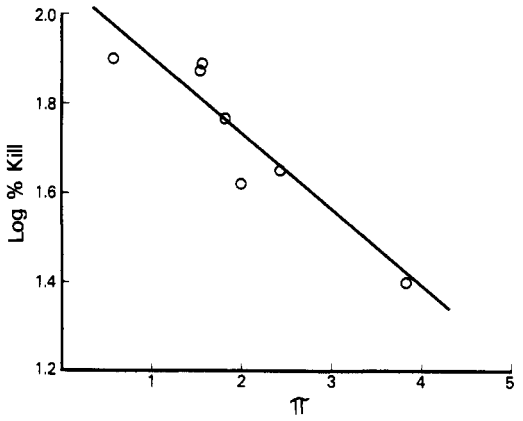

Figure 2. Correlation between log percent kill and the hydrophobic constant π for 0.1% 3-*p*-CO_2R in soybean oil.

elimination procedure (Draper and Smith, 1966). The correlation with π was poor for all eight substituents as shown in eq 2, where the correlation coefficient, r, was only 0.736. However, if the ethyl ester was omitted, an excellent correlation was found with π as shown in eq 3. The ethyl ester may be hydrolyzed easier than the other esters with the corresponding loss of toxicity. The coefficient of π is negative in these correlations, meaning that the most hydrophobic R's are the poorest toxicants. This is one of the first correlations of the toxicity of a phosphate–phosphorothionate type insecticide where the hydrophobic effect was found to be the dominant effect. It should be emphasized that the reason the electronic effects were not more prominent in this study is that they were purposely kept constant. Figure 2 graphically illustrates that this correlation of log percent kill with π is fairly respectable for an in vivo study.

The methyl and ethyl esters were studied in the ortho, meta, and para positions of phosphorothionate **3**. In each case, the para ester was found to be most toxic, the ortho ester second, and the meta ester the least toxic. An insufficient number of substituents were studied to know if this is a general trend. The Allen test data confirmed this trend with the exception of the *o*-CO_2Me group, which was found to be more toxic than the para isomer.

Diethyl carboalkoxyphenyl phosphorothionates show good potential as insecticides for the imported fire ant and the most promising substituents are the para esters with low hydrophobic character.

ACKNOWLEDGMENT

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Peanut Uptake and Metabolism of [^{14}C]Oxadiazon from Soil

Samuel W. Bingham,* Richard L. Shaver, and Charles L. Guyton

Peanuts were planted in 25-cm diameter pots, and [^{14}C]oxadiazon was mixed into the surface 0.64 cm of the soil. Cotyledons contained about 0.27 ppmw oxadiazon equivalent at 30 days after planting, while hypocotyls had a 0.10 ppmw concentration. After 61 days, pegs and immature nuts which were in direct contact with [^{14}C]oxadiazon-treated soil contained the highest concentration of herbicide. Hulls contained about 1 ppmw oxadiazon equivalent after 131 days (similar to a field commercial harvest); however, nuts accumulated only about 0.12 ppmw. The total peanut plant had an average of 0.59 ppmw oxadiazon equivalent at maturity. Hulls were found to have at least three ^{14}C-labeled compounds other than [^{14}C]oxadiazon. All other plant parts contained some [^{14}C]oxadiazon, but degradation products were below the level of detection, using TLC and autoradiography.

Oxadiazon [2-*tert*-butyl-4-[2,4-dichloro-5-(isopropoxy)-phenyl]-Δ^2-1,3,4-oxadiazolin-5-one] provided preemergence control of annual grasses and certain broadleaf weeds in peanuts, soybeans, rice, ornamentals, orchards, and turfgrasses (Burgaud et al., 1969). Oxadiazon was among a group of oxadiazole compounds discovered to have herbicidal properties in 1963 in the research laboratories of the Societe Usines Chimigues Rhone-Poulenc (Boesch and Metivier, 1965). Absorption, translocation, and metabo-

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lism of oxadiazon in rice revealed the major degradation products to be carboxylic acids, alcohols, and dealkylated derivatives (Hirata and Ishizuka, 1975; Ishizuka et al., 1974, 1975). Preemergence application of [^{14}C]oxadiazon in soybean showed relatively high amounts of ^{14}C in cotyledons and hypocotyls which passed through the treated soil surface (Ambrosi and Desmoras, 1974). Longer periods were required for translocation of ^{14}C to other portions of the shoot in amounts of up to 1.11 ppmw oxadiazon. The highest concentration of ^{14}C was found in the first trifoliate leaf and progressively less was found in succeeding leaves.

