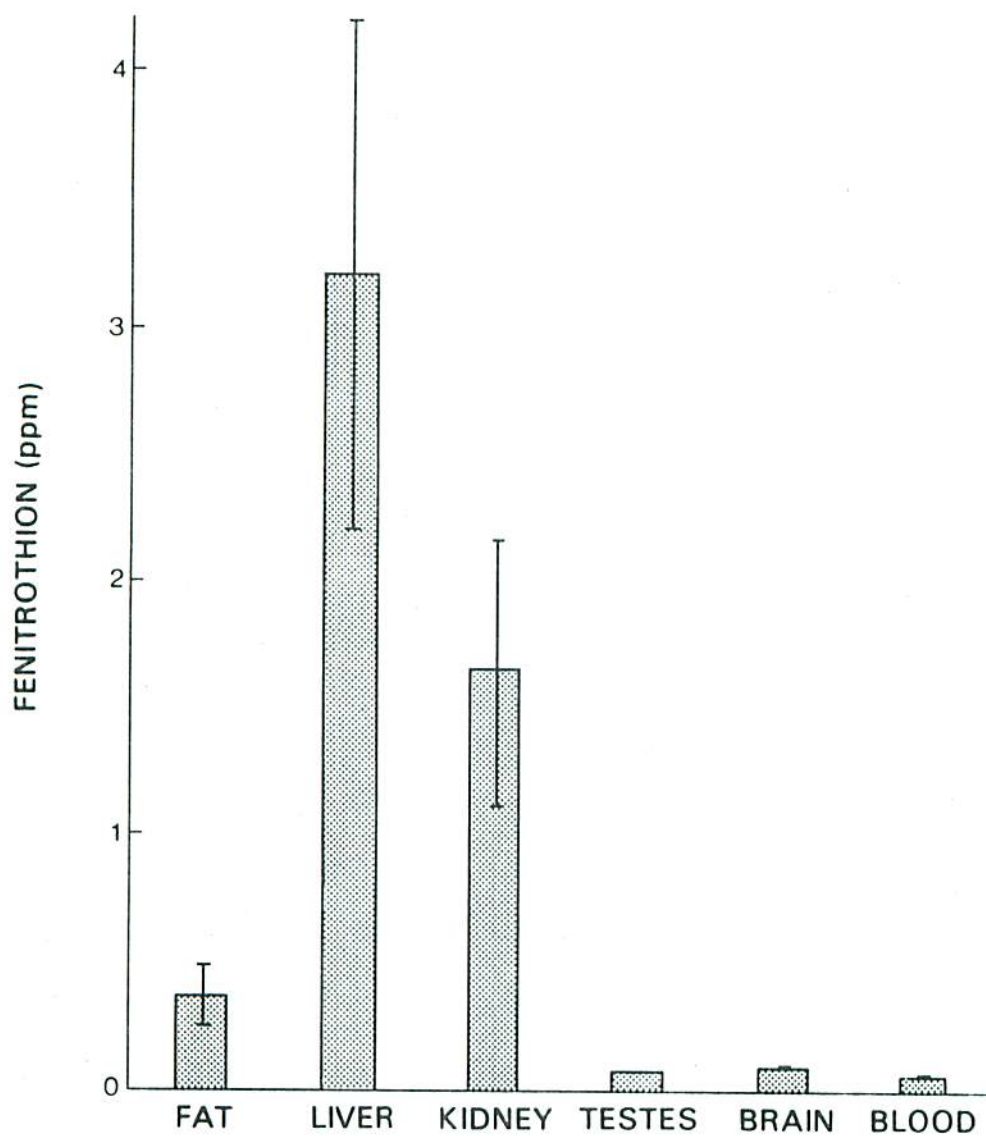Figure 14: Input-Output of Fenitrothion by Rats.

