

THE FATE OF ENDOSULFAN (THIODAN[®]) DEPOSITED ON
PLANT LEAVES AND GLASS SURFACES

by

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ABSTRACT

Fates of the two endosulfan isomers and three related compounds were investigated under controlled and semi-controlled environmental conditions. Glass plates, bean plants, and sugar beet plants were used as substrates upon which the compounds were deposited and the rate of dissipation from each surface was measured.

Metabolism of the five compounds was investigated and was found to be greater under semi-controlled environmental conditions where temperatures and humidities were higher. In the laboratory where the experimental environment was closely controlled, only traces of any metabolites were observed.

Penetration of endosulfan I, II, ether, diol and the sulfate into plant tissue and translocation of the five compounds from the leaves into the roots was observed in both bean and sugar beet plants. The greatest amount of endosulfan translocation occurred in those plants exposed to higher temperatures and humidities.

INTRODUCTION

Endosulfan (Thiodan[®]), 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide, is widely used in the control of various insect pests on fruits, vegetables, and forage crops (Gunther and Jeppson 1960). The technical material is a mixture composed of 10% endosulfan ether and diol; the remaining 90% is a mixture of two stereoisomers: endosulfan I and II with melting points between 108°-110°C and 208°-210°C respectively (Reimschneider 1963). Another compound known to occur as a metabolite of endosulfan is the sulfate.

Residue research has shown that a significant quantity of technical endosulfan, applied to the surfaces of fruits, berries, and fruit tree leaves, is converted to endosulfan sulfate (Harrison, Holmes, Roburn, and Tatton 1967; Harrison, Ruzicka, and Tatton 1967). The sulfate has also been identified as a metabolite commonly found after treatment of alfalfa with the technical material and according to Ware (1967) traces of endosulfan ether and diol were also found to occur in the residues on alfalfa plants. Ware also found that endosulfan disappears from field crops at a logarithmic rate as do other similar organochlorine insecticides. Cassil and Drummond (1965) attempted to find residues of endosulfan I and II and an

unknown metabolite on the inner portions of several food crops but concluded that none of the three compounds penetrated the cuticular surface of the fruits and vegetables.

Since little reference is made in the literature to the fate of endosulfan under controlled conditions, the following research was undertaken to determine fates of the five known endosulfan compounds applied to glass and leaf surfaces maintained under conditions of constant light intensity, temperature, and humidity. Loss-rate curves were determined for each compound from the surfaces and the occurrence of metabolites was investigated. Penetration of the compounds into plant tissue and translocation of the endosulfan from the leaves to the roots was determined.

Residue levels, metabolism, penetration, and translocation were investigated in endosulfan treated plants maintained under semi-controlled conditions where temperatures and humidities were relatively high. The fate of endosulfan weathered in both environments was compared.

MATERIALS AND METHODS

Chemicals

The two endosulfans and three related compounds were obtained from Niagara Chemical Co., Middleport, New York. Purity of each compound was: diol 100%, ether 100%, sulfate 100%, endosulfan I 99.3%, and endosulfan II 97.8%. Nuchar-Attaclay was obtained from Varian Aerograph Co., Walnut Creek, California. Reagent grade hexamethyldisilazane and trimethylchlorosilane were purchased from Pierce Chemical Co., Rockford, Illinois. Triton X-150 was obtained from Rohm and Haas Co., Philadelphia, Pennsylvania. Baker analyzed grade ethylene glycol was obtained from J. T. Baker Chemical Co., Phillipsburg, New Jersey. High purity acetone, alcohol, ether, and hexane were obtained by re-distilling, in an all glass apparatus, pure grade solvents purchased from local distributors. All other chemicals used in the experiment were obtained from commercially available sources and were of reagent grade.

Plants

Two plant species were used in this study. Bean plants, Phaseolus sp., and sugar beet plants, Beta vulgaris L., were grown from seed in the laboratory under insecticide-free conditions. Six week old bean plants and

16 week old sugar beet plants, of uniform size, were selected for treatment with endosulfan.

Air Scrubbing Apparatus

The arrangement of the air scrubbing apparatus is shown in Figure 1. Treated petri dish halves or plants were enclosed in the sample chamber and scrubbed with 26°C air at a rate of 2 liters per minute. Air from the chamber was pulled through two 250 ml air scrubbing bottles (Pyrex 16405) fitted with coarse fritted disks. Each air scrubber was charged with 200 ml of ethylene glycol which served as the insecticide trapping medium. Gelman pressure/vacuum pumps (No. 13152) were used to pull the air through the systems and Matheson air flow meters (No. R-2-15-B) were used to monitor the flow rates.

Constant temperature ($26^{\circ}\text{C} \pm 1^{\circ}\text{C}$) was maintained by conducting the experiment in a Percival climate control cabinet (No. PGW-132); the temperature was recorded on an Electric Auto-Lite recording thermometer (Model 1000).

Humidity was maintained at approximately 33% by passing the incoming air through two 250 ml Erlenmeyer flasks containing 75 ml of a magnesium chloride solution, 140 gm MgCl_2 /50 ml distilled water (O'Brien 1948). The humidity was monitored by exposing strips of Whatman #1 filter paper, impregnated with cobalt chloride (12.5 gm CoCl_2 /50 ml distilled water), to the atmosphere inside

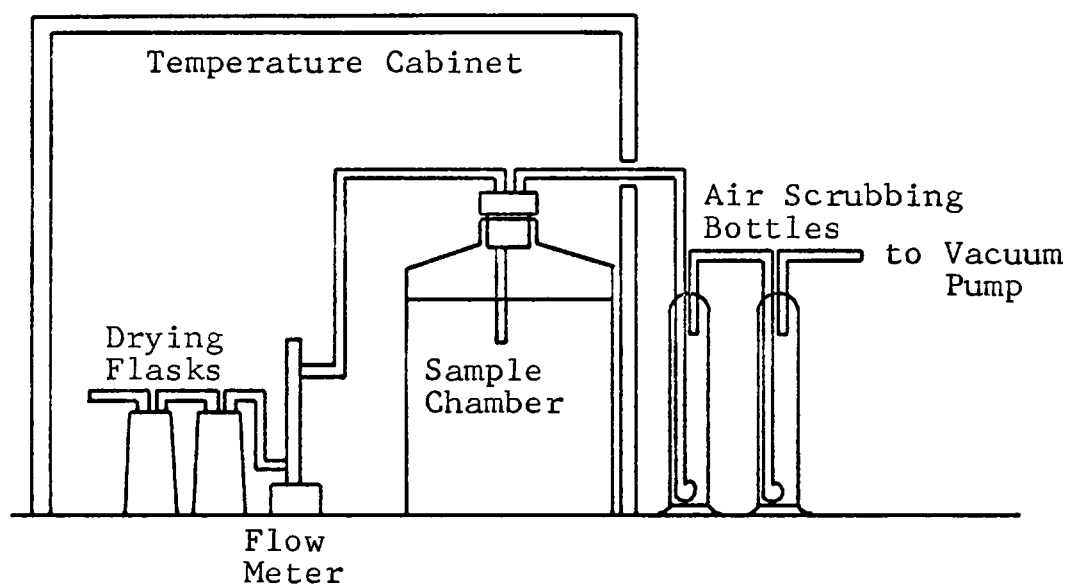


Figure 1. Air Scrubbing Apparatus

Air is introduced through two drying flasks into the sample chamber. The insecticide laden air is pulled from the sample chamber into two air scrubbing bottles where the insecticide is absorbed in ethylene glycol.

the sample chamber and then comparing those papers to similar papers maintained in atmospheres of known humidity (Solomon 1945).

Sample chambers containing plants were lighted by four 4 ft. Sylvania GRO-LUX bulbs. The chambers containing glass plates were kept in darkness throughout the experiment.

The plants were watered at four day intervals by pipetting 100 ml of water onto the soil without wetting the leaf surfaces.

Scrubbing Efficiency

Scrubbing efficiency was determined by measuring the per cent recovery of 100 ug deposits of each endosulfan compound from the sample chambers and air scrubbing bottles. One milliliter of a hexane solution (0.1 ug/1.0 ul) of each compound was deposited in separate petri dish halves, air dried, and sealed in separate sample chambers. The temperature was maintained at 26°C with no air movement through the chamber. After 16 hours each chamber was opened and the petri dish and chamber walls rinsed with 50 ml of acetone followed by 250 ml of hexane. The solvent was combined and stored.