The residue of oxadiazon in soybeans was evaluated by Craine (Craine, 1975, 1976). Small amounts (0.22 ppmw) of oxadiazon were found in the bean and higher residues were located in leaves and stems. Some of the ^{14}C residues was attributed to air-borne transfer from the soil to aerial portions of the plant with larger ^{14}C concentrations in plant parts closer to the soil. The ^{14}C in soybean plants increased slowly until physiological maturity and reached a total of 0.84 ppmw (when calculated as oxadiazon) at 90 days after planting. Further evaluation revealed that approximately 46% of the residue in the foliage of soybean was [^{14}C]oxadiazon. A hydroxy metabolite (9%) was also reported. Hydrazine, DIMTH, detected by the Japanese workers (Hirata and Ishizuka, 1975; Ishizuka et al., 1974, 1975) in rice was not observed in soybean; however, 14 ^{14}C-labeled products were shown, using TLC analysis of soybean extracts.

Oxadiazon was rapidly and strongly bound to soil colloids (three soils used) after treatment (Ambrosi and Desmoras, 1973) and was postulated to preferentially adsorb on hydrophobic areas in soil organic matter (Carringer et al., 1975). Ambrosi et al. (1977) showed that oxadiazon degraded slowly in all soils, and at least three metabolites were detected, a phenolic, a carboxylic acid, and a dealkylated derivative.

The purpose of this study was to evaluate the uptake of [^{14}C]oxadiazon in the tissues of peanuts and to determine the metabolic products as ^{14}C components in various peanut tissues.

METHODS AND MATERIALS

Growth and Treatment of Peanut Plants. A Woodston sandy loam soil with bulk density of 1.32 g/cm^3 was obtained from Holland, VA, which is in the peanut production area of Virginia. Particle size analysis for sand, silt, and clay showed 75, 19, and 6%, respectively. The soil with 1.1% organic matter was high in magnesium and phosphate, medium in potash, and low in calcium. The pH was 5.9. Calcium sulfate was added to the top 3.8 cm of each pot at the rate of 1345 kg/ha to insure formation of peanuts in the soil. Peanut seed (*Arachis hypogaea* L. "Virginia bunch NC 17") were moistened in wet paper towels for 24 h before planting on July 15, 1976. Five seeds were placed 2.54 cm deep in the soil of each pot.

The top 0.64 cm of soil in each pot was mixed with enough oxadiazon (240 g/L of formulation batch no. 75–11) and [^{14}C]oxadiazon (74.76 μCi/mg, phenyl ring ^{14}C labeled, supplied by Rhone-Poulenc, Inc., Monmouth Junction, NJ) for 2.2 kg/ha and 3 μCi per pot, respectively. The [^{14}C]-oxadiazon was above 99% purity as shown by TLC, using benzene as developing solvent. Watering of pots was by subirrigation, except two–three times monthly 1.27 cm was applied, simulating rainfall from the top of the pot. Dicofol [1,1-bis(chlorophenyl-2,2,2-trichloroethanol)] or Plictran (tricyclohexyltin hydroxide) was applied for red spider mites and other insects at 5, 6, 7, and 10 weeks after planting the peanuts. These pesticides were used as wetting sprays on the foliage of the peanut. Growing