Figure 15: Concentrations of Fenitrothion in Rat Organs Fed
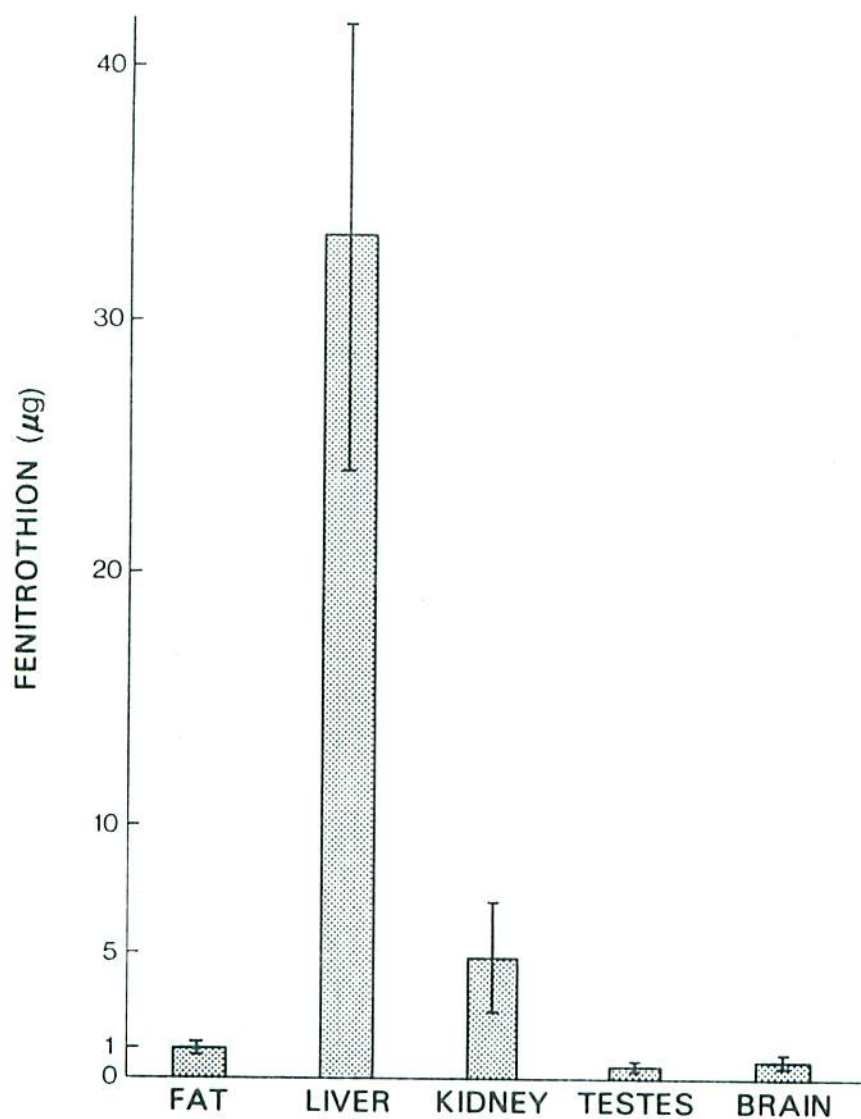With Diets Contaminated with 10 ppm of the Insecticide.

Figure 16: Absolute Amounts of Fenitrothion in each Organs of Rats after Fed with Diets Fortified with 10 ppm of the Insecticide.

DISCUSSION

White pine seeds absorbed fenitrothion from an aqueous solution during the course of stratification and germination. The fenitrothion was metabolized primarily into desmethyl fenitrothion, with lower levels of fenitro-oxon and S-methyl fenitrothion. These metabolites became evident in significant amounts in the rapidly growing embryo at later stages of germination (12 to 14 days). The experiments were carried out in the dark at neutral pH, at temperatures between 10 and 30°C; under these conditions fenitrothion is very stable (Zitko and Cunningham, 1974). These metabolites were not found in the aqueous media in which the seeds were germinated but in the seed coat, and endosperm of the seeds. The seeds were pre-sterilized to inhibit metabolism of the fenitrothion by bacteria and fungi (The primary product of microbial metabolism is amino-fenitrothion, none was found present). It is evident that the -oxon, S- and desmethyl fenitrothion were therefore formed biologically by enzymatic action in the growing embryos.

Fenitro-oxon and desmethyl fenitrothion have been shown previously to be metabolites in rice plants sprayed at the preheading stage of growth (Miyamoto et al, 1963,1965). The oxidation of thiol groups of phosphorothionate insecticides is well documented in the literature. Oxons are formed by microsomal oxidase enzymes such as lipoxidase enzymes found in wheat germ (Rowlands, 1966, 1968, 1970; Little and O'Brien, 1968). Activity is linked to electron transport (cytochrome P 450) and with the normal aerobic peroxidation of un-saturated fats in the embryo (Gardner and Inglett, 1968).