Five air washing bottles were each filled with 200 ml of ethylene glycol and charged with 100 ug of an endosulfan compound. The bottles were sealed, permitting

no air movement, and the temperature adjusted to 26°C. After 16 hours, aliquots of the trapping solvent were removed and stored.

The ability of ethylene glycol to retain endosulfan after initial trapping was also determined. Five air scrubbers, filled with 200 ml of ethylene glycol, were treated with 100 ug of an endosulfan compound. Each air scrubber was connected, in tandem, to a second scrubber filled with 200 ml of the trapping solvent. After 8 hours of scrubbing with 26°C air at 2 liters/min, aliquots of ethylene glycol were removed from each air scrubbing bottle and stored.

Treatment of Glass Plates and Plants for Exposure in Controlled Environments

Pyrex glass petri dishes, 9 cm. in diameter, were treated by applying 1000 ug of one endosulfan compound to each dish. The compounds were dissolved in hexane (1 ug/ul) and 1.0 ml of the solution was pipetted into a dish, spread evenly over the surface, and evaporated with a gentle jet of dry air.

Treated plants were prepared by immersing the above-soil portions in an emulsion of endosulfan dissolved in acetone and diluted in distilled water. An emulsifier, Triton X-150 was added:

<u>Compound</u>	<u>Weight (gm)</u>	<u>Triton X-150 (ml)</u>	<u>Acetone (ml)</u>	<u>Final volume (ml)</u>
Isomer I	0.6105	0.225	2.0	61.0
Isomer II	0.6007	0.225	2.0	60.0
Ether	0.6017	0.225	2.0	60.2
Diol	0.5994	0.225	2.0	59.9
Sulfate	0.1568	0.050	2.0	15.7

Three plants of each species were immersed in an emulsion of one of the compounds, drained, and air dried. Three untreated plants of each species were maintained throughout the study as controls.

Treatment of Plants for Exposure in
Semi-controlled Environments

A second group of laboratory grown bean plants and field grown sugar beet plants were treated with the following endosulfan emulsions:

<u>Compound</u>	<u>Weight (gm)</u>	<u>Triton X-150 (ml)</u>	<u>Acetone (ml)</u>	<u>Final volume (ml)</u>
Isomer I	0.5147	5.0	2.0	250.4
Isomer II	0.5055	5.0	2.0	245.8
Ether	0.5059	5.0	2.0	245.9
Diol	0.5222	5.0	2.0	254.1
Sulfate	0.5108	5.0	2.0	248.4

Six plants of each species were immersed in one of the five solutions, drained and air dried. Six untreated plants of each species were maintained as controls. The

potted plants were placed in one inch deep steel trays and weathered in a greenhouse in which the temperature and humidity were relatively high. At four day intervals, the trays were filled with water, exercising care not to wet the plant leaves.

Sample Collection

Samples were collected from the air scrubbing bottles by removing aliquots of the trapping solvent from each bottle. The air scrubbers used to trap vapors from the glass plates were sampled at 0, 1, 2, 4, 6, 12, and 24 hours and again at 2, 4, 7, and 10 day intervals. After each 10 ml sample was removed, the volumes in the scrubbers were restored to 200 ml by adding clean ethylene glycol.

The air scrubbers used to collect endosulfan from the scrubbed plants were sampled by removing the entire volume of solvent from each bottle at 0, 1, 4, 8, 12, and 16 day intervals. The scrubbers were rinsed with acetone and again filled with 200 ml of clean ethylene glycol.

Upon completion of a scrubbing experiment, the sample chamber was opened and rinsed with hexane. Treated petri dishes were also rinsed with hexane and the rinse from the chambers and dishes collected in separate glass bottles and stored. Scrubbed plants were clipped at ground level and weighed before rinsing the leaf surfaces for several minutes in hexane. The leaves were then air dried

and stored. A 100 gm sample of soil was removed from the plant roots and stored in a polyethylene bag. The roots were then washed, dried, and weighed before storage.

The greenhouse weathered plants were sampled at 0, 1, 4, 8, 12, and 16 day intervals by harvesting one plant of each species from each of the treatments. The plants were weighed and rinsed with hexane before storage. A soil sample was collected and the roots were washed, dried, weighed, and stored.

Sample Storage

Solvent samples from the scrubber efficiency study, ethylene glycol from the air washing bottles, and the leaf surface rinses were stored in glass bottles and refrigerated. Plant material samples and soil samples were sealed in polyethylene bags, labeled, and frozen.

Extraction of Samples

Samples containing endosulfan diol were treated with a silane reagent (pyridine, hexamethyldisilazane, and trimethylchlorosilane mixed in the ratio 20:2:1) as described by Ludwig and Korte (1965). Solvents from the air scrubbing bottles and the plant surface extracts were mixed with equal volumes of the reagent, warmed to 50°C, and allowed to stand for one hour at room temperature. Water was then added and the samples extracted three times

with 50 ml volumes of hexane. The hexane extract was consolidated and dried over anhydrous sodium sulfate.

Plants having diol residues were macerated in an Omni mixer with 200 ml of hexane, 100 ml of 95% alcohol, and 100 ml of petroleum ether for three minutes. Each sample was then treated with the silane reagent and an aliquot cleaned with Nuchar-Attaclay (Burke and Mills 1963; Butler, Maitlen, and Fahey 1962; Coulson and Barnes 1963).

An endosulfan diol standard (1 ug/ul) prepared in hexane was silane treated. Subsequent standard solutions used in the analytical procedures were diluted from this stock solution.

Solvent samples containing endosulfan I, II, ether, and sulfate were not silane treated. These samples were extracted in hexane, 3 parts hexane to 1 part sample, dried, and stored. The plant samples not treated with diol were not treated with the silane reagent but were extracted, dried, and stored.

Extraction recoveries were conducted for each of the endosulfan compounds. Measured quantities of the materials were added to ethylene glycol and extracted with hexane. Per cent recoveries were calculated for each compound.

Gas Chromatography

Sample volumes were adjusted by evaporation or dilution with hexane before injection into a Jarrell-Ash Model 700 Universal Chromatograph with a tritium source, electron capture detector. Injection block, column, and detector temperatures were 220°C, 140°C, and 210°C, respectively. A 2' x 1/4" ID Pyrex glass column, packed with 5% SF-96 on 60/80 mesh, iron free, Chromosorb W, was used to separate the compounds. The samples were injected from a 10 ul Hamilton syringe and eluted from the chromatographic column with dry nitrogen at 50 ml/min.

RESULTS AND DISCUSSION

Development of an Apparatus for the Recovery of Endosulfan

Before investigating the fate of endosulfan under controlled conditions, it was necessary to develop an apparatus from which the insecticide and its metabolites could be quantitatively recovered. A large sample chamber was chosen to accommodate the plants and several sizes of air scrubbing bottles with different trapping solvents were compared before selection of a 250 ml trap charged with ethylene glycol. This scrubber was a convenient size and afforded adequate recovery of the insecticide. Evidence of high recovery of the compounds from the sample chamber portion of the system is presented in Table 1. As seen from these data, an average recovery of two 100 ug deposits of each compound range from 89.7% for the diol to 108.1% for endosulfan II.

Recovery of endosulfan from the air scrubbing bottles was measured under both static, no air movement, and dynamic conditions, 26°C air pulled through the scrubbers for 8 hours at a flow rate of 2 liters/min. Tables 2 and 3 show that under both conditions the recovery of each compound was 90% or greater. Table 3 also shows that a small amount of the original deposit of endosulfan from

Table 1. Average values for two recoveries of endosulfan and related compounds from sample chambers.

Expt. No.	Endosulfan compound	Treatment ug	% Recovery	Average % recovery
1	Isomer I ^a	100	101.3	97.0
2	Isomer I	100	92.6	
3	Isomer II ^b	100	107.1	108.1
4	Isomer II	100	109.1	
5	Ether	100	108.1	105.0
6	Ether	100	101.9	
7	Diol	100	91.5	89.7
8	Diol	100	87.9	
9	Sulfate	100	102.0	97.9
10	Sulfate	100	93.8	

^aLow melting point isomer (108°-110°C).

^bHigh melting point isomer (208°-210°C).