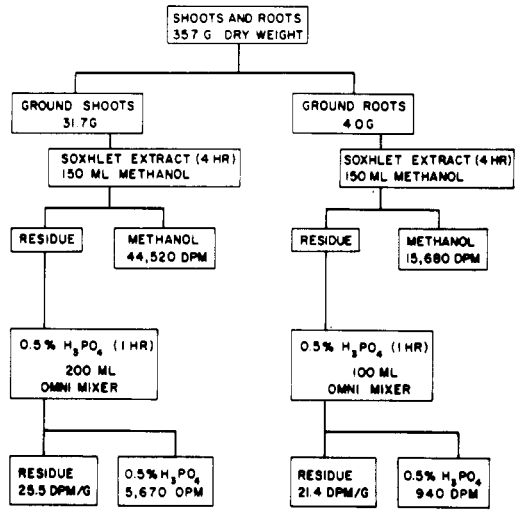

Figure 1. Extraction of [^{14}C]oxadiazon and ^{14}C metabolites from peanut plants at 61 days after planting and treating with the herbicide.

conditions included natural sunlight with temperatures ranging from a low of 25 to 37 °C and dark temperatures from 18 to 21 °C.

Six replications in a completely random design were utilized. After emergence of the peanut seedlings, plants were thinned to one per pot. Each treatment included one plant for autoradiography and two plants for combustion for ^{14}C liquid scintillation analyses at 30, 61, and 131 days after planting. Another 86 plants were utilized for residue analysis and evaluation of metabolism of [^{14}C]oxadiazon after 131 days.

Harvest and Radioassay of Peanut Plants. For autoradiography, plants were clipped off at the soil surface. Roots were removed from the soil and washed with water. Autoradiography was similar to procedures described by Crafts and Yamaguchi (1964). For ^{14}C analysis of plant parts, each plant was separated into shoots, roots, hypocotyls, and cotyledons at 30 days, shoots, roots, and immature nuts at 61 days and shoots, roots, peanut hulls, and nuts at 131 days after planting. After drying at 40 °C for 48 h in a forced air oven, plant parts were ground in a Wiley mill with a 20-mesh screen. Five subsamples were combusted with ^{14}C trapped in Riech (Rapkin and Riech, 1972) solution [mixture of 400 mL of toluene, 330 mL of phenylethylamine, 200 mL of methanol, 50 mL of distilled water and 7 g of 2-(4-biphenyl)-5-(*p-tert*-butylphenyl)-1,3,4-oxadiazole (Butyl-PBD)] in liquid scintillation vials (IN 4101 LS sample oxidizer). Liquid scintillation counting (Beckman LS 250) was utilized, and counts per minute (cpm) were corrected to disintegrations per minute (dpm). Each subsample was radioassayed for 10 min or to a 1% counting error. Untreated check plants were harvested and assayed in the same manner.

The 86 plants used for residue and metabolism of [^{14}C]oxadiazon were harvested similar to 131 day plants and frozen immediately until utilized for extraction of ^{14}C-labeled compounds.

Analysis for ^{14}C-Labeled Compounds. Details for extraction of [^{14}C]oxadiazon and ^{14}C-labeled metabolites from peanut plant materials are described in Figures 1, 2, 3, and 4. Methanol was the main extraction solvent during grinding in an Ommi mixer or of ground plant material from a Wiley mill. To remove additional ^{14}C from plant material, acetone, hexane, phosphoric acid, and ethyl acetate were selected. Millipore filters were used to separate plant material and solvents after extraction.