O-dealkylation of organophosphorous insecticides to the corres-

ponding diester is recognized as one of the main mechanisms for detoxi-
cation in animals, and has been implicated in the development of resist-
ance by certain insects to organophosphorous insecticides. Bull (1972)
indicated three possible biochemical mechanisms by which O-dealkylation
could take place. The first involves an oxidative process mediated by
NADPH requiring microsomal enzymes. Donninger et al (1966) showed that
chlorfenvinphos was desethylated by microsomal preparations of rabbit
liver, requiring NADPH and oxygen to give desethyl chlorfenvinphos and
acetaldehyde. Hollingworth (1969) showed that dealkylation of fenitro-
thion did not require microsomal oxidase enzymes requiring an energy
source.

The second mechanism involves a glutathione dependent alkyl
transferase enzyme. Fukami and Shishido (1963) showed that methyl para-
thion was demethylated by the supernatant fraction of rat liver microsomal
preparations. Hutson et al (1967) showed the reaction involved alkylation
of the reactive SH of glutathione by isolating S-methyl glutathione and
desmethyl gardona using pig liver supernatant. Miyamoto et al (1968)
showed that both fenitrothion and fenitro-oxon were dealkylated by rat
liver homogenase in vitro. Hollingworth et al (1973) further showed that
the glutathione S-alkyl transferase enzyme found in liver homogenase favour
methyl versus ethyl esters.

The third mechanism involves hydrolytic cleavage of the alkyl
phosphate bond to give the O-dealkylated product and an alcohol. Nolan
and O'Brien (1970) found ethanol as a major metabolite from the in vivo
dealkylation of paraoxon by certain strains of house flies.

A glutathione transferase system has been shown in plants for

the detoxification of triazine herbicides such as atrazine. An atrazine-glutathione conjugate has been shown to form in corn (Shimabukuro et al, 1970) and sorghum leaf sections (Lamoureux et al, 1970).

In the germinating pine seeds treated with $OC^{14}H_3$ labelled fenitrothion, $S-C^{14}H_3$ glutathione was recovered along with fenitro-oxon, and S-methyl fenitrothion which were similarly labelled with C^{14} radio-activity. The tree seedlings were capable of O-dealkylation of fenitro-thion via the glutathione S-alkyl transferase system to form desmethyl fenitrothion and S-methyl glutathione as products. They were also cap-able of activating the pesticide into the more toxic oxon form. The bio-logical formation of the toxic S-alkyl isomer has not however been shown previously.

S-alkyl isomers of organophosphorous insecticides are readily formed by a number of reactions. Artificial irradiation of parathion with u.v. light produced bis paraoxon and S-ethyl parathion (Cook and Pugh, 1957). Joiner and Baetcke (1974) isolated 10 more products on irradiation of parathion. Irradiation by sunlight of the pesticide on cotton leaves sprayed in the field produced both S-ethyl, and S-phenyl parathion (Joiner and Baetcke, 1973). All seed germination studies were carried out in the dark. Therefore in the present study, isomerization by irradiation was impossible.

Heat isomerization is another method of forming S-alkyl isomers. Jaglan and Gunther (1970) showed that methyl parathion would yield 50% of the S-methyl isomer after 7 hours at 125°C. Such extreme temperatures were not approached during the germination studies.

A third method employs the action of alkylating agents. Kovacicova et al (1973) used dimethyl sulphate to alkylate the potassium salt of

desmethyl fenitrothion to prepare S-methyl fenitrothion.

Methyl phosphate esters are also alkylating agents. Eto et
al (1968) reported that when fenitrothion was left in dimethyl formamide
for 30 days, half of the parent compound disappeared and both desmethyl
fenitrothion, and S-methyl fenitrothion were produced. They showed that
a mixture of fenitrothion and desmethyl fenitrothion in acetone produced
16% S-methyl fenitrothion after 14 hours at 60°C. A similar study showed
a higher recovery of S-methyl fenitrothion when the two reactants are in-
cubated in DMSO, a more polar solvent. Fenitro-oxon also exhibited the
ability to realkylate desmethyl fenitrothion. The alkylation reaction is
second order and as such is concentration dependent.