Table 2. Average values for two recoveries of endosulfan and related compounds from gas scrubbing bottles.^a

Expt. No.	Endosulfan compound	Treatment ug	% Recovery	Average % recovery
1	Isomer I	100	105.0	104.5
2	Isomer I	100	104.0	
3	Isomer II	100	91.2	91.6
4	Isomer II	100	92.0	
5	Ether	100	97.0	98.5
6	Ether	100	100.0	
7	Diol	100	90.4	91.3
8	Diol	100	92.1	
9	Sulfate	100	99.3	97.3
10	Sulfate	100	95.3	

^aNo air movement through gas scrubbing bottles.

Table 3. Retention of endosulfan and related compounds by ethylene glycol^a in gas scrubbing bottles.^b

Expt. No.	Endosulfan compound	Treatment ug	% Recovery	
			Air scrubber 1	Air scrubber 2
1	Isomer I	100	90.0	trace ^c
2	Isomer II	100	112.0	trace
3	Ether	100	96.3	0.07
4	Diol	100	94.2	0.10
5	Sulfate	100	106.0	trace

^a200 ml of ethylene glycol per gas scrubbing bottle.

^b26°C at 2 liters/min pulled through scrubber 1 into scrubber 2 for 8 hours.

^cIndicates peak height on gas chromatogram less than 2 mm.

scrubber 1 was transferred to scrubber 2 where it was trapped. The quantity of the diol and ether present in the second scrubber was small but measurable (0.10 and 0.07% respectively) but only traces of the sulfate, isomer I, and isomer II were found.

Next, a hexane-water extraction procedure was developed to recover the ether, isomer I, isomer II, and sulfate from the ethylene glycol trapping solvent. It was found that the volume of hexane used to extract the compounds from the trapping solvent was not critical if the ratio of hexane to ethylene glycol was 3:1 or greater.

Recovery of the diol posed a different problem. Because of the unstable nature of this compound and the ease of chemical substitution at the -OH sites, it was necessary to silanize all diol samples before the extraction with hexane to insure high recovery of the material from the trapping solvent. If the extraction was attempted before the silanizing process, the effective recovery was from 20 to 30% of the initial deposit but was increased to above 60% after treatment. The silanizing process also caused pronounced changes in the chromatographic response of the endosulfan alcohol. Before treatment, several peaks with variable heights and retention times were observed. After silanizing, however, only one peak with reproducible response and constant retention time resulted as illustrated in Figures 2 and 3. An additional increase in per

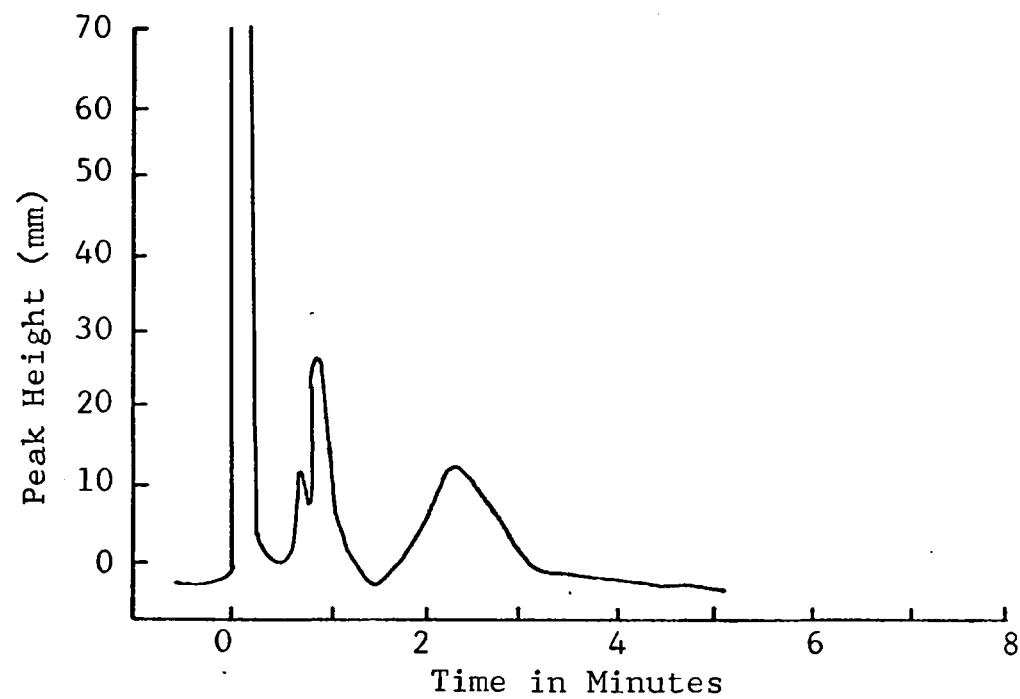


Figure 2. Gas Chromatogram of Un-silanized Endosulfan Diol
Curve represents 0.22 ng of the compound; column temperature 160°C.

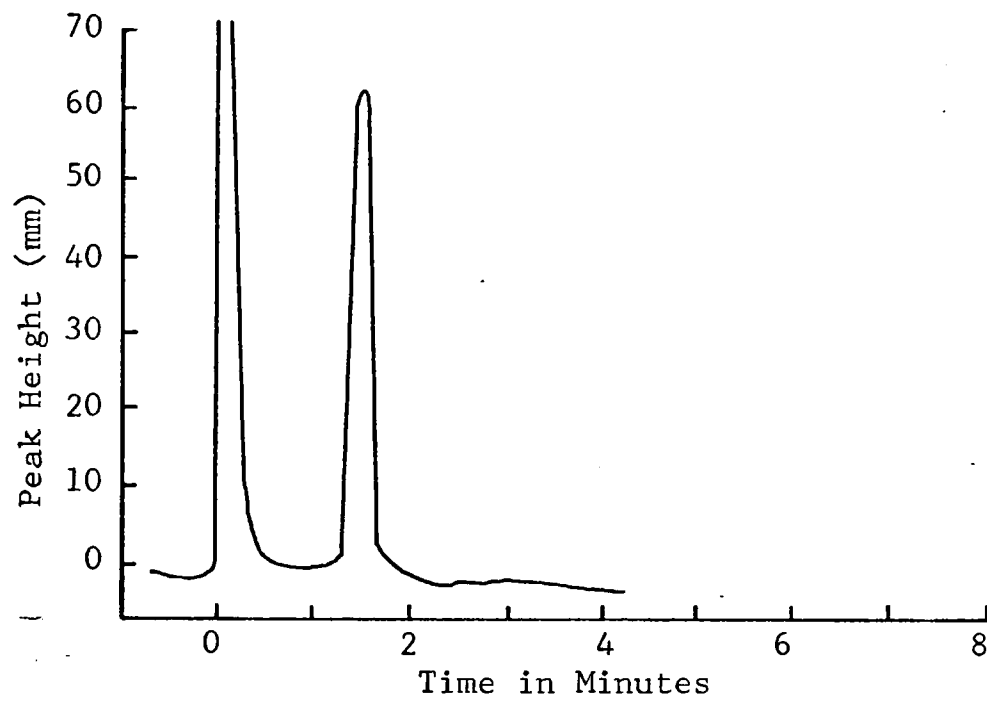


Figure 3. Gas Chromatogram of Silanized Endosulfan Diol
Curve represents 0.22 ng of the compound; column temperature 160°C.

cent recovery of endosulfan alcohol from ethylene glycol was accomplished by using 40°C distilled water in the extraction process. This increased the total recovery of the diol to above 70%.

The recovery of endosulfan and related materials from ethylene glycol was measured for each compound and the results of seven of these recovery experiments are summarized in Table 4.

Finally, the recovery of endosulfan from Nuchar-Attaclay was determined. Wet plant extracts containing known quantities of the compounds were mixed with the charcoal, filtered through glass wool, and dried over anhydrous sodium sulfate. Again it was found that silane treatment of the diol samples was necessary before addition of the Nuchar-Attaclay to insure adequate recoveries. The results of a typical experiment are summarized in Table 5.

Exposure of Endosulfan Treated Surfaces in a Controlled Environment

After development of an efficient scrubbing apparatus and establishment of suitably high recoveries of endosulfan from the sample extraction and clean-up processes, bean plants, sugar beet plants, and glass plates were treated with the endosulfan compounds and weathered in an environment where the light intensity, temperature, and humidity were held constant.

Table 4. Average values for seven extraction^a recoveries of endosulfan and related compounds from ethylene glycol.

