# Fate of Endosulfan on Plants and Glass

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Fates of the two isomers of endosulfan and three related compounds were investigated under controlled and semicontrolled conditions. Bean plants, sugar beet plants, and glass plates were used as substrates and the rate of dissipation from each surface was measured. Loss rates of isomers I and II and the ether were consistent in both environments and apparently substrate-dependent: glass > sugar beets > beans. In the controlled environment losses from glass and plant surfaces were: ether > isomer I > isomer II > sulfate > diol. In the greenhouse, under semicontrolled conditions, the sequence of loss from plant surfaces was: isomer I > ether > isomer II > diol > sulfate. High greenhouse temperatures appeared to facilitate atmospheric translocation of isomer I. Metabolism was greater under greenhouse conditions where temperature and humidity fluctuated. The only metabolite detected in the greenhouse was the sulfate, and this primarily as a translocated product. Volatile metabolites found under controlled conditions were the sulfate and ether. Penetration of the compounds into plant tissue and translocation from leaves to roots were observed in both bean and sugar beet plants. Translocation occurred at a higher rate in greenhouse plants and in the sequence: isomer II > sulfate > ether > isomer I > diol.

Endosulfan (Thiodan), 6,7,8,9,10,10-hexachloro-1,5,5*a*,6,9,9*a*-hexahydro-6,9-methano-2,4,3-benzodioxathiepin-3-oxide, is widely used in the control of various insect pests on fruits and vegetables (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 108–10° C. and 208–10° C., respectively (Reimschneider, 1963). The fifth compound, known to occur as a metabolite of endosulfan, is the sulfate.

A large proportion of technical endosulfan, applied to the surfaces of fruits, berries, and fruit tree leaves, is converted to endosulfan sulfate (Harrison *et al.*, 1967a, b). The sulfate, diol, and ether have also been identified as residues on alfalfa after treatment with the technical material, which disappeared from alfalfa at a logarithmic rate similar to other organochlorine insecticides (Ware, 1967). Cassil and Drummond (1965) attempted to find residues of endosulfan I and II and a metabolite on the inner portions of several food crops, but concluded that none of the three compounds penetrated the cuticular surface.

Little reference is made in the literature to the fate of endosulfan under controlled conditions; thus the following study was undertaken to determine fates of the five known endosulfan compounds applied to glass and leaf surfaces under controlled and semicontrolled conditions of light, temperature, and humidity.

## MATERIALS AND METHODS

**Insecticides.** The two stereoisomers of endosulfan and three related compounds were obtained from the Niagara Chemical Co., Middleport, N. Y. Their purities were: endosulfan I, 99.3%; endosulfan II, 97.8%; diol, 100%; ether, 100%; and sulfate, 100%.

**Reagents.** Reagent grade hexamethyldisilazane and trimethylchlorosilane were purchased from the Pierce Chemical Co., Rockford, Ill. High purity acetone, alcohol, ether, and hexane were obtained by redistilling, in an all-glass apparatus. All other chemicals were reagent grade, obtained from commercial sources.

**Scrubbing Apparatus.** The all-glass collection system and scrubbing apparatus were similar to those described by Abbot *et al.* (1966), as modified by Starr and Johnsen (1968) in which ethylene glycol was used as the solvent. Scrubbing efficiency was determined by measuring the per cent recovery of 100-$\mu$g. deposits of each compound from sample chambers and air scrubbing bottles. The retention of the endosulfan compounds by ethylene glycol after initial trapping was also determined.

**Controlled Environment Studies.** GLASS PLATES. Standard 9-cm. borosilicate glass Petri dishes were treated by applying 1000 $\mu$g. of a test compound in 1 ml. of hexane solution to each dish, spread evenly over the surface, and evaporated to dryness.

PLANTS. Two plant species were used: bean plants, *Phaseolus vulgaris* (Top Crop variety), and sugar beet plants, *Beta vulgaris* L., grown from seed in the laboratory under insecticide-free conditions. Bean plants, 6 weeks of age, and 16-week-old sugar beet plants, of uniform size, were treated with the five compounds and used in the controlled environment.

Treated plants for controlled exposures were prepared by immersing the above-soil portions in an emulsion containing 0.6 gram of toxicant and 0.225 gram of Triton X-150, dissolved in 2.0 ml. of acetone diluted to 60 ml. with distilled water. The greenhouse plants were dipped in emulsions containing 0.5 gram of toxicant and 5 grams of X-150 dissolved in 2.0 ml. of acetone diluted to 250 ml.

Three plants of each species were immersed in one of the five compound emulsions, drained horizontally

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to avoid soil contamination, and air-dried. Three untreated plants of each species were maintained throughout the study as controls.

The three plants were sealed in the two-piece, 5-gallon carboy, which was placed in a Percival climate control cabinet at 72° ± 1° F., and subjected to air of 33% relative humidity (RH) (O'Brien, 1948) exchanged at the rate of 2 liters per minute. The plants were maintained in the closed system under constant light and the effluent air was scrubbed continuously for 16 days.

Scrubbers used to collect insecticide vapors from treated plants were sampled by removing all of the solvent from each bottle at 0-, 1-, 4-, 8-, 12-, and 16-day intervals. Upon completion of an experiment, the chambers were rinsed with hexane, and the resulting solutions analyzed by electron-capture gas-liquid chromatography (EC GLC). The results were included in the final loss and recovery calculations.

Air scrubbers trapping vapors from the glass plate effluent were sampled at 0, 1, 2, 4, 6, 12, and 24 hours and again at 2-, 4-, 7-, and 10-day intervals, by removing aliquots of the trapping solvent.

**Semicontrolled Studies.** For the second, semicontrolled greenhouse study, laboratory-grown bean plants and field-grown sugar beet plants were treated similarly with the described emulsions.

Six plants of each species were immersed individually in one of the five emulsions, drained horizontally, and air-dried. Six untreated plants of each species were maintained as controls. The potted plants were weathered in a greenhouse in which the temperature ranged from 49° to 103° F. while the humidity varied from 47 to 71%.

These were sampled by harvesting one plant of both species from each of the treatments at 0-, 1-, 4-, 8-, 12-, and 16-day intervals. However, for sake of brevity only data for 4 and 16 days are presented. Plant material or roots were sealed separately in polyethylene bags, labeled, and frozen.

**Extraction.** Plants from both studies were clipped at soil level, weighed, and stripped of surface residues by rinsing in hexane for approximately 2 minutes. After air-drying, the leaves were frozen for subsequent extraction.

Leaf or root samples were extracted by macerating in an Omnimixer with 400 ml. of hexane, ethanol, and petroleum ether (2:1:1) for 3 minutes. The ethanol was partitioned into water and the remaining wet petroleum fraction cleaned with Nuchar Attaclay (Burke and Mills, 1963; Butler *et al.*, 1962; Coulson and Barnes, 1963). Plants bearing known diol residues were similarly extracted without removing the ethanol, followed by conversion to the disilane derivative by treating with a silylating reagent described by Ludwig and Korte (1965). Solvents from the air-scrubbing bottles and plant surface rinses containing the diol were mixed with equal volumes of the reagent, warmed to 50° C., and allowed to remain at room temperature for 1 hour. Water was then added to the silanized or to the normal scrubbing solvent and the samples were extracted three times with 50-ml. volumes of hexane. The hexane extracts were consolidated and dried over anhydrous sodium sulfate.

**Gas Chromatography.** Sample volumes were adjusted by evaporation or dilution with hexane before analysis by EC GLC using a Jarrell-Ash Model 700 Universal chromatograph. Injection block, column, and detector temperatures were generally 220°, 140°, and 210° C., respectively. A 2-foot × ¼-inch O.D. borosilicate glass column, packed with 5% SF-96 on 60- to 80-mesh, iron-free Chromosorb W, was used to separate the compounds eluted with nitrogen at 50 ml. per minute.

## RESULTS AND DISCUSSION

Analytical recovery studies of the compounds from sealed sample chambers after 16 hours at 26° C. indicated for isomer I, 97%; isomer II, 108%; ether, 105%; diol, 90%; and sulfate, 98%.

Recovery of the compounds from ethylene glycol in the air-scrubbing bottles was determined after 8 hours. Under static conditions of no air flow, recovery for isomer I was 105%; isomer II, 92%; ether, 99%; diol, 91%; and sulfate, 97%. Under an air-flow rate of 2 liters per minute recovery for isomer I was 90%; isomer II, 112%; ether, 96%; diol 94%; and sulfate, 106%.

Recovery from the Nuchar Attaclay cleanup method for isomer I was 90%; isomer II, 97%; ether, 98%; diol, 101%; and sulfate, 94%.

The loss data for endosulfan residues on glass surfaces under controlled conditions are presented in Figure 1. Endosulfan ether, the most volatile of the five compounds studied, dissipated most rapidly, with only 17% of the initial deposit remaining 10 days after treatment, followed by isomer I, 57%. Most of the isomer II, 96%, remained as a residue on the glass plate after 10 days. There were no measurable changes in the diol or sulfate.

The total recovery of each compound from the chamber, scrubber, and plates was relatively high except for the diol, only 78% of which was recovered. For the others recovery was: isomer I, 86%; isomer II, 95%; ether, 97%; and sulfate, 101%.

Initial deposits for compounds on plant surfaces were calculated using the total recovered from plant extraction, scrubbers, and sample chamber as 100%. Data from the treated bean plant scrubbing experiments are illustrated in Figure 2, closely resembling the dissipation pattern from glass plates. Again the ether was lost most rapidly, with 56% remaining after 10 days, followed by isomer I, 86%, and isomer II, 99%. Diol was not detected in the scrubbing solvent, and after 16 days only 0.2% of the sulfate was trapped.

Losses of endosulfan from sugar beet plants are presented in Figure 3, again resembling the glass and bean plant loss patterns. The ether volatilized most rapidly, with 36% remaining after 10 days, followed by isomer I, 58%. Isomer II remaining on beet plants after 10 days was 98% of the initial deposit. Small amounts of sulfate, 0.73%, and diol, 0.02%, were apparently volatilized and recovered in the scrubbing solvent.

Endosulfan ether was lost most rapidly from all three

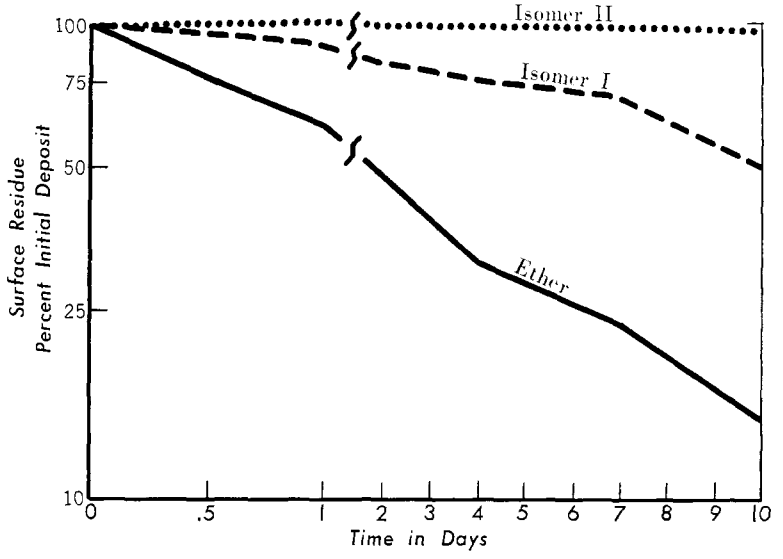


**Figure 1. Loss of endosulfan and related compounds from glass surfaces in controlled environments**

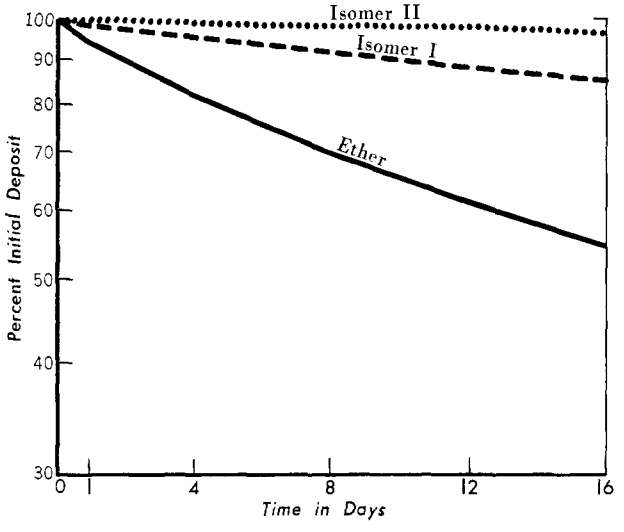


**Figure 2. Loss of endosulfan and related compounds from bean plants in controlled environments**

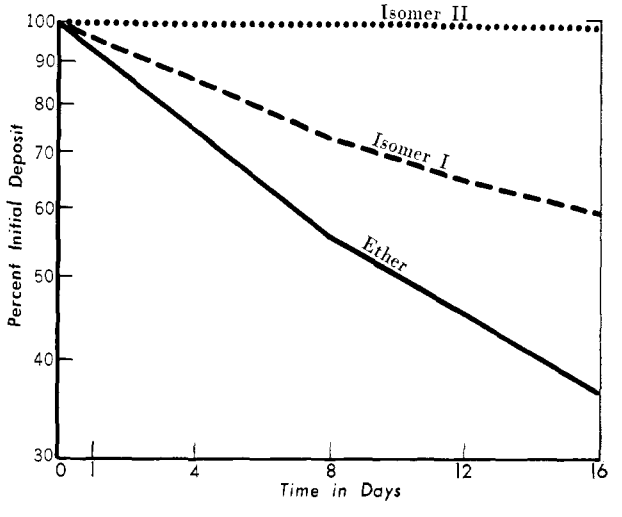


**Figure 3. Loss of endosulfan and related compounds from sugar beet plants in controlled environments**

surfaces, followed by isomer I and isomer II. Only small amounts of diol and sulfate were dissipated from the leaves. Figures 1, 2, and 3 indicate that higher percentages of isomers I and II and ether were lost from glass than from plants, and the losses from sugar beets were greater than from beans.

Penetration into plant tissues and translocation to the roots occurred in both beans and sugar beets under controlled conditions (Table I). With the exception of the diol, all compounds were identified in the roots of both plant species. These residues represent translocated material and were of a higher magnitude in sugar beets than in beans. The sulfate and isomer II were translocated in greatest amounts. In all but the diol-treated beans, the plant surface residue was greater than within the tissue and, except for the sulfate, penetration into bean leaves was greater than in beets.

Metabolism was insignificant for most endosulfan compounds on glass and plant surfaces exposed to controlled conditions. No metabolites were found in the glass plate studies. In each case only the applied compound was recovered.

The sulfate was found after 16 days only in the sample chamber from which endosulfan I–treated sugar beets were scrubbed. Endosulfan ether was found regularly in the scrubbers attached to the isomer I–treated sugar beets. Microgram quantities found at each sampling were: 0 day, 0.0; 1 day, 0.3; 4 days, 0.81; 8 days, 1.31; 12 days, 1.38; and 16 days, 1.59 $\mu$g. As in the studies of endosulfan metabolism on bean plants by Terranova and Ware (1963), no diol was found on plant surfaces.

In the second group of plants, treated and weathered in the greenhouse, residues were measured at 4-day inter-

**Table I. Distribution of Endosulfan and Related Compounds in Plants Exposed to a Controlled Environment**

| Location | Per Cent Initial Deposit | | | | |
|---|---|---|---|---|---|
| | Isomer I | Isomer II | Ether | Diol | Sulfate |
| **Bean** | | | | | |
| 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 | Trace | 0.01 | 0.01 | 0.00 | 0.02 |
| Total | 85.8 | 97.1 | 55.5 | 94.7 | 97.7 |
| **Sugar Beet** | | | | | |
| 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 | 53.8 | 96.5 | 34.7 | 93.9 | 97.7 |

[a] Rinsed from plant surfaces with hexane. [b] Extract of macerated plant.

vals. Total residues (parts per million) found on bean plants are illustrated in Figure 4. The 0-day residues were found to vary greatly. Comparison of the curves in Figure 4, however, indicates that isomer I is lost most rapidly, followed by the ether and isomer II.

Losses of the compounds from sugar beets followed much the same pattern as from the beans. Figure 5 shows that the ether and isomer I disappeared rapidly, followed by isomer II. Diol and sulfate were lost very slowly from both plants, but more rapidly from beets than from beans. However, the rates of loss for isomers I and II and the ether were greater from beans than from beets. The order of endosulfan dissipation from both plant surfaces based on the residues remaining 16 days was: isomer I > ether > isomer II > diol > sulfate.

The loss of endosulfan from plants was greater and more rapid under greenhouse conditions of high temperature and humidity. Lyon and Davidson (1965) found that high humidity is responsible for rapid vaporization of insecticides and the authors believe this explains the greater losses in the greenhouse than the laboratory.

Metabolism was also more evident in the greenhouse than in the controlled environment. Table II lists the residues of sulfate found in bean and sugar beet plants treated with isomer I. Only traces of sulfate were found in the bean roots or on bean leaves. However, in beets, higher levels of sulfate were found both in the roots, as expected based on the root-storage characteristic of the plant, and on the leaves.

Residues of isomer I and the sulfate were found on beet leaves, in leaf extracts, and on roots of beet plants treated with isomer II (Table III). Residues of the compounds were also identified from bean leaves. The bean roots, however, contained only endosulfan I. Since conversion of one isomeric form to another is unlikely, isomer I found in these plants was presumed relocated by volatilization. The sulfate, however, is considered a metabolite. Residues of isomer I and sulfate were higher within the plant than on the surface.

On plants treated with the sulfate, isomer I was again

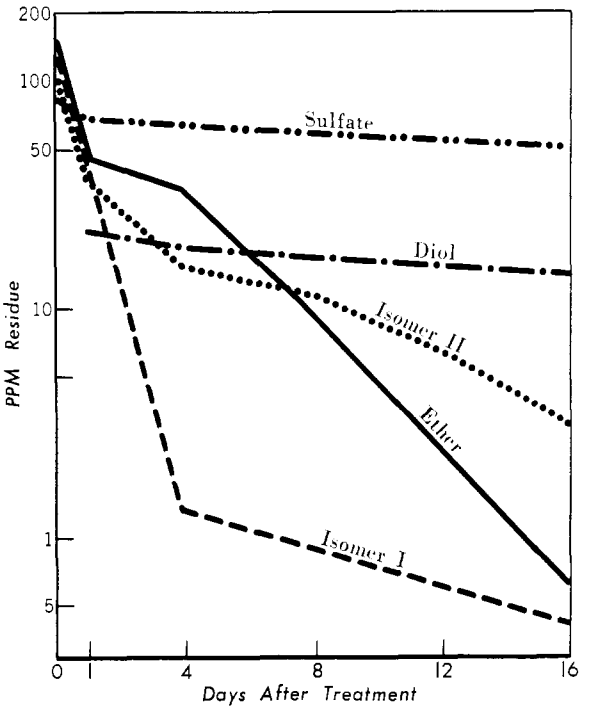


**Figure 4. Loss of endosulfan and related compounds from bean plants exposed in semi-controlled environments**

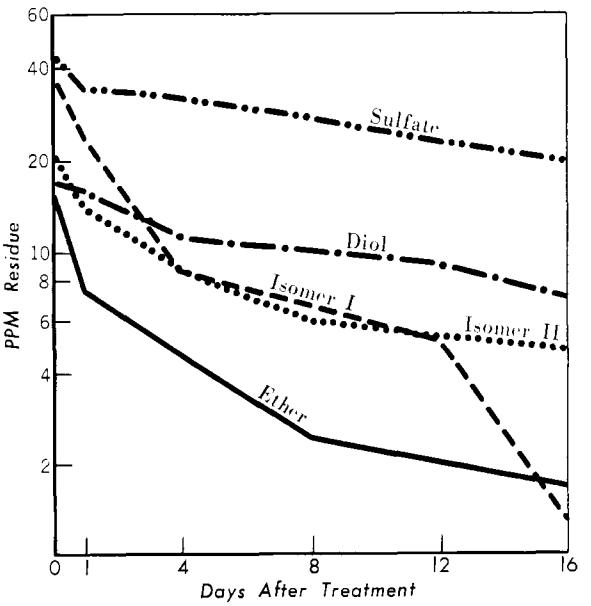


**Figure 5. Loss of endosulfan and related compounds from sugar beet plants exposed in semi-controlled environments**

found as a contaminant, with the highest level occurring in the leaf tissue. More of isomer I was found on bean leaves than on beets, with only traces present in roots. More sulfate was detected in sugar beet roots than in bean roots when treated with isomer I or II, but not with the sulfate *per se*.

The distribution of endosulfan residues in bean and sugar beet plants at 4 and 16 days is shown in Tables IV and V. In both beets and beans, the greatest residue was in the plant extracts, indicating penetration of the

**Table II. Endosulfan Sulfate Residues in Plants Treated with Isomer I and Held under Greenhouse Conditions**

| Days | P.P.M. Endosulfan Sulfate | | |
|---|---|---|---|
| | Surface | Extract | Root |
| | BEAN | | |
| 4 | 0.00 | 0.12 | 0.00 |
| 16 | 0.00 | 0.25 | 0.00 |
| | SUGAR BEET | | |
| 4 | 0.05 | 0.95 | 0.23 |
| 16 | Trace | 0.87 | Trace |

**Table III. Endosulfan Isomer I and Sulfate Residues in Plants Treated with Isomer II and Held under Greenhouse Conditions**
**(Parts per million)**

| Days | Surface | | Extract | | Root | |
|---|---|---|---|---|---|---|
| | Isomer I | Sulfate | Isomer I | Sulfate | Isomer I | Sulfate |
| | BEAN | | | | | |
| 4 | 0.09 | 0.02 | 1.00 | Trace | Trace | 0.00 |
| 16 | 0.01 | 0.03 | 0.13 | 0.07 | Trace | 0.00 |
| | SUGAR BEET | | | | | |
| 4 | Trace | 0.00 | 0.21 | 0.02 | 0.05 | 0.01 |
| 16 | 0.02 | 0.00 | 0.12 | 0.02 | 0.04 | 0.03 |

**Table IV. Distribution of Endosulfan and Related Compounds in Bean Plants Exposed to Greenhouse Conditions**
**(Parts per million)**

| Days | Isomer I | Isomer II | Ether | Diol | Sulfate |
|---|---|---|---|---|---|
| | SURFACE | | | | |
| 4 | 0.15 | 1.05 | 6.90 | 6.14 | 30.90 |
| 16 | Trace | 0.69 | 0.05 | 1.70 | 45.40 |
| | EXTRACT | | | | |
| 4 | 1.15 | 14.30 | 26.70 | 12.70 | 7.66 |
| 16 | 0.36 | 1.70 | 0.53 | 11.50 | 3.65 |
| | ROOT | | | | |
| 4 | 0.00 | 0.28 | 0.18 | 0.00 | 0.08 |
| 16 | 0.05 | 0.63 | Trace | Trace | 0.10 |

materials. The next largest deposit was on leaves with only low levels in roots.

Residues on and in leaf surfaces and tissues diminished during the course of the experiment while those in the roots increased. In bean plants translocation was: isomer II > sulfate > ether. Only traces of isomer I and the diol appeared in bean roots. In sugar beet roots, residues were: isomer I > isomer II > ether > sulfate > diol.

The small quantities of sulfate and ether found throughout the study indicate that metabolism of endosulfan to these was slight. Since the diol is comparatively nonvolatile, but disappears at a constant rate from plant

**Table V. Distribution of Endosulfan and Related Compounds in Sugar Beet Plants Exposed to Greenhouse Conditions**
**(Parts per million)**

| Days | Isomer I | Isomer II | Ether | Diol | Sulfate |
|---|---|---|---|---|---|
| | SURFACE | | | | |
| 4 | 0.6 | 0.3 | 1.2 | 6.1 | 24.2 |
| 16 | 0.1 | 2.9 | 0.3 | 2.8 | 8.9 |
| | EXTRACT | | | | |
| 4 | 2.8 | 7.7 | 3.2 | 3.8 | 7.7 |
| 16 | 1.0 | 1.6 | 1.2 | 4.3 | 10.7 |
| | ROOTS | | | | |
| 4 | 2.8 | 0.5 | 0.1 | 0.0 | 0.1 |
| 16 | 0.2 | 0.2 | 0.3 | Trace | 0.2 |

surfaces, it is probably absorbed and metabolized to nondetectable products. The higher sulfate residues in bean and beet leaf extracts from plants treated with isomer I indicate that isomer I is more readily oxidized than isomer II.

The initial deposits on the two plant species were grossly different, which may be a function of the characteristics of plant morphology and the actual physical nature of the initial deposit. The different loss rates are probably also explained in the differences in leaf surfaces and the actual site of residue deposition. The pubescent nature and thinner wax layer on the bean leaf provide a much larger active surface area accounting for both the increase in initial deposit and the high loss rate.

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