Metabolic and detoxication pathways of fenitrothion in pine
seeds are shown in Figure 17. Primary deactivation probably occurs through
0-dealkylation by means of a glutathione dependent S-alkyl transferase
to produce desmethyl fenitrothion, and in addition, hydrolysis involving
cleavage of the P-0 aryl bond occurs. Fenitrothion was activated to the
more toxic fenitro-oxon. Activation may also take place by alkylation
of desmethyl fenitrothion by fenitrothion to give S-methyl fenitrothion.

Birch seedlings treated in a similar fashion absorbed consider-
ably more fenitrothion than pine. This is likely due to their small size.
Equivalent amounts by weight of birch seeds would present a larger sur-
face area for adsorption of the pesticide than would pine or spruce.
Significantly higher concentrations of toxic fenitro-oxon were found early
in germination of birch. Higher amounts of desmethyl fenitrothion were
also produced. The higher rate of absorption and metabolism of fenitro-
thion at high concentrations may account for the toxicity to the birch
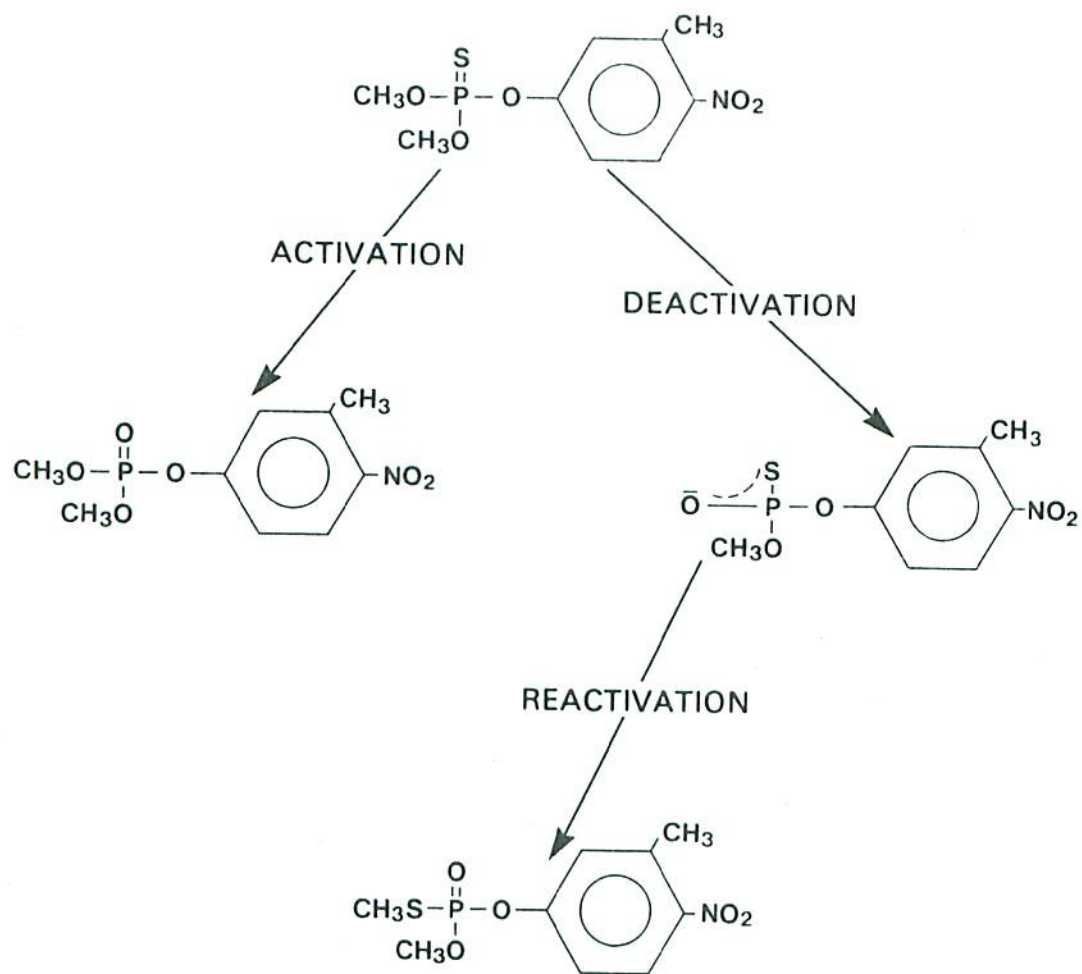
Figure 17: Metabolic Pathways of Fenitrothion in White Pine Seeds.