Endosulfan compound	Range of % recoveries	Average % recovery
Isomer I	81.7-93.0	87.4
Isomer II	89.5-106.5	99.0
Ether	89.0-102.0	94.4
Diol	67.0-91.0	77.5
Sulfate	72.7-99.2	88.1

^aExtracted samples contained from 10-1000 ug of the endosulfan compounds.

Table 5. Recovery of endosulfan and related compounds from Nuchar-Attaclay

Expt. No.	Endosulfan compound	Treatment ug	% Recovery
1	Isomer I	250	90.2
2	Isomer II	250	96.5
3	Ether	250	97.8
4	Diol	100	100.8
5	Sulfate	250	93.5

The recovery data for endosulfan residues on glass surfaces is presented in Table 6 and Figure 4. Endosulfan ether, the most volatile of the five compounds studied, dissipated from the glass surface most rapidly, with 16.9% of the initial deposit remaining 10 days after treatment, followed by isomer I, 57.1% of initial deposit at 10 days. Most of the isomer II, 96.1%, remained as a residue on the glass plate. Since no diol or sulfate were found in the air scrubbers at the sampling periods, it is thought that only small amounts below analytical sensitivity were transferred from the glass surface to the air scrubbers. Analysis of solvent rinse from the sample chambers after 10 days of scrubbing showed the amount of residue of each compound remaining within the chamber.

The total recovery of each compound is also given in Table 6 and was found to be relatively high for all compounds except the diol, from which 77.9% initial deposit was recovered. The reduced recovery of this compound reflects the lower recovery efficiency of the alcohol from the extraction and clean-up processes. Another possible cause for low recovery of the diol is conversion of this compound into some unrecognizable metabolite or material which produces low response by electron capture gas chromatography.

Samples collected from scrubbing untreated glass plates show several peaks which were identified as solvent

Table 6. Residues of endosulfan on glass plates after exposure in a controlled environment.

Sample time	% Initial deposit ^a				
	Isomer I	Isomer II	Ether	Diol	Sulfate
0 hr	100.0	100.0	100.0	100.0	100.0
1	100.0	100.0	99.8	100.0	100.0
2	100.0	100.0	98.0	100.0	100.0
4	100.0	100.0	94.4	100.0	100.0
6	99.4	100.0	89.5	100.0	100.0
12	96.8	100.0	78.5	100.0	100.0
1 day	91.5	100.0	62.9	100.0	100.0
2	86.0	99.9	49.7	100.0	100.0
4	78.7	98.5	33.7	100.0	100.0
7	73.6	97.8	25.6	100.0	100.0
10	57.1	96.1	16.9	100.0	100.0
Sample chamber 10 day	43.2	91.2	14.0	77.9	101.4
Total % recovery	86.1	95.1	97.1	77.9	101.4

^aInitial deposits on glass plates equal 1000 ug. The per cent initial deposit of endosulfan remaining on the glass plates at each sample time is calculated by setting the total per cent recovery equal to 100.0%.

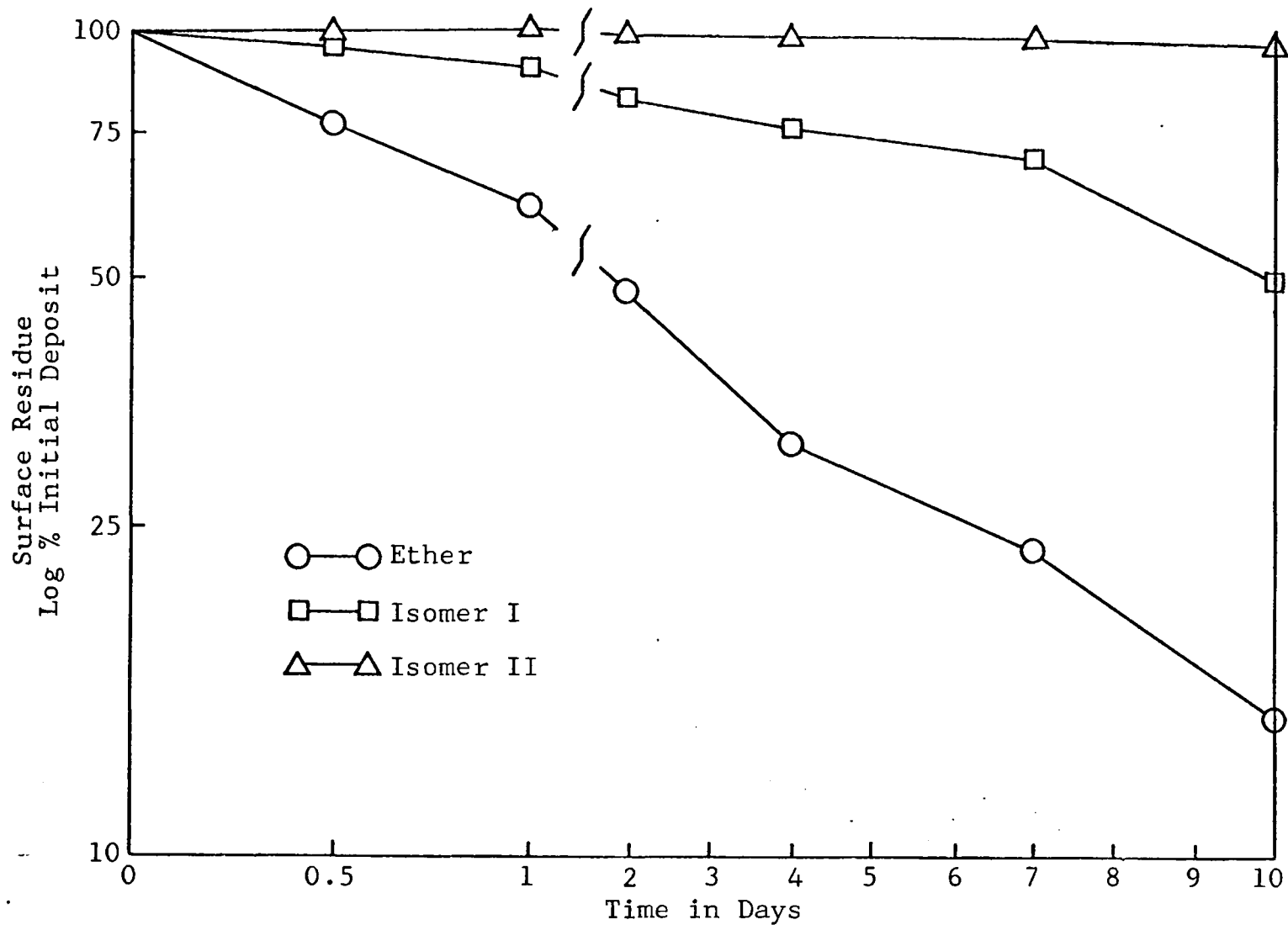


Figure 4. Loss of Endosulfan and Related Compounds from Glass Surfaces in Controlled Environments

contaminants. Since only small random unknown peaks occurred during the study, no correction for background contamination was necessary.

The per cent initial deposits of compounds on the plant surfaces are calculated using the total microgram recovery as 100%. Data from the treated bean plant scrubbing experiment are found in Table 7 and Figure 5. The dissipation pattern of endosulfan from the bean plant surfaces closely resembles that from the glass plates. Endosulfan ether is again lost most rapidly from the surfaces with 56.0% of the initial deposit remaining after 10 days, followed by isomer I, 86.2%, and isomer II, 99.2%. Again diol was not found in the scrubbing bottles and after 16 days only 0.17% of the sulfate deposit had been lost from the plants and trapped in the air scrubbers.

Data indicating loss of endosulfan from the sugar beet plants is found in Table 8 and Figure 6. The same general pattern is seen in loss of the compounds from the sugar beets as found in losses from bean plants and glass plates. The ether was most rapidly lost with 35.7% initial deposit remaining after 10 days, followed by isomer I, 58.4%. The residue of isomer II on the sugar beets at 10 days was 97.92% of the initial deposit. Small amounts of sulfate, 0.73%, and the diol, 0.02%, were found in the air scrubbing apparatus during the experiment.

Table 7. Residues of endosulfan and related compounds on bean plants after exposure in a controlled environment.