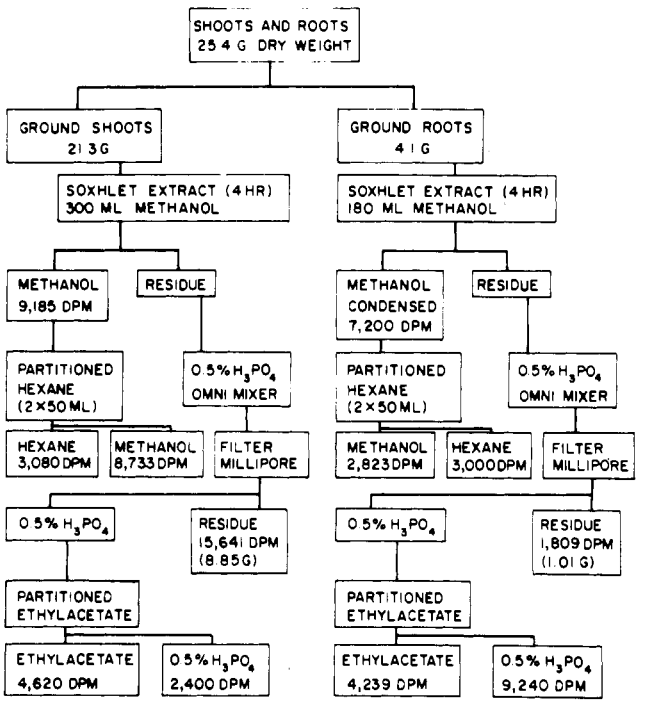

Figure 2. Extraction of [^{14}C]oxadiazon and ^{14}C metabolites from peanut plants at 131 days after planting and treating with the herbicide.

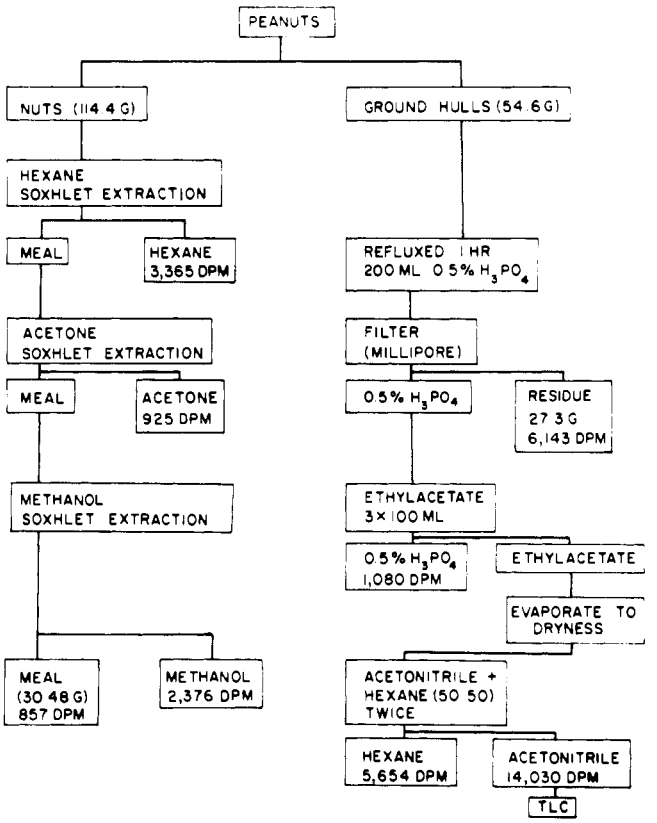

Figure 3. Extraction of [^{14}C]oxadiazon and ^{14}C metabolites from peanut hulls and nuts at 131 days after planting and treating with the herbicide.

Chemicals and Solvents Used for TLC Comparisons. Major metabolites of oxadiazon found in plants were obtained for TLC comparative evaluation in this study (Figure 5).

The solvents used for ascending development of TLC included (1) dichloromethane and methanol (90:10, v/v), (2) benzene (distilled), and (3) hexane and acetone (4:1, v/v). Silica gel TLC plates (50 μm) with fluorescent indicator were used to determine location of unlabeled oxadiazon (I, oxadiazon) and related compounds (II, alcohol; III, acid; IV, methoxy; and V, phenol; Figure 5), using ultraviolet light. Autoradiography of TLC plates was utilized to locate ^{14}C-labeled compounds. Liquid scintillation counting of silica gel fractions was utilized to quantitate respective metabolites.

Gas–liquid chromatography (Tracor Model 550 with ^{63}Ni electron-capture detector) was utilized to confirm the presence of oxadiazon in various extracts as observed during TLC.