seeds during germination. The higher amount of pesticide absorbed may affect the plant directly via chlolinesterase inhibition or by providing more substrate for metabolism. High fenitro-oxon levels may also affect germination as an antichlolinesterase agent or by affecting microsomal enzymes such as lipoxidases. O-dealkylation could be the major inhibiting factor. Glutathione levels are severely depleted in the birch treated with 1000 ppm of fenitrothion. Glutathione has been implicated in the induction of enzyme synthesis key metabolic pathways in both plant and animal cells (Webb, 1966; Boyland and Chasseaud, 1969). The higher glutathione levels found in the two coniferous species were affected only slightly by treatment with 1000 ppm fenitrothion.

Neither fenitrothion, nor any of the derivatives tested caused mutagenic activity in the bacterial strain sensitive to alkylators. In animal tissues, the pesticide should not therefore exhibit the teratogenic and carcinogenic effects such as those exhibited by the fungicide captan (Verret et al, 1969).

Fenitrothion was absorbed by rats fed a diet treated with 10 ppm fenitrothion. Significant amounts of the pesticide were found in the liver and kidney. No toxic or non-toxic metabolites were however found in detectable levels in the organ tested. A considerable amount of the pesticide was detoxified by O-dealkylation in the kidney and was excreted as desmethyl fenitrothion in the urine. The excretory system of the animal was more efficient at disposing of this water soluble metabolite than were the germinating seedlings. It seems that desmethyl fenitrothion does not persist long enough in the animal to be realkylated to form the more toxic S-methyl isomer found in the germinating seeds.

The rats did accumulate a significant amount of pesticide in the parent form during the 7 day feeding experiment, but neither the parent compound nor the metabolites had any adverse effects upon their growth and survival. Moreover, under field conditions the rodents would hardly ever be exposed to such a high concentration and therefore there is no likelihood of any deleterious impact resulting from operational sprayings.

SUMMARY AND CONCLUSIONS

Fenitrothion (0,0-dimethyl-0-(4-nitro-m-tolyl) phosphorothioate
was absorbed by germinating seeds of eastern white pine; *Pinus strobus* L.,
white spruce; *Picea glauca* Voss/Meunch, and yellow birch; *Betula
alleghaniensis* Britt, from an aqueous solution containing 10 ppm of the
pesticide. The fenitrothion was detoxified in white pine via a glutathione
dependent S-alkyl transferase enzyme to form desmethyl fenitrothion and
S-methyl glutathione. The pesticide was also activated to form fenitro-
oxon, which is more toxic than the parent compound. The desmethyl form
was also shown to be reactivated via alkylation by fenitrothion to form
S-methyl fenitrothion which is also a more potent cholinesterase inhibitor
than the parent compound.

Treatment at higher concentrations of 100 ppm fenitrothion
inhibited germination of yellow birch seeds but not white pine or white
spruce. Severe depletion of tissue glutathione levels was shown to be
related to toxicity in yellow birch which absorbed much higher levels of
pesticide than the two coniferous species.

Fenitrothion and seven derivatives of the compound were tested
for mutagenicity via alkylation of replicating DNA in an indicator strain
of *Salmonella typhimurium*, sensitive to base-pair substitutions caused by
alkylating agents. No mutagenic activity was evident for fenitrothion or
its derivatives in this system.

Young rats were fed a diet containing 10 ppm of fenitrothion
for 7 days. No differences in feeding and drinking habits or weight
gain were shown between control and treated rats. Fenitrothion was
detoxified in the kidney via 0-dealkylation and desmethyl fenitrothion

was excreted in the urine. No detectable metabolites were evident in the brain, liver, kidney, omental fat, blood, or testes. Unaltered fenitrothion was detected however in all of these tissues. Significantly high levels were found in liver and kidney. There was no apparent physiological effects of the pesticide on the animals. Because of the nature of these investigations which were carried out under laboratory conditions, no extrapolation can be made to explain the impact of fenitrothion sprayings under field conditions. However, it can be safely concluded that at the normal dosage applied in the forests (4 ozs/acre or ca. 10 ppm), fenitrothion is least likely to cause any environmental problems to major ecosystems inhabiting the mixed forests of Canada.

ACKNOWLEDGEMENTS

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