Sample time	% Initial deposit				
	Isomer I	Isomer II	Ether	Diol	Sulfate
0 day	100.0	100.0	100.0	100.0	100.0
1	99.2	99.97	95.2	100.0	99.99
4	96.6	99.82	82.9	100.0	99.95
8	92.8	99.68	71.1	100.0	99.91
12	89.2	99.47	62.3	100.0	99.88
16	86.2	99.23	56.0	100.0	99.83
Total ug ^a recovery	34016.5	29318.5	29896.3	20984.0	23576.2

^a% initial deposits calculated using total ug recoveries as 100% of the initial deposit.

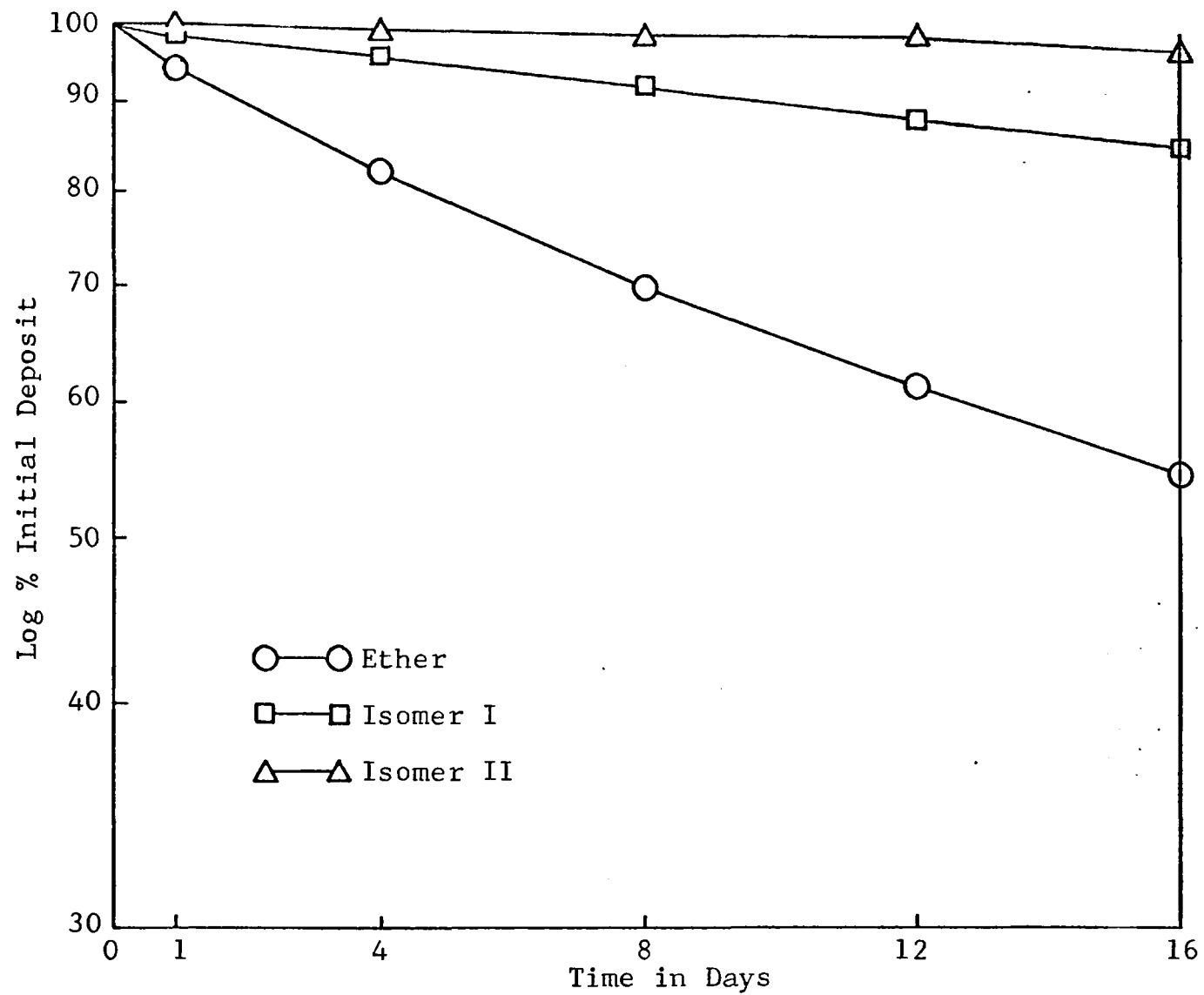


Figure 5. Loss of Endosulfan and Related Compounds from Bean Plants in Controlled Environments

Table 8. Residues of endosulfan and related compounds on sugar beet plants after exposure in a controlled environment.

Sample time	% Initial deposit				
	Isomer I	Isomer II	Ether	Diol	Sulfate
0 day	100.0	100.0	100.0	100.0	100.0
1	95.5	99.89	93.3	99.99	99.98
4	85.0	99.49	73.2	99.99	99.87
8	72.8	98.98	55.2	99.99	99.69
12	64.3	98.44	45.0	99.98	99.48
16	58.4	97.92	35.7	99.98	99.27
Total ug ^a recovery	12515.0	9402.3	17130.0	14423.5	9698.0

^a% initial deposits calculated using total ug recoveries as 100% of the initial deposit.

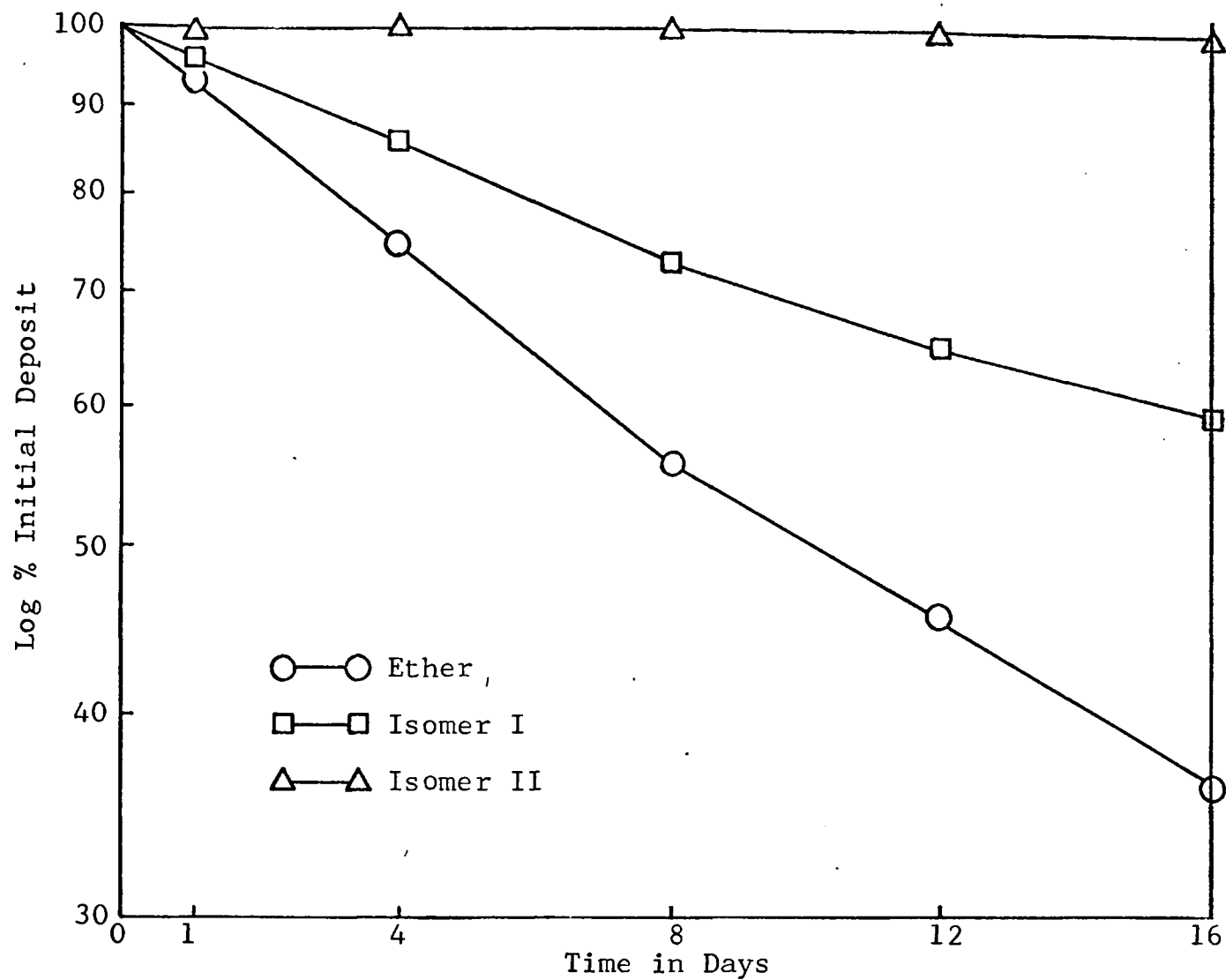


Figure 6. Loss of Endosulfan and Related Compounds from Sugar Beet Plants in Controlled Environments

A comparison of losses from the three surfaces shows that endosulfan ether is lost rapidly from all surfaces followed by isomer I and isomer II. Only small amounts of diol and sulfate were dissipated from the leaves during the scrubbing periods. Also Figures 4, 5, and 6 show that more endosulfan I, II, and ether is lost from the glass surface than from the plant surfaces. The per cent loss of endosulfan from the sugar beets was also greater than from the bean plants. It was expected that endosulfan would be lost from the glass plates faster than from the plants. It was not expected, however, that the compounds would dissipate more rapidly from the large waxy sugar beet leaves than from the bean plants.