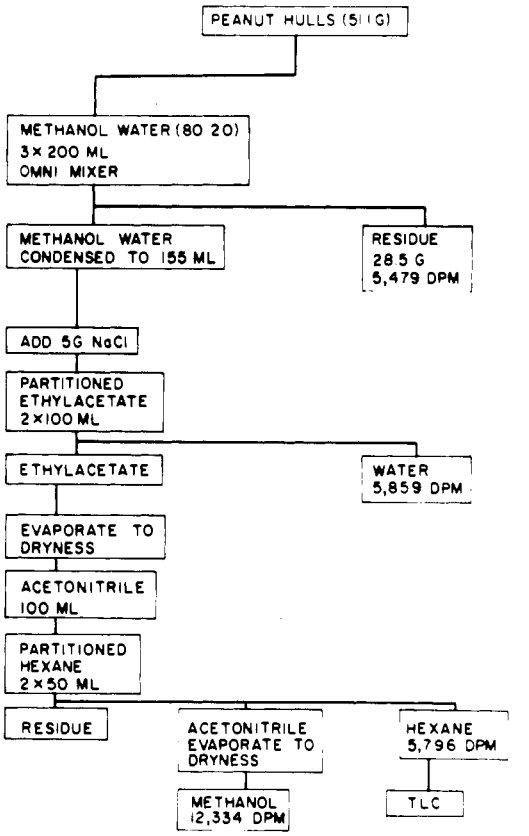

Figure 4. Extraction of [^{14}C]oxadiazon and ^{14}C metabolites from peanut hulls at 131 days after planting and treating with the herbicide.

RESULTS AND DISCUSSION

[^{14}C]Oxadiazon Uptake and Translocation in Peanut Plants. The ^{14}C-labeled herbicide was placed in the surface soil, and watering was mainly from subirrigation. As the peanut seed germinated, the cotyledons and hypocotyl passed through the [^{14}C]oxadiazon treated soil and picked up some ^{14}C by direct contact with the herbicide in the soil. At 30 days after planting, only the hypocotyls and cotyledons contained ^{14}C at significant levels for detection through autoradiography and combustion of tissue for liquid scintillation counting (Table I). Cotyledons absorbed enough to reach about $^1/_4$ ppmw of herbicide, while hypocotyls absorbed less chemical.

As more water (similating rainfall) was used and roots of the plants grew nearer the surface of the soil, [^{14}C]oxadiazon uptake and translocation to the foliage increased, giving a light image on autoradiographs. About $^1/_3$ ppmw of herbicide was detected in the foliage at 61 days after planting peanuts. Roots, pegs, and immature nuts contained approximately twice the concentration of herbicide as the foliage, although the shoot accounted for three-fourths of the plant material and a larger part of the ^{14}C

Table I. Uptake and Accumulation of [^{14}C]Oxadiazon in Various Parts of Peanut Plants after Preemergence Treatments

	30-day harvest		61-day harvest		131-day harvest	
plant part	fresh wt, g/plant	oxadiazon[a] equiv, ppmw	fresh wt, g/plant	oxadiazon[a] equiv, ppmw	fresh wt, g/plant	oxadiazon[a] equiv, ppmw
treated plants						
shoots	4.99	0.00	61.08	0.32	76.23	0.65
roots	1.22	0.00	8.54	0.63	11.82	0.65
hypocotyl	0.16	0.10				
cotyledon	0.19	0.27				
pegs and immature nuts			6.03	0.72		
hulls					24.36	0.98
nuts					32.92	0.12
total	6.56	0.01	75.65	0.39	145.37	0.59
untreated plants						
shoots	9.15	0.00	55.66	0.05	106.03	0.02
roots	1.75	0.00	8.68	0.02	20.03	0.01
hypocotyl	0.32	0.02				
cotyledon	0.39	0.00				
pegs and immature nuts			2.93	0.05		
hulls					38.82	0.00
nuts					42.30	0.01
total	11.61	0.00	67.27	0.05	207.18	0.01

[a] Oxadiazon equivalent in the various plant parts was calculated from dpm obtained after combustion, trapping of $^{14}CO_2$, and counting by liquid scintillation.

at this stage. Aerial transmission of [^{14}C]oxadiazon was not apparent since younger leaves accumulated as much or more ^{14}C than that accumulated by older leaves. The radioactivity found in the foliage of untreated control plants located in a separate section of the greenhouse was not explicable.

Hulls were in direct contact with [^{14}C]oxadiazon-treated soil and absorbed some of the herbicide. Surveys of the soil (scintillation counting of soil in Aquasol) showed the presence of ^{14}C to a depth of 5 cm (approximately 0.034 ppmw oxadiazon equivalent at 5 cm) after 131 days. After 131 days of harvest and mixing the top 5 cm of soil, surveys indicated approximately 1.74 ppmw oxadiazon equivalent in this layer of soil. The peanut pod developed and matured in this layer of soil. The herbicide continued to be absorbed and moved to the foliage during maturation of peanut plants from 61 days to 131 days. The shoot increased about 25% in fresh weight and contained twice the concentration of ^{14}C at the later stage. Root growth was almost complete at 61 days and the level of ^{14}C in roots was about the same until 131 days. The nuts accumulated little ^{14}C during maturation and had the least concentration of ^{14}C of the plant parts.

Isolation and Characterization of ^{14}C from [^{14}C]-Oxadiazon-Treated Plants. At 61 days after planting peanuts, methanol extracted 90% of the ^{14}C from the plant material (Figure 1). Dilute aqueous H_3PO_4 removed the remaining ^{14}C residue, leaving about 1% of the radioactivity in the plant residue. Shoots of peanut contained only three times as much ^{14}C as roots; however, the shoot weighed eight times more than the root.

After 131 days or mature peanut plants, methanol extracted about 28% of the ^{14}C from plant root tissue (Figure 2). Dilute aqueous H_3PO_4 removed another 64% of the radiolabeled compounds, leaving about 8% of the ^{14}C in the plant root residue. For shoot tissue, methanol extracted 34% of the ^{14}C, dilute H_3PO_4 removed 20% of the ^{14}C, and 45% of the ^{14}C remained in the shoot residue. Very little [^{14}C]oxadiazon is partitioned from the methanol with hexane which removes the nonpolar metabolites (Table II). Metabolites in hexane accounted for one-third of the methanol extractable ^{14}C from plant material (shoots and roots). After hexane partitioning, methanol contained oxadiazon and polar metabolites which represented about two-thirds of the ^{14}C in the methanol extract or one-fifth of the total radioactivity in the plant (roots and shoots).

Table II. Effectiveness of Various Solvents for Extraction of ^{14}C from [^{14}C]Oxadiazon-Treated Plants at Maturity (131 Days) or for Partitioning from Other Solvents (See Figure 2)

description of extract	% of ^{14}C found in plant sample	oxadiazon equiv, ppmw in fresh wt
shoots		
methanol (oxadiazon and polar metabolites)	25.3	0.16
hexane (nonpolar metabolites)	8.9	0.06
organosolubles after acid treatment	13.4	0.09
aqueous	7.0	0.05
nonextractable residue	45.4	0.30
roots		
methanol (oxadiazon and polar metabolites)	13.3	0.09
hexane (nonpolar metabolites)	14.2	0.09
organosolubles after acid treatment	20.1	0.13
aqueous	43.8	0.28
nonextractable residue	8.6	0.06

Table III. Effectiveness of Various Solvents for Extraction of ^{14}C from [^{14}C]Oxadiazon-Treated Peanut Hulls (54.58 g) and Nuts (114.4 g) of Mature Plants (131 Days) or for Partitioning from Other Solvents (See Figure 3)

type of extract	% of ^{14}C found	oxadiazon equiv, ppmw in fresh wt
hulls		
acetonitrile	52	0.44
hexane	21	0.18
aqueous (0.5% H_3PO_4)	4	0.03
residue (nonextracted)	23	0.19
total for hulls	100	0.84
nuts		
hexane (nonpolar metabolites)	45	0.05
acetone-methanol (polar metabolites)	44	0.05
residue (nonextracted)	11	0.01
total for nuts	100	0.11

Table IV. Separation of ^{14}C Metabolites in Acetonitrile Fraction from Hull Extracts on Thin-Layer Chromatograms Developed with Benzene and Dichloromethane-Methanol (90:10)

TLC developing solvent	R_f of metabolite	% ^{14}C on TLC plate	oxadiazon equiv, ppmw in fresh wt	% of total residue	identification
benzene	0.77	1	<0.005		
	0.45	66	0.29	30	oxadiazon
	0.30	3	0.01	2	unknown
	0.16	5	0.02	2	unknown
	origin to 0.07	23	0.12	12	alcohol and phenol
dichloromethane-methanol (90:10)	0.93	74	0.33	32	oxadiazon
	0.67	9	0.04	4	unknown
	0.37	7	0.03	3	phenol
	origin to 0.13	10	0.04	4	alcohol

Table V. Separation of ^{14}C Metabolites in Hexane Fraction from Hull Extracts on Thin-Layer Chromatograms Developed with Dichloromethane-Methanol (90:10) and Hexane-Acetone (4:1)

TLC developing solvent	R_f of ^{14}C metabolite	% ^{14}C on TLC plate	oxadiazon equiv, ppmw in fresh wt	% of total residue	corresponding TLC location of standards
dichloromethane-methanol (90:10)	0.95	1.2	0.002	0.2	
	0.90	1.2	0.002	0.2	oxadiazon
	0.80	84.3	0.163	16.7	oxadiazon plus plant extract
	0.53	6.5	0.012	1.2	
	0.33	3.8	0.007	0.7	phenol derivative
	origin to 0.07	2.7	0.005	0.5	alcohol (hydroxy derivative)
hexane-acetone (4:1)	0.60	59	0.115	11.7	oxadiazon
	0.40	20	0.039	4.0	phenol
	0.23	15	0.029	3.0	alcohol
	origin to 0.10	04	0.012	1.2	acid

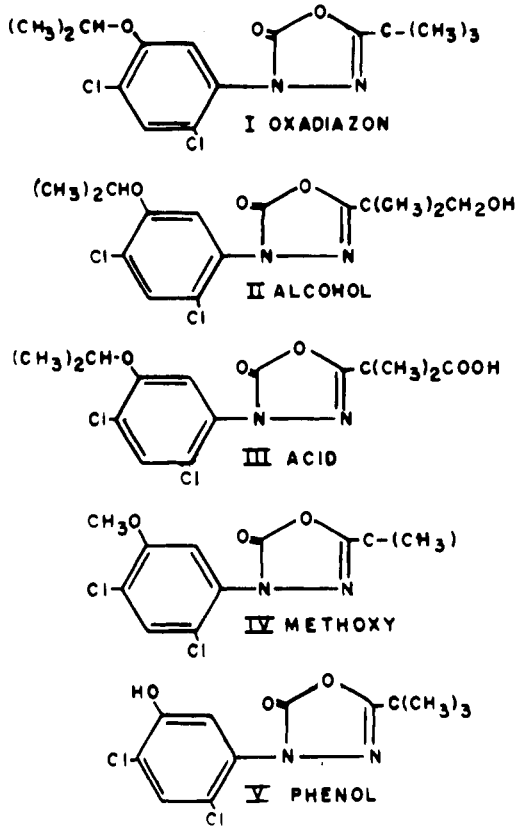

Figure 5. Authentic chemicals utilized for TLC comparison with extracted ^{14}C-labeled compounds (I, oxadiazon; II, alcohol; III, acid; IV, methoxy; and V, phenol derivatives).

The hull was the only part containing large enough concentrations of ^{14}C for separation and/or identification of metabolites (Table III). After fractionation into acetonitrile, hexane, aqueous, and plant residue portions, 0.84

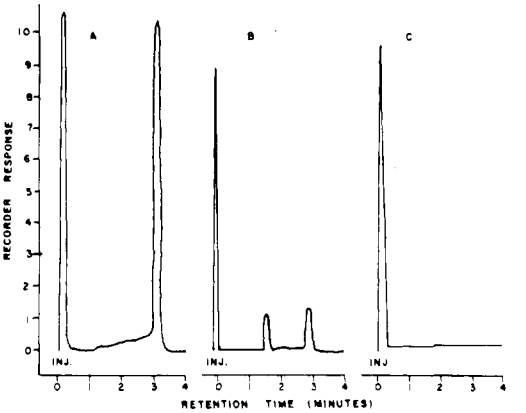

Figure 6. Representative chromatograms of standard oxadiazon (a), acetonitrile extract from hulls of oxadiazon treated plants (b), and acetonitrile extract from hulls of untreated check plants (c) harvested at 131 days.

ppmw of oxadiazon equivalent was observed. This compares well with 0.98 ppmw equivalent from combustion of hulls, collection of $^{14}CO_2$ and evaluation by liquid scintillation techniques (Table I). The presence of ^{14}C in the hexane fraction indicated that nonpolar metabolites were present. The large fraction of ^{14}C in the acetonitrile was indicative of [^{14}C]oxadiazon and/or polar metabolites. Gas chromatographic analysis confirmed the presence of oxadiazon in this fraction (Figure 6).

Dilute H_3PO_4 extracted 77% of the ^{14}C in peanut hulls (Figure 3) which contained approximately 0.84 ppmw oxadiazon equivalent (includes all metabolites). About 52% was partitioned into acetonitrile and 21% went into the hexane fraction, leaving 4% in aqueous solution. TLC revealed that the acetonitrile fraction contained enough [^{14}C]oxadiazon for 0.29 ppmw of herbicide in the hulls (Table IV and Figure 3). The alcohol (II) and phenol (V) derivatives of oxadiazon were left at the origin, where 12%

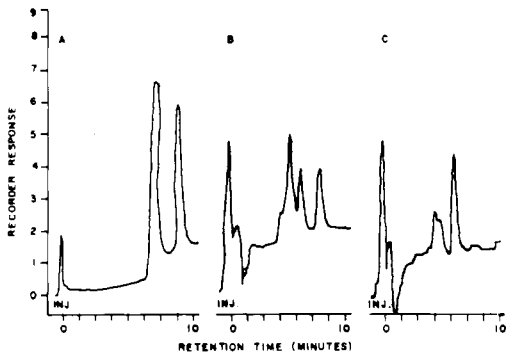

Figure 7. Representative chromatogram of standard oxadiazon and methoxy derivative (a) and hexane partition solvent from acetonitrile–hull extract of oxadiazon treated peanut plants (b) and hexane partition solvent from acetonitrile–hull extract of untreated peanut plants (c) harvested at 131 days.

of the ^{14}C residue remained, with benzene used as developing solvent for TLC. From a different sample, methanol and water (80:20, v/v) extracted 81% of the ^{14}C from peanut hulls (Figure 4).

About 20% of ^{14}C was partitioned into hexane and chromatographed as four or more metabolites, including phenol and alcohol metabolites (II and V) at concentrations approximating 0.01 to 0.04 ppmw each (Table V and Figure 4) in the hulls.

The hexane fraction from the hull extracts contained some [^{14}C]oxadiazon (Table V). The [^{14}C]oxadiazon with plant extract moved slower on TLC plates than standard [^{14}C]oxadiazon as verified by overspotting on TLC plates and GLC analysis (Figure 7). The methoxy derivative (IV) was not detected by TLC or GLC.

Peanut plants absorbed relatively low amounts of [^{14}C]oxadiazon from the soil during the 131 days of growth to maturity. Approximately 0.74% of the applied oxadiazon equivalent was located in the plants at maturity. This amount was distributed throughout the plant material with the largest concentration in or on the peanut hulls. The nuts contained the lowest concentration of oxadiazon equivalent of the plant parts.

Oxadiazon metabolism was apparently slow in the peanut hull, with approximately 0.3 ppmw remaining as oxadiazon. The major pathway of metabolism involved oxidation and dealkylation to phenol and alcohol derivatives, which accounted for less than 0.16 ppmw of the residue. Other unidentified material extracted accounted for 0.3 ppmw of the residue and 0.23 ppmw of the residue was not extracted.

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Metabolic Fate of *cis*-Photochlordane in the Rat. 1. Excretion