Translocation and Metabolism of Perfluidone (1,1,1-Trifluoro-*N*-[2-methyl-4-(phenylsulfonyl)phenyl]methanesulfonamide) in Peanuts

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[^{14}C]Perfluidone was slowly absorbed by the roots of peanuts grown in nutrient solution containing 0.5 to 5 μM [^{14}C]perfluidone, and from 22.4 to 41.2% of the absorbed ^{14}C was translocated to the shoots. Slight injury to the roots was observed with peanuts treated with 5 μM [^{14}C]perfluidone. [^{14}C]Perfluidone was the only radioactive product detected in the initial chloroform extracts from any of the treated tissues. In excised peanut leaves, the β-*O*-D-glucoside of 1,1,1-trifluoro-*N*-[4-[(3-hydroxyphenyl)sulfonyl]-2-methylphenyl]methanesulfonamide and products that liberated [^{14}C]perfluidone upon mild acid treatment were the major water-soluble metabolites present. These products were present in much lower concentrations in intact seedlings where the treatment period was longer. Unidentified aqueous 80% methanol-soluble products (37.4%) and aqueous 80% methanol-insoluble products (41.9%) were the major radioactive components in seedlings harvested 22 days after treatment. Seedlings treated with [^{14}C]perfluidone did not evolve an experimentally significant amount of $^{14}CO_2$.

Perfluidone (1,1,1-trifluoro-*N*-[2-methyl-4-(phenylsulfonyl)phenyl]methanesulfonamide) is an experimental preemergence herbicide that selectively controls nutsedges (*Cyperus*) and other weeds in cotton (*Gossypium hirsutum* L.), peanuts (*Arachis hypogea* L.), soybeans (*Glycine max* L.), and other crops (3M, 1972; Gentner, 1973). Since the nutsedges represent a serious worldwide weed problem, any herbicide that selectively controls nutsedges is potentially of great agricultural importance, and it is therefore desirable to study the behavior of such herbicides in pertinent biological systems. Studies have been reported on the metabolism of perfluidone in a lactating cow (Ivie, 1975) and in the chicken and rat (Paulson, 1977). The effects of perfluidone on cotton and yellow nutsedge (*Cyperus esculentus*) have also been reported (Davis and Dusbabek, 1975). The purpose of this study was to determine if a resistant crop species, peanuts (*Arachis hypogea* L. Spanish), could absorb [^{14}C]perfluidone through the roots, translocate the herbicide or its metabolites to the shoots, and/or metabolize the herbicide to readily identifiable hydroxylated analogues, hydrolysis products, or conjugates.

MATERIALS AND METHODS

Chemicals. [^{14}C]Perfluidone, uniformly labeled in the trisubstituted phenyl ring, was provided by the 3M Company at specific activities of 0.210 mCi/mmol and 5.20 mCi/mmol. Thin-layer chromatography (TLC) in solvents A, B, and C indicated the radiochemical purity of both preparations to be >99%. Except where noted, tissue was treated with the higher specific activity [^{14}C]perfluidone. The 3M Company also provided nonradioactive analogues of perfluidone that were considered potential metabolites. These analogues were hydroxylated either in the 3, 2′, 3′ or 4′ positions; or the 2-methyl group was oxidized to a carboxylic acid; or the SO_2CF_3 group was replaced by H; or the monosubstituted phenyl ring was replaced by an -OH (Figure 1).

Thin-Layer Chromatography. Thin-layer chromatograms were developed to a 15-cm solvent front on glass plates coated with either a 250-μm or a 500-μm layer of silica gel HF_{254} (Merck). Chromatograms were developed in one of the following solvents: (A) chloroform–acetic acid (90:10); (B) toluene–acetic acid–water (50:50:5); (C) toluene–chloroform–acetic acid (70:20:10); (D) ethyl acetate–xylene–formic acid–water (25:1:2:2); and (E) ethyl acetate–acetic acid–water (23:1:1).

Assay of Radioactivity. Radioactive zones on thin-layer plates were detected with a radiochromatogram scanner or by autoradiography. All column effluents, except those from high-pressure liquid chromatography (HPLC), were continuously monitored for ^{14}C with a radioactive flow monitor. Quantitative determinations of ^{14}C in reaction mixtures, plant extracts, column effluent fractions, thin-layer plates, etc. were made by liquid scintillation counting. The ^{14}C contained in insoluble plant residues, or in highly colored chloroform extracts, was determined by liquid scintillation counting after the sample was converted to $^{14}CO_2$ in a Model 306 Packard Tri-Carb sample oxidizer. Residues of ^{14}C in tissues are reported either as parts per million perfluidone equivalents on a fresh weight basis or as microgram equivalents of perfluidone.