Metabolism did not play a major role in the degradation of most endosulfan compounds on glass and plant surfaces exposed to the controlled conditions. In only one instance was a material resembling a metabolite found on glass plates. After one hour of scrubbing, a trace amount of endosulfan sulfate was found in the trapping solvent of the air scrubber used to collect vapors from endosulfan I-treated plates. However, this is thought to be a contaminant rather than a metabolite. Endosulfan sulfate, 7.66 ug, was also found in the sample chamber in which endosulfan I-treated sugar beet plants were scrubbed.

Endosulfan ether was found in the air scrubbers used to trap the vapors from endosulfan I-treated sugar

beet plants. The micrograms quantities found at each sampling time were: 0 day--0, 1 day--0.3, 4 day--0.81, 8 day--1.31, 12 day--1.38, and 16 day--1.59. As in the studies of endosulfan metabolism on bean plants done by Terranova and Ware (1963), no diol was found among the residues on the plant surfaces. A material with a retention time close to that of diol was observed but was identified as a contaminant coming from the reagents used to extract the samples. Several other peaks occurred in the air scrubber solvent and plant samples. None of these materials were identified as metabolites, however.

Penetration of endosulfan into the plant tissue and translocation of the compounds from the leaves to the roots occurred in both bean and sugar beet plants under laboratory conditions. The data from these studies are found in Table 9. With the exception of the diol, all of the endosulfan compounds were identified in the roots of both species of plants. These residues represent the amount of material translocated from the leaves to the roots and were found to be greater in sugar beets than in beans. The sulfate and endosulfan II were translocated in larger amounts than the other compounds and since these materials are also the most persistent residues on the plant surfaces, it is thought that the amount of translocation is in some way related to the length of time the materials reside on the surface. In all but the diol-treated beans, the amount

Table 9. Distribution of endosulfan and related compounds in plants exposed in a controlled environment.

Plant Location	Endosulfan (% initial deposit)				
	Isomer I	Isomer II	Ether	Diol	Sulfate
	<u>Bean</u>				
Surface ^a	72.4	75.9	41.8	35.5	90.8
Extract ^b	13.4	21.2	13.7	59.2	6.9
Roots	c	0.01	0.01	0.00	0.02
Total residue on plants	85.8	97.1	55.5	94.7	97.7
	<u>Sugar beet</u>				
Surface	51.5	88.8	32.1	65.9	83.3
Extract	2.3	7.7	2.6	28.0	14.3
Roots	0.01	0.04	0.01	0.00	0.13
Total residue on plants	53.8	96.5	34.7	93.9	97.7

^aPer cent of initial deposit rinsed from surface of plant leaves and stems.

^bPer cent of initial deposit in hexane extract of plant leaves and stems.

^cTrace amount of compound.

of residue found on the surface of the plants was greater than that found within the plant tissue and, except for the sulfate, more of the endosulfan was found to penetrate into the bean leaves than into the sugar beet leaves. This observation is not consistent with other observations where more residue was found in sugar beet roots than in bean roots.

Exposure of Endosulfan Treated Plants in a Semi-controlled Environment

A second group of bean and sugar beet plants was treated with endosulfan and weathered under semi-controlled environmental conditions in a greenhouse. The temperature range inside the study area was 49°-103°C and the humidity varied from 47-71%. No attempt was made to alter the light intensity or day length from that occurring naturally.

Residue levels on the plants were measured at four day intervals after treatment and were found to be erratic. The reasons for the non-uniformity of residue levels are thought to be due to differences in plant size at the time of treatment and the wide variation in environmental conditions in the study area. Total residues expressed as parts per million (PPM) found on bean plants are listed in Table 10. The 0-day residues represent initial deposits and were found to vary greatly. Comparison of the curves in Figure 7, however, indicates that endosulfan I is lost most rapidly from the plants followed by the ether and

Table 10. Residues of endosulfan and related compounds on bean plants after exposure in a semi-controlled environment.

Sample time	PPM				
	Isomer I	Isomer II	Ether	Diol	Sulfate
0 day	145.0	113.9	150.7	14.0	83.2
1	44.3	38.6	46.8	22.8	68.2
4	1.3	15.7	33.8	18.8	38.6
8	0.9	11.1	9.3	16.5	56.7
12	1.3	6.4	0.6	14.9	47.2
16	0.4	3.1	0.6	13.2	49.0

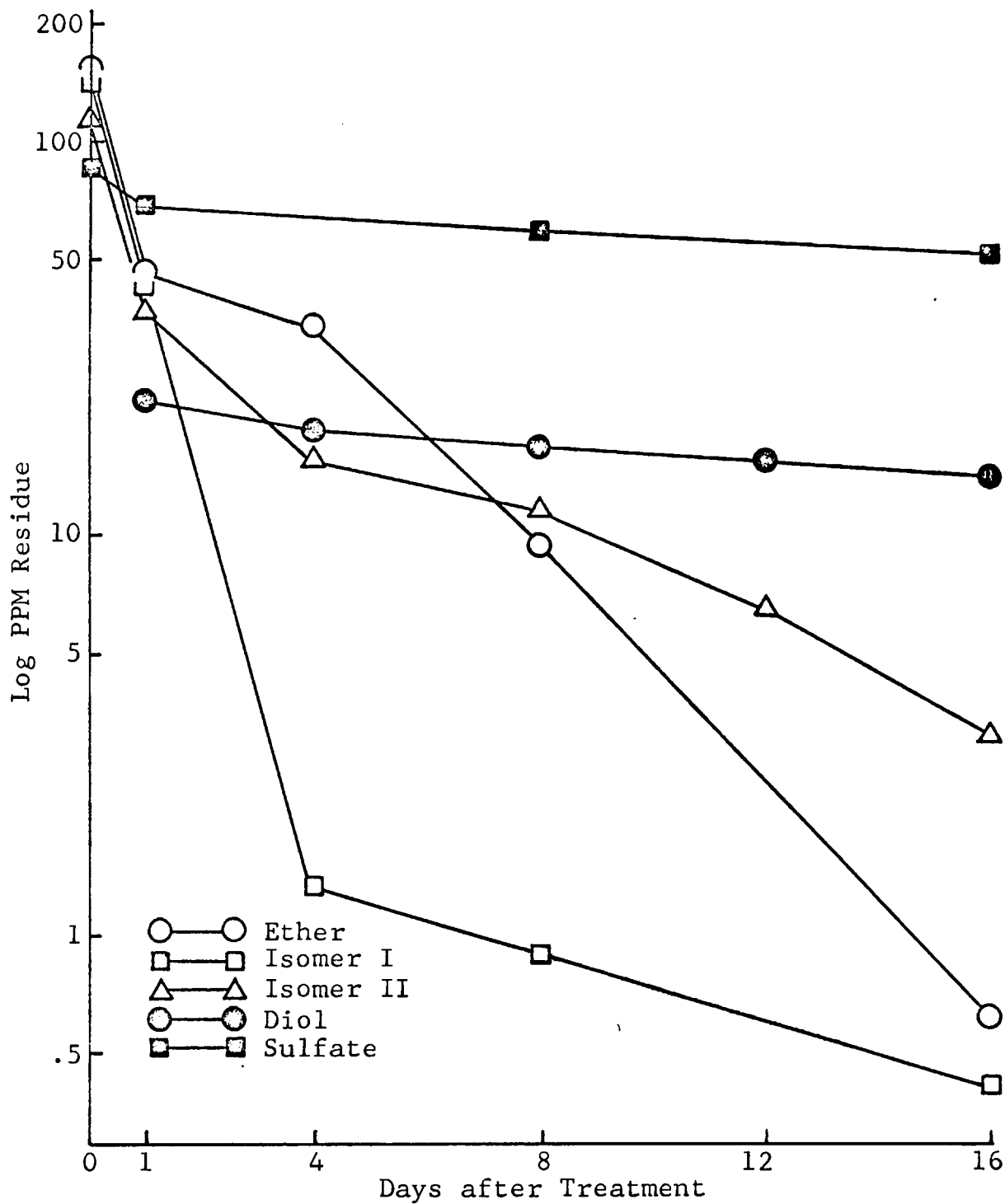


Figure 7. Loss of Endosulfan and Related Compounds from Bean Plants Exposed in Semi-controlled Environments

endosulfan II. For some unknown reasons the initial deposits of the sulfate and diol were relatively low but Figure 7 shows that the rate of dissipation of these compounds was slow with little to moderate reduction in residue levels 16 days after treatment.

Loss of endosulfan from the sugar beet plants follows much the same pattern as losses from the bean plants. Table 11 and Figure 8 show that the ether and endosulfan I residues disappear rapidly followed by endosulfan II. Diol and sulfate were found to be lost from the plants very slowly. The data show that these two compounds are lost faster from sugar beet plants than from bean plants but the rates of loss of endosulfan I, II, and ether were found to be greater from the bean plants than from the sugar beets. The order of dissipation of endosulfan from both plant surfaces, based on the residues remaining 16 days after treatment, is: endosulfan I > ether > endosulfan II > diol > sulfate.

It was found that the loss of endosulfan from the plants was greater and more rapid under the conditions of high temperature and humidity. Lyon and Davidson (1965) have found that high humidity is responsible for rapid vaporization of insecticides and it is thought that this is the major reason why losses were greater in the greenhouse than in the laboratory.

Table 11. Residues of endosulfan and related compounds on sugar beet plants after exposure in a semi-controlled environment.

Sample time	PPM				
	Isomer I	Isomer II	Ether	Diol	Sulfate
0 day	37.9	20.5	15.2	16.3	43.0
1	23.3	13.8	7.4	15.3	33.2
4	8.5	8.5	4.6	10.8	31.9
8	12.6	5.8	2.4	6.7	27.7
12	5.0	6.4	1.1	9.1	23.0
16	1.3	4.7	1.7	7.1	19.7

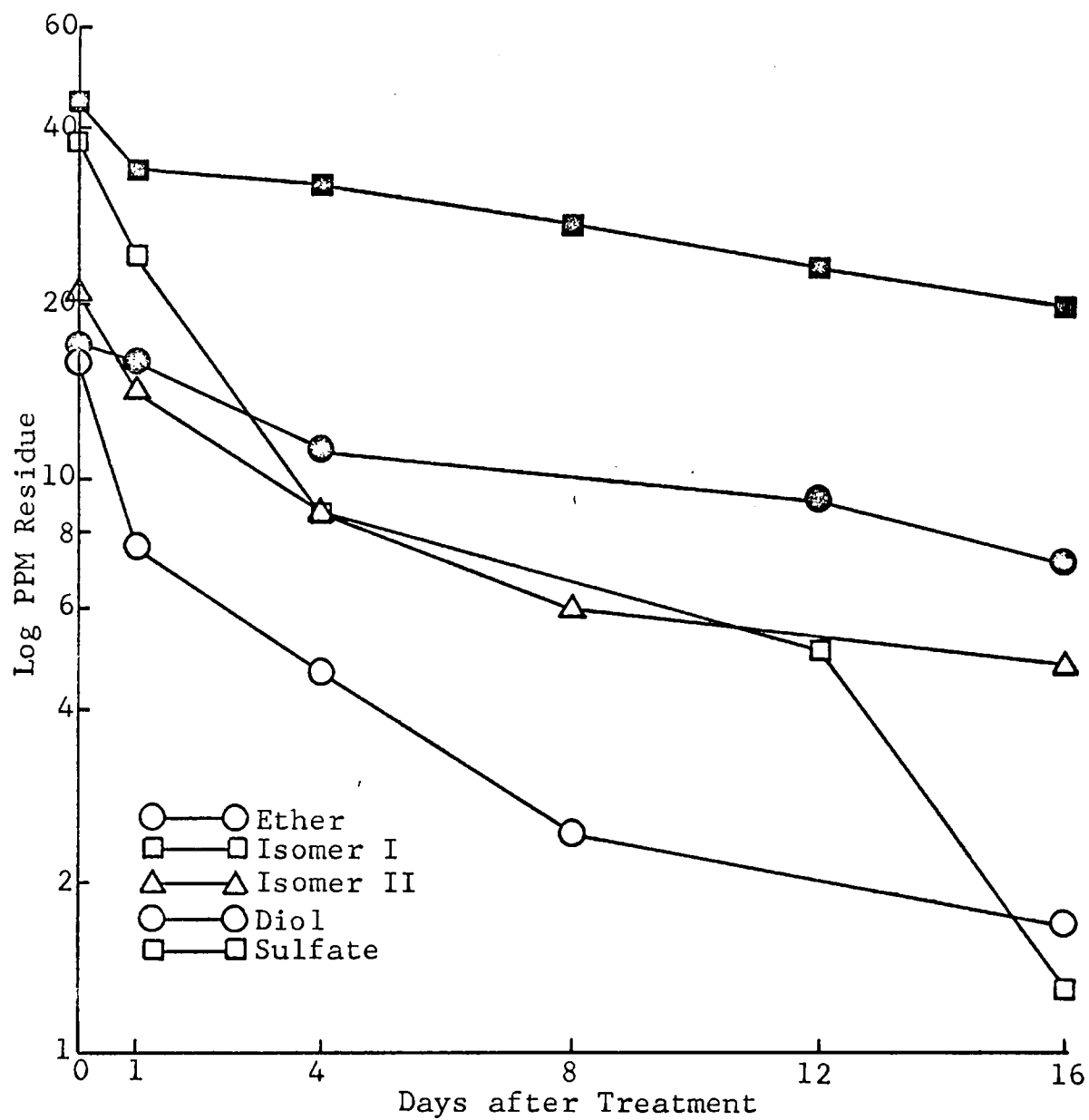


Figure 8. Loss of Endosulfan and Related Compounds from Sugar Beet Plants Exposed in Semi-controlled Environments

Metabolism was more evident under the conditions of high temperature and humidity in the greenhouse than in the milder, controlled environment. Table 12 lists the residues of endosulfan sulfate found in the extracts of endosulfan I-treated bean and sugar beet plants. Only traces of sulfate were found in the bean roots or on the bean leaf surfaces but in the sugar beets, larger residues of sulfate were found both in the roots and on the surfaces of the plants. These data indicate that the sites of metabolism are within the leaf tissue or in the roots with little conversion on the surface of the plants. Also contributing to the high level of sulfate found in the sugar beet roots is the translocation of the material to the roots from the leaves.

Two compounds were found in both bean and sugar beet plants treated with endosulfan II, Table 13. Residues of isomer I and the sulfate were found on the sugar beet leaf surfaces, in the leaf extracts, and in the roots of the sugar beet plants. Residues of the compounds were also identified from bean leaf surfaces and extracts. The bean roots, however, contained only endosulfan I. Since conversion of one isomeric form to another is unlikely, endosulfan I found in these plants was probably relocated from the endosulfan I treated plants. The sulfate, however, is considered a metabolite. Residues of endosulfan I and the sulfate metabolite were larger within the plant tissue than

Table 12. Degradation of endosulfan I on plants exposed in a semi-controlled environment.

Time days	PPM degradation products		
	Surface	Extract	Root
	Sulfate	Sulfate	Sulfate
<u>Bean plant</u>			
0	0.00	0.00	0.00
1	0.00	0.71	0.00
4	0.00	0.12	0.00
8	a	0.25	a
12	a	0.32	0.00
16	0.00	0.25	0.00
<u>Sugar beet plant</u>			
0	0.00	0.00	a
1	0.02	0.54	a
4	0.05	0.95	0.23
8	0.02	0.86	0.24
12	a	2.18	0.08
16	a	0.87	a

^aTrace amount of compound.

Table 13. Degradation of endosulfan II on plants exposed in a semi-controlled environment.

Time days	PPM degradation products					
	Surface		Extract		Root	
	Isomer I ^a	Sulfate	Isomer I ^a	Sulfate	Isomer I ^a	Sulfate
	<u>Bean plant</u>					
0	b	b	1.24	0.00	0.00	0.00
1	0.06	0.01	1.46	0.00	0.00	0.00
4	0.09	0.02	1.00	c	c	0.00
8	0.08	0.01	0.74	0.21	0.02	0.00
12	0.04	0.09	0.15	0.23	0.00	0.00
16	0.01	0.03	0.13	0.07	c	0.00
	<u>Sugar beet plant</u>					
0	b	b	0.25	0.00	0.03	c
1	c	0.00	0.38	0.00	0.02	0.01
4	c	0.00	0.21	0.02	0.05	0.01
8	0.02	c	0.20	0.06	c	c
12	0.03	0.00	0.16	0.08	0.04	c
16	0.02	0.00	0.12	0.02	0.04	0.03

^aEndosulfan I is a contaminant, not a metabolite.

^bSample not taken.

^cTrace amount of compound.

on the surface. This suggests that endosulfan I is absorbed by the plants and that the major site of sulfate metabolism lies within the plant tissue.

Table 14 presents the data collected from the degradation of sulfate on bean and sugar beet plants exposed in the semi-controlled environment. One compound, endosulfan I, was found in both plant species, the greatest amount occurring in the leaf tissue. More of this material was found on the leaf surfaces of the bean plants than on the sugar beets and only traces of the material were present in the roots. Again, endosulfan I is thought to be a contaminant rather than a metabolite.

Generally, more metabolite was observed in the tissue of the sugar beet roots than in the bean roots. Slightly more of the conversion products were found on the leaf surfaces of both beans and sugar beets than were found in the roots of the plants. The greatest amount of metabolism occurred in the leaf tissue of the plants with approximately the same amount of metabolic residue in both species.

The distribution of endosulfan residues in the bean and sugar beet plants at four day intervals for 16 days is shown in Tables 15 and 16. In both sugar beets and beans the greatest amount of residue was found in the plant extract samples, indicating considerable penetration of endosulfan into the leaf tissue. The next largest deposit

Table 14. Degradation of endosulfan sulfate on plants exposed in a semi-controlled environment.

Time days	PPM degradation products		
	Surface	Extract	Root
	Isomer I ^a	Isomer I ^a	Isomer I ^a
	<u>Bean plant</u>		
0	0.00	0.35	b
1	0.16	0.13	b
4	0.12	0.04	b
8	0.23	0.26	b
12	0.09	b	b
16	0.10	0.05	b
	<u>Sugar beet plant</u>		
0	0.09	0.08	0.00
1	0.00	0.07	0.02
4	0.05	0.18	b
8	0.03	0.13	b
12	0.04	0.18	0.00
16	0.01	0.16	b

^aEndosulfan I is a contaminant, not a metabolite.

^bTrace amount of compound.

Table 15. Distribution of endosulfan and related compounds in bean plants exposed in a semi-controlled environment.

Time days	PPM				
	Isomer I	Isomer II	Ether	Diol	Sulfate
	<u>Leaf surface</u>				
0	32.20	a	39.00	5.54	66.20
1	14.70	0.85	6.40	5.94	56.40
4	0.15	1.05	6.90	6.14	30.90
8	0.03	0.82	3.70	3.50	49.80
12	0.06	1.80	0.33	2.64	40.00
16	c	0.69	0.05	1.70	45.40
	<u>Leaf extract</u>				
0	113.00	113.80	111.90	8.65	17.00
1	29.60	37.70	40.20	16.90	11.80
4	1.15	14.30	26.70	12.70	7.66
8	0.85	10.10	5.50	13.00	6.64
12	1.24	4.20	0.26	12.30	5.33
16	0.36	1.70	0.53	11.50	3.65
	<u>Roots</u>				
0	0.00	0.00	b	0.00	0.00
1	0.00	0.00	0.26	0.00	0.01
4	0.00	0.28	0.18	0.00	0.08
8	0.05	0.19	0.08	0.00	0.15
12	0.00	0.38	c	c	1.98
16	0.05	0.63	c	c	0.10

^aSample not taken.

^bSample lost.

^cTrace amount of compound.

Table 16. Distribution of endosulfan and related compounds in sugar beet plants exposed in a semi-controlled environment.

Time days.	PPM				
	Isomer I	Isomer II	Ether	Diol	Sulfate
	<u>Leaf surface</u>				
0	2.9	a	5.6	7.6	31.1
1	0.5	0.7	1.7	8.7	16.3
4	0.6	0.3	1.2	6.1	24.2
8	0.3	1.5	0.3	1.2	13.7
12	0.4	2.7	0.2	3.6	18.6
16	0.1	2.9	0.3	2.8	8.9
	<u>Leaf extract</u>				
0	37.9	20.0	9.6	8.7	10.7
1	20.0	12.8	5.6	6.6	16.9
4	2.8	7.7	3.2	3.8	7.7
8	3.4	4.2	1.9	5.5	13.7
12	2.0	3.0	0.8	5.5	4.1
16	1.0	1.6	1.2	4.3	10.7
	<u>Roots</u>				
0	0.03	0.0	b	0.0	0.07
1	1.6	0.3	0.04	0.0	c
4	2.8	0.5	0.1	0.0	0.1
8	4.6	0.04	0.2	0.0	0.2
12	1.6	0.8	0.1	0.0	0.3
16	0.2	0.2	0.3	c	0.2

^aSample not taken.

^bSample lost.

^cTrace amount of compound.

was on the leaf surfaces and small quantities of the compounds were found in the roots of the plants.

The residues on the surfaces and in the tissues of the plants tend to diminish during the course of the experiment while those in the roots increase. This is strong evidence that translocation of the materials from the leaves to the roots occurs. The data indicate that, in bean plants, endosulfan II is translocated to the greatest extent followed by sulfate and ether. Only traces of endosulfan I and the diol were found in the bean roots. In the sugar beet roots, endosulfan I and II are the most abundant residues and smaller residues of ether and sulfate are present. Again only trace amounts of diol were found.

Several unidentified peaks were observed in the samples. Most of these peaks were found to come from contamination in the extraction solvents or from unknown compounds in the soil in which the plants were grown. Control samples collected from untreated plants held in the greenhouse indicate that endosulfan II and ether were released into the air and re-deposited on the plants treated with the other compounds. These contaminants would have concealed small conversions of the other endosulfan compounds to these materials. The low levels of sulfate and ether found throughout the study indicate that metabolism of endosulfan to these materials was very small.

SUMMARY

A summary of the observations and conclusions drawn from this study is as follows:

1. Endosulfan was lost from the glass and plant surfaces at a predictable rate. The losses from glass plate were greater than from the plant surfaces. For plants, the loss was greatest from surfaces exposed to high temperatures and humidities.
2. The loss of endosulfan from bean and sugar beet plants exposed in semi-controlled environments was in the order: endosulfan I > ether > endosulfan II > diol > sulfate. For plants in controlled environments the order was: ether > endosulfan I > endosulfan II > sulfate > diol. The loss of endosulfan from glass plates exposed to controlled environmental conditions was: ether > endosulfan I > endosulfan II > diol > sulfate.
3. Metabolism of endosulfan occurred on both glass and plant surfaces. The amount of metabolism appeared to be greatest on those plants exposed to the semi-controlled environmental conditions.
4. The major site of metabolism in the plants appeared to be within the leaf tissue.

5. Endosulfan was found to penetrate into the plant leaves of both beans and sugar beets. More penetration occurred at higher temperatures and humidities.
6. Endosulfan I, II, ether, diol, and sulfate are translocated from the leaves to the roots of the plants. The compounds most persistent on the leaf surfaces appear to be translocated to the greatest degree.
7. The metabolism of endosulfan on the plants was as follows: on sugar beets held under controlled conditions endosulfan I was converted to the sulfate and the ether, and on both beans and sugar beets held under semi-controlled conditions endosulfan I was converted to the sulfate.

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