Plant Material. Spanish peanut seeds (*Arachis hypogaea* L.) were germinated and grown in vermiculite under greenhouse conditions (Blankendaal et al., 1972). After the plants reached an age of 30–60 days, they were used as a source of excised leaves. Peanut seedlings used for root-uptake studies were germinated and grown under similar conditions; however, these seedlings were transferred to one-third strength Hoagland's solution immediately after germination. They were grown in a greenhouse for 15 days prior to treatment. The average conditions in the greenhouse during these experiments were: 13 h photoperiod, 31 °C day temperature, 22 °C night temperature, and 44% relative humidity.

$^{14}CO_2$ Evolution. $^{14}CO_2$ evolution from two peanut plants (42.6 g fresh weight at harvest) treated for 5 days with 4.22×10^6 dpm of [^{14}C]perfluidone in 150 mL of one-third strength nutrient solution was monitored over the 5-day treatment and 10-day posttreatment periods. At the conclusion of the experiment, the plants were harvested, extracted, and subjected to chromatographic analysis as described in the following section. The peanut seedlings were housed in a 4-L glass metabolism chamber

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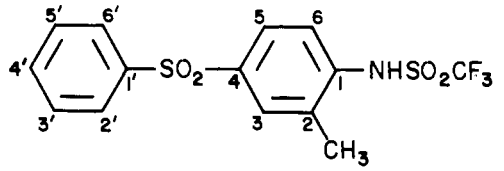

Figure 1. Structure of perfluidone showing numbering system used in this paper.

in a greenhouse. Air was drawn through the chamber at 50 mL/min under slightly reduced pressure and exhausted through a calcium sulfate drying tube and two CO_2-trapping tubes. Each trapping tube contained 25 mL of ethanolamine:methyl Cellosolve (7:1) and was recharged every 24 h when aliquots were removed for liquid scintillation counting. The drying tube was recharged as needed. The trapping efficiency of the chamber for $^{14}CO_2$ under the described conditions was 98.6%.

Absorption, Translocation, and Metabolism of [^{14}C]Perfluidone by Intact Peanut Seedlings. Six lots of peanut seedlings (three per lot) were placed in beakers of 150 mL of 5.17 μM [^{14}C]perfluidone in one-third strength nutrient solution and three lots of seedlings (three per lot) were placed in beakers of 200 mL of 0.512 μM [^{14}C]perfluidone in one-third strength nutrient solution. The beakers were wrapped with foil to exclude light from the nutrient solution and returned to a greenhouse under the previously described conditions. The nutrient solution in each beaker was continuously aerated and the volume readjusted with water each day to make up for transpiration and evaporation losses. After 8 days, the seedlings were removed from the treating solutions and their roots were rinsed with water. Three lots of seedlings treated with 5.17 μM [^{14}C]perfluidone were then placed in perfluidone-free nutrient solution and grown for an additional 22 days. One lot of seedlings treated with 0.512 μM [^{14}C]perfluidone and one lot treated with 5.17 μM [^{14}C]perfluidone were pressed, freeze-dried, and placed on x-ray film for 1 month. Each of the two remaining lots was subdivided into root and shoot tissue and homogenized in an Omni Mixer with a 10:1 (v:w) ratio of cold aqueous 80% methanol. The homogenates were filtered and the resulting residues were extracted two additional times in the same manner. The three methanolic extracts from each lot of tissue were combined, concentrated, dissolved in water, and partitioned three times with chloroform. The aqueous phases were adjusted to pH 1 with 0.5 N HCl and partitioned three times with chloroform. The acidified aqueous phases were then concentrated to dryness under vacuum at 37 °C, dissolved in 0.2 N HCl, and partitioned three times with chloroform. The resulting aqueous phases were concentrated to dryness and the process (treatment with 0.2 N HCl, partitioning with chloroform, and concentration to dryness) repeated three additional times. The final aqueous extracts were concentrated, hydrolyzed in 2 N HCl at 105 °C for 2 h, cooled, and partitioned three times with equal volumes of chloroform. The three chloroform extracts from each partitioning step with each lot of tissue were combined and analyzed for ^{14}C content. Extracts containing significant ^{14}C were further analyzed by TLC in solvents A and B and by HPLC on a 2.2-mm i.d. × 2-m column of Biosil A (Bio Rad Laboratories) eluted with hexane–chloroform–acetic acid (25:65:10) at 1 mL/min. The aqueous extracts obtained after treatment with 0.2 N HCl and after hydrolysis in 2 N HCl were examined by TLC in solvents D and E.

The three lots of peanut seedlings that were grown for an additional 22 days after treatment were used to prepare

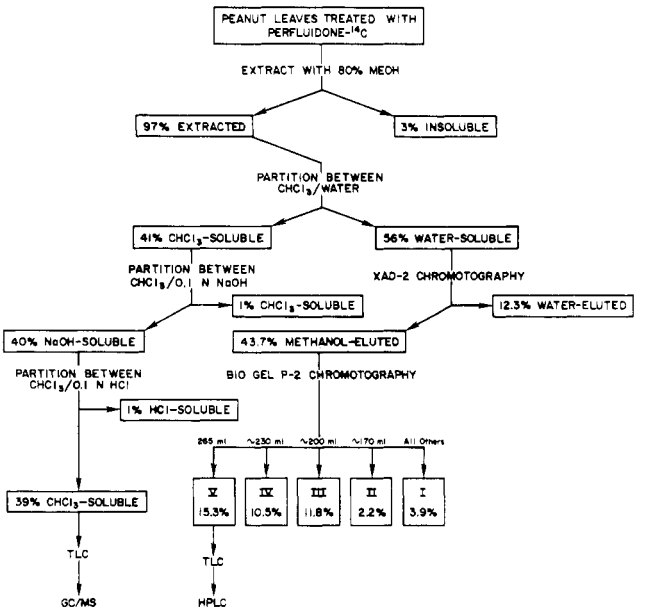

Figure 2. Separation of metabolites produced by excised leaves.

autoradiograms, extracts, and chromatograms exactly as described for the seedlings harvested after 8 days.

Uptake and Metabolism of [^{14}C]Perfluidone by Excised Peanut Leaves. Peanut leaves were excised near the base of the petiole and placed in 15 mL of 0.10 μM [^{14}C]perfluidone (0.114 mCi/mmol) treating solution (four leaves per 20-mL vial). During treatment, the excised leaves were held in a growth chamber with a 14-h photoperiod (20 000 lux) at 31 °C, a 10-h nyctoperiod at 22 °C, and a constant relative humidity of 40%. Water was added to the vials as needed to make up for transpiration and evaporation losses, and after 48 h the leaves were removed, homogenized, and extracted three times with a 5:1 (v:w) ratio of cold aqueous 80% methanol. The extracts were concentrated to near dryness and partitioned between water and chloroform as described previously for the intact tissue. A total of four lots of excised peanut leaves (3.0 kg of tissue, approximately 3000 leaves) were treated in this manner.

The chloroform-soluble extracts from the four treatments of excised leaves were subjected to TLC in solvents A, B and C, and to HPLC with Biosil A as previously described. These chromatographic procedures indicated the presence of a single radioactive product in each of the chloroform-soluble extracts. The chloroform-soluble product was quantitatively extracted into 0.1 N aqueous sodium hydroxide. The pH of the basic extract was adjusted to pH ~1, and the product was reextracted into chloroform and further purified by TLC in solvents A and B. After each chromatographic step, the product was eluted from the gel with ethyl acetate. The final product was treated with dizaomethane and subjected to GC/MS (gas chromatography/mass spectrometry) analysis under conditions similar to those described by Ivie (1975).

The water-soluble extracts from the four treatments of excised leaves were applied to 2.4-cm × 60-cm water-jacketed (5 °C) columns of water-equilibrated XAD-2 resin (Mallinckrodt) and successively eluted with 1 L of cold water and 1 L of cold methanol. The methanol eluates were concentrated to dryness, dissolved in water, applied to 1.75-X 105-cm water-jacketed (15 °C) columns of Bio Gel P-2 (Bio Rad Laboratories) and eluted with water at 0.5 mL/min. Fractions were collected as indicated in Figure 2. Fraction V was concentrated to dryness and

Table I. Selective Hydrolysis of Aqueous Fractions from Excised Peanut Leaves

Method of treatment	Percent ^{14}C rendered chloroform-soluble by treatment[a]			
	XAD-2	II	III	IV
1. Adjusted to pH 1	12.3	8.7	14.0	11.3
2. Evaporated in 0.2 N HCl	21.4	27.8	30.8	33.1
3. Evaporated in 0.2 N HCl		9.2	7.5	6.7
4. Evaporated in 0.2 N HCl		2.6	3.3	4.2
5. 2 N HCl hydrolysis	13.0	22.8	12.2	19.3
6. 6 N HCl hydrolysis		3.1	6.0	3.1
Total ^{14}C rendered $CHCl_3$-soluble	41.8	74.2	73.8	77.7

[a] The XAD-2 fraction contained 12.3% of the ^{14}C isolated from the excised leaves; fraction II, 2.2%; fraction III, 11.8%; and fraction IV contained 10.5%.

further purified by successive TLC in solvents D, E, and D. After each thin-layer chromatographic step, the product was eluted from the gel with aqueous 80% methanol. The product was then dissolved in 50% methanol and chromatographed with 50% methanol (1 mL/min) by HPLC on a 6.4-mm × 30-cm micro Bondapak C_{18} column (Waters Associates). A UV detector was used. The radioactive product (metabolite V) was eluted as a single peak between 9 and 15 min. After each chromatographic separation, the column was washed with methanol and reequilibrated with 50% methanol. The elution time of the product shifted from 9–15 min during early chromatographic separations to 7–10 min during the later separations. These differences were probably the result of incomplete regeneration of the column.

Characterization of Metabolite Fractions II, III, IV, and V. Metabolite V (Figure 2) (0.050 μmol) was hydrolyzed with 1 mL of 1 N HCl at 80 °C for 18 h in a sealed tube. The chloroform extract from the hydrolysate was compared with the standards by TLC in solvents A and B, and by HPLC on a 2.2-mm i.d. × 2-m column of Biosil A (Bio Rad Laboratories) eluted with hexane–chloroform–acetic acid (25:65:10). After preliminary thin-layer chromatographic purification with solvents A and B, the chloroform-soluble hydrolysate was methylated and subjected to GC/MS as described for the original chloroform extract from the excised leaves. The aqueous phase from the acid hydrolysate was concentrated to dryness under high vacuum and incubated for 15 min with 120 μL of a 10:2:1 mixture of pyridine–hexamethyldisilazane–trimethylchlorosilane. The reaction mixture was analyzed for carbohydrates by GC on both an SE 52 column and an EGS polyester column by the method of Sweeley et al. (1963). Metabolite V was also treated with β-glucosidase (almond emulsion) in a reaction mixture that contained 0.100 μmol of metabolite V, 4 mL of 0.01 M pH 5.0 phosphate buffer, and 1 mg of enzyme. A no-enzyme control was prepared in a similar manner. The control and reaction mixtures were incubated for 16 h at 37 °C, adjusted to pH 2, and partitioned five times with chloroform. The chloroform phase from the enzyme reaction was analyzed by TLC in solvents A and B and by HPLC as described above.

The water eluate from the XAD-2 column and fractions II, III, and IV from the Bio Gel P-2 column (Figure 2) were systematically treated with HCl as described above for intact peanut seedlings, but a final hydrolysis in 6 N HCl at 105 °C for 2 h was added as the last step in this procedure. Each of the chloroform-soluble extracts from the hydrolysates was examined by TLC in solvents A and B. In each case, chloroform extracts 2–4 of fractions II, III, and IV (Table I) were combined, purified, and subjected to GC/MS as described for the chloroform-soluble acid hydrolysate of V.

RESULTS AND DISCUSSION

$^{14}CO_2$ Evolution. Peanut seedlings grown in nutrient solution with [^{14}C]perfluidone did not evolve a significant amount of $^{14}CO_2$ during the 5 days the seedlings were exposed to the herbicide or during the 10 days immediately after treatment was terminated. Only 1.05% of the ^{14}C originally in the treating solution was recovered in the CO_2 traps. The apparent uptake of ^{14}C by the seedlings was 29.5%, and the total recovery of ^{14}C was 98.2%. The distribution of ^{14}C in the seedlings and the products detected were comparable with those reported in the following section. After 5 days, when $^{14}CO_2$ evolution from the treating solution and the seedlings was measured separately, nearly equal amounts of ^{14}C were collected from the traps over the seedlings (0.043%/day) and over the treating solution (0.060%/day). Since $^{14}CO_2$ evolution from the treating solution was slightly greater than that from the seedlings, and since it was found that ^{14}C in the roots was leached back into the nutrient solution (Table II), it was not possible to attribute the ^{14}C in the CO_2 traps to plant metabolism. The very low levels of ^{14}C evolved, however, indicated that the trisubstituted phenyl ring of perfluidone was quite resistant to metabolism to CO_2 under the conditions described.

Absorption and Translocation. Peanut seedlings grown for 8 days in a solution of 0.5 μM [^{14}C]perfluidone absorbed only 21.9% of the herbicide and had an overall tissue concentration of 0.27 ppm (expressed as perfluidone on a fresh weight basis). When the concentration of [^{14}C]perfluidone was increased to 5 μM, the seedlings absorbed 25% of the dose and an overall tissue concentration of 3.25 ppm was observed. Some slight injury to the roots was observed at the 5 μM concentration, and no attempt was made to study perfluidone uptake at higher levels. Over the narrow concentration range examined, perfluidone uptake appeared to be directly related to perfluidone concentration. Autoradiograms of the seedlings that were root-treated for 8 days showed that ^{14}C was translocated throughout the seedlings. The roots contained the highest levels of ^{14}C and were uniformly labeled. Seedlings treated for 8 days and then grown for an additional 22 days yielded similar results; however, the blossoms and foliar portions that developed after the plants had been transferred to ^{14}C-free nutrient solution contained only trace levels or no detectable ^{14}C.

The quantitative data from the translocation studies and the distribution of ^{14}C into water-soluble, chloroform-soluble, and 80% methanol-insoluble fractions are shown in Table II. Between the 8th and 30th day, there was no significant change in the amount of ^{14}C present in the shoots; however, there was a decrease in the amount of chloroform-soluble ^{14}C and an increase in the amount of water-soluble and 80% methanol-insoluble ^{14}C. These changes indicated that the herbicide was being metabolized during this period. In the roots, there was a net loss of 14 ± 7 μg of perfluidone equivalents between the 8th and 30th days. There was a decrease in the amount of ^{14}C in the chloroform-soluble and water-soluble fractions and a small increase in the amount of ^{14}C in the 80% methanol-insoluble fraction. At the end of the study, 24.9 ± 4.0 μg of ^{14}C (as perfluidone) was recovered in the post-nutrient solution. Most of this (74.5%) was recovered during the first 4 days following treatment. Partitioning and TLC in solvents A, B, and C indicated that approximately 90% of the ^{14}C in the post-nutrient solutions was [^{14}C]per-

Table II. Distribution of ^{14}C in [^{14}C]Perfluidone-Treated Peanut Seedlings (μg equiv of Perfluidone[a])

Perfluidone treatment	Initial T-sol[b]	Post T-sol (8 days)	Post N-sol[c] (30 days)	Roots				Shoots			
				$CHCl_3$	Water	Insol[d]	Total	$CHCl_3$	Water	Insol	Total
8 days 0.5 μM	38.8	28.3 ± 0.6	NA	1.7 ± 0.2	3.5 ± 0.3	1.5 ± 0.2	6.6 ± 0.7	1.1 ± 0.6	0.56 ± 0.07	0.24 ± 0.01	1.9 ± 0.5
8 days 5 μM	294	194 ± 19	NA	12 ± 2	20 ± 5	12 ± 1	44 ± 6	8.9 ± 0.2	9.7 ± 4.4	1.6 ± 0.3	20 ± 5
30 days 5 μM	294	187 ± 7	24.9 ± 4.0	1.6 ± 1.1	12 ± 2	17 ± 2	30 ± 1	5.2 ± 2.2	12 ± 3	4.3 ± 1.0	21 ± 7

[a] The fresh weights of the roots and shoots at harvest were: (0.512 μM treatment) 8 days, 11.2 g and 20.0 g, respectively; (5.17 μM treatment) 8 days, 9.0 g and 13.7 g, respectively; 5.17 μM treatment for 8 days with a 22-day posttreatment period (30 days), 23.1 g and 31.8 g, respectively. The total percent recovery of ^{14}C was 95.4% for the 0.512 μM treatment and 87.8 and 89.4% for the 5.17 μM 8- and 30-day studies. [b] T-sol, treating solution. [c] N-sol, nutrient solution. [d] Insoluble in aqueous 80% methanol.

fluidone. These changes indicated that some metabolism of perfluidone or its metabolites occurred in the roots between the 8th and 30th day. Loss of perfluidone to the post-nutrient solution was also a major process. The percent distribution of ^{14}C between the roots and shoots of peanuts was similar to that observed in cotton (Davis and Dusbabek, 1975).

Analysis of the Extracts from Peanut Seedlings. HPLC and TLC indicated that after 8 and 30 days, all or nearly all of the ^{14}C in the chloroform extracts from the roots and shoots was in the form of perfluidone. The corresponding aqueous extracts from the roots and shoots were acid-labile and liberated chloroform-soluble ^{14}C in moderately high yields when treated with HCl under successively more rigorous conditions (Table III). The chloroform-soluble ^{14}C liberated when the samples were adjusted to pH 1 appeared to be in the form of perfluidone and several more polar products. That liberated by repetitively evaporating the samples to dryness in 0.2 N HCl appeared to be perfluidone (60 to 90%) and from four to seven more polar compounds. The ^{14}C liberated by hydrolysis in 2 N HCl was perfluidone, 3′-hydroxyperfluidone, and more polar products. Based on TLC and HPLC of these extracts, the percent distribution of ^{14}C into perfluidone, perfluidone conjugates, etc. is shown in Table IV. The unidentified radioactivity in the chloroform- and water-soluble fractions consisted of numerous products. Because of the low concentrations of the individual products involved, no further attempt was made to isolate and identify them.

Analysis of the Chloroform-Soluble Products from Excised Leaves. The low percentage uptake and the perfluidone-induced injury to the roots made it difficult to study the metabolism of perfluidone in intact peanuts. In replicated experiments with excised peanut leaves treated for 48 h with 5 μM perfluidone, moderate amounts of [^{14}C]perfluidone were absorbed. When the extracts from these leaves were examined by partitioning and by TLC in solvents A, B, and C, it was found that 85% of the ^{14}C was in the form of water-soluble products. All subsequent studies on the metabolism of perfluidone were conducted with excised peanut leaves treated for 48 h with 10 μM [^{14}C]perfluidone. The average uptake of perfluidone was 62% and the average concentration of ^{14}C in the tissue was 8.6 ppm. The radioactive products in the excised leaves were resolved by the methods described in Figure 2. The final chloroform-soluble fraction was examined by TLC, HPLC, and by GC/MS of the methylated derivative. Each method of analysis showed that [^{14}C]perfluidone was the only radioactive compound present. These results were similar to those obtained with the root and foliar tissues of intact peanut seedlings.

Analysis of the Water-Soluble Products from Excised Leaves. During the fractionation of the water-soluble ^{14}C, a large peak was consistantly eluted from the Bio Gel P-2 column with 265 mL of water. This peak accounted for about 35% of the ^{14}C recovered from the column. Considerable variation in the number and elution volumes of the other products eluted from the Bio-Gel P-2 column was observed, and these peaks were divided into crude fractions as shown in Figure 2. Strong acid hydrolysis of fractions II, III, and IV converted approximately 75% of the ^{14}C to chloroform-soluble products. TLC indicated that about 75% of the ^{14}C in each of these chloroform-soluble fractions was perfluidone. When fractions II, III, and IV were subsequently treated with HCl in a systematic manner, most of the ^{14}C was converted to chloroform-soluble products under comparatively mild

Table III. Selective Hydrolysis of Aqueous Extracts from Peanut Seedlings

Sample source	Percent chloroform-soluble ^{14}C after treatment: Adjusted to pH 1	Concentrated in 0.2 N HCl	Hyd in 2 N HCl	Total	Percent ^{14}C water-soluble after final treatment
Roots, 8 days	11.4	35.4	15.9	62.7	37.3
Roots, 30 days	8.8	14.6	29.2	52.6	47.4
Shoots, 8 days	17.9	23.0	11.1	52.0	48.0
Shoots, 30 days	15.7	16.2	12.4	44.3	55.7

Table IV. Percent Distribution of ^{14}C in Peanut Seedlings

Chemical form of product[a]	Roots, % 8 days	Roots, % 30 days	Shoots, % 8 days	Shoots, % 30 days
Perfluidone	18.5	3.3	14.3	9.8
Conjugates of perfluidone	8.5	2.9	1.6	1.4
Conjugates of 3′-hydroxyperfluidone	2.8	2.3	0.9	1.0
Unidentified $CHCl_3$-soluble products from hydrolysis	7.8	7.1	4.9	7.1
Products remaining water-soluble after hydrolysis	11.4	11.2	6.9	12.0
Insoluble in 80% methanol	19.9	33.5	2.5	8.4
Total in tissue	68.9	60.3	31.1	39.7

[a] All products were characterized by chromatographic methods.

conditions (Table I). TLC indicated that after the first acid treatment, the radioactivity in the chloroform extracts of fractions II, III, and IV was in the form of compounds other than perfluidone; however, strong acid hydrolysis of the first chloroform extract from fraction IV liberated about 75% of the ^{14}C in a form that co-chromatographed with perfluidone. Strong acid hydrolysis of the first chloroform-soluble extracts from fractions II and III did not liberate perfluidone.

TLC indicated that the primary chloroform-soluble product in chloroform extracts 2 to 4 of fractions II, III, and IV was perfluidone, and from 75 to 100% of the ^{14}C in the chloroform extracts of the 2 N HCl hydrolysates of fractions II, III, and IV also appeared to be perfluidone. Chloroform extracts 2 to 4 of fraction II were combined, purified by TLC, derivatized with diazomethane, and subjected to GC. A single ^{14}C-labeled product with the same elution volume as the methylated derivative of perfluidone was eluted. The mass spectrum of this product was identical with that of the methylated derivative of perfluidone. Chloroform extracts 2 to 4 of fractions III and IV were examined by GC/MS in the same manner with identical results.

The water eluate from the XAD-2 column contained 12.3% of the ^{14}C originally present in the leaves. When this fraction was treated with acid under successively stronger conditions (Table I), 41.8% of the ^{14}C was rendered chloroform-soluble. Of the ^{14}C in the chloroform-soluble fraction, 16.8% appeared to be 3′-hydroxyperfluidone, 35.6% perfluidone, and 47.6% did not correspond to any of the standards by TLC.

Metabolite V from the Bio-Gel P-2 column was the 80% methanol-soluble metabolite present in the highest concentration. When it was treated with β-glucosidase, 95% of the ^{14}C was converted to a chloroform-soluble product as compared with 4% conversion with the no-enzyme control. Acid hydrolysis also liberated a chloroform-soluble product in 95% yield. In each case, HPLC and TLC indicated that the single chloroform-soluble radioactive product was 3′-hydroxyperfluidone. The structure was confirmed as 3′-hydroxyperfluidone by GC/MS comparison of the methylated hydrolysate with the methylated

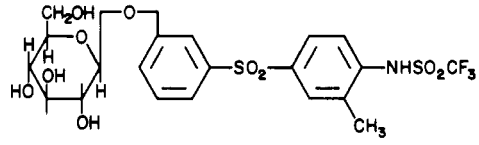

Figure 3. Proposed structure of metabolite V.

Table V. Percent Distribution of ^{14}C in Excised Peanut Leaves and the Shoots of Intact Peanut Seedlings[a]

Form of radioactivity	Excised leaves, %	Shoots of intact seedlings, %
Perfluidone	41	46
Perfluidone conjugates	16	5
3′-Hydroxyperfluidone conjugates	15	3
Unidentified $CHCl_3$-soluble hydrolysis products	7	16
Products remaining water-soluble after hydrolysis	18	22
80% methanol-insoluble residue	3	8

[a] Excised peanut leaves were treated for 48 h with 10 μM perfluidone, and peanut seedlings were treated for 8 days with 5 μM perfluidone.

standard. When the aqueous phase from the acid hydrolysate was analyzed for carbohydrates on both an SE 52 column and an EGS polyester column, the only components detected were α- and β-glucose. Quantitative analysis indicated that the combined molar ratio of α- and β-glucose to 3′-hydroxyperfluidone was 0.97:1. Based on these results, we concluded that metabolite V was the β-*O*-D-glucopyranoside with the structure shown in Figure 3. In an earlier report in which a different purification procedure was used, 3′-hydroxyperfluidone was liberated by β-glucosidase hydrolysis, but glucose was not detected in the reaction mixture until it was refluxed with methanol (Lamoureux and Stafford, 1974). The molar ratio of glucose to 3′-hydroxyperfluidone liberated from this earlier preparation by acid hydrolysis was 2.0:1. The differences between these two preparations may have been due to an artifact produced during the purification of the earlier sample under the more acidic conditions involving HPLC on a column of Biosil A with ethyl acetate:formic acid.

Although significant quantitative differences were observed between the products in the excised leaves and those in intact seedlings (Table V), the overall distribution of ^{14}C between different solubility classes was quite similar. The quantitative differences may have been due to alterations in metabolism caused by the much higher tissue levels of [^{14}C]perfluidone present in the excised leaves. The percent chloroform-soluble radioactivity in the excised leaves was similar to that in the leaves of the intact seedlings. In both cases nearly all of the radioactivity in the chloroform-soluble fraction was [^{14}C]perfluidone. Excised leaves contained a higher percentage of water-soluble metabolites that could be hydrolyzed to perfluidone or 3′-hydroxyperfluidone than the shoots of root-treated intact plants. This difference was also noted by TLC. The β-glucoside of 3′-hydroxyperfluidone was present in only

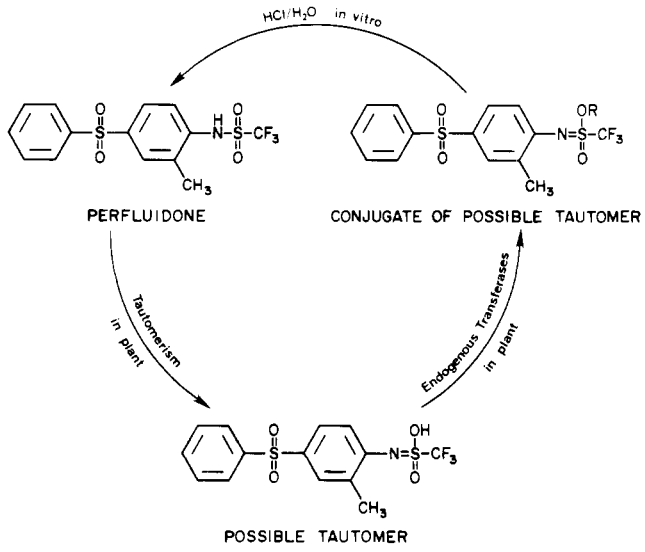

Figure 4. Possible explanation for water-soluble metabolites that liberate perfluidone on hydrolysis.

small amounts in intact shoots, but it accounted for 35% of the ^{14}C in the water-soluble fractions recovered from Bio-Gel P-2 chromatography of the extracts from excised leaves. The chemical nature of the water-soluble metabolites that released perfluidone during mild acid treatment was not established. Salt solutions of perfluidone were readily extracted into chloroform at pH 2, and perfluidone also readily partitioned into chloroform from tissue that was spiked with perfluidone immediately before homogenation and extraction. Attempts to form chelates of perfluidone with Mg^{2+} and Ca^{2+} were not successful. These results indicated that the water-soluble products in question were not simple salts or metal chelates.

Molecular models of perfluidone indicated that the sulfonamide nitrogen was too hindered to form conjugates readily with large molecules such as glucose. However, a tautomeric form of perfluidone, with a more exposed reactive site, might be expected to form an acid-labile conjugate. The trifluoromethylsulfonamide group of perfluidone appears to be an ideal system for tautomerism, and a possible tautomeric form of perfluidone and its corresponding conjugate(s) are shown in Figure 4. This tautomeric form of perfluidone was previously suggested by Lamoureux and Stafford (1974) and by Trepka et al. (1974). A conjugate of this nature would explain the presence of the acid-labile water-soluble products detected in high concentration in the excised leaves. These acid-labile products might also arise via conjugation of the *N*-hydroxy analogue of perfluidone. Although no evidence for this moiety was observed in this study, strong evidence for its existence in animals has been presented by Paulson et al. (1977).

ACKNOWLEDGMENT

The authors thank R. G. Zaylskie for his expert assistance in mass spectrometry. [^{14}C]Perfluidone and the various nonradioactive analogues were generously supplied by the 3M Company.

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Received for review August 23, 1976. Accepted December 29, 1976. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

Metabolism and Degradation of Glyphosate in Soil and Water

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Complete and rapid degradation of glyphosate [*N*-phosphonomethylglycine (**1**)] occurs in soil and/or water microbiologically and not by chemical action. Using soil/water shake flasks, up to 50% of each carbon of **1**-^{14}C was evolved as $^{14}CO_2$ in 28 days. In two of the three soils examined, **1** was 90% dissipated in less than 12 weeks. Aminomethylphosphonic acid (**2**), the only significant soil metabolite of **1**, also undergoes rapid degradation in soil. Short-term shake flask metabolism experiments with both ^{13}C- and ^{14}C-labeled **1** were carried out in order to permit facile, unequivocal spectral identification of **1** and its transient metabolite aminomethylphosphonic acid (**2**). Comparison of the metabolic samples to both reference standards and the spiked controls by means of ^{1}H, ^{31}P, and ^{13}C NMR, mass spectral analysis, ion-exchange chromatography, and thin-layer chromatography has unequivocally characterized both bound and unbound **1** and **2** in soil. The parent herbicide **1** has also been shown to be stable to sunlight, nonleachable in soil, to have a low propensity for runoff, and to have a minimal effect on microflora.

Glyphosate [*N*-phosphonomethylglycine (**1**), Figure 1] formulated (Roundup is a registered trademark of Monsanto Company, St. Louis, Mo.) as the isopropylamine salt is a new broad spectrum herbicide characterized by high unit activity, effective destruction of both annual and perennial herbaceous plants, inactivation by soil components, and favorable toxicology (Baird et al., 1971). This herbicide will be applied either preplant (foliar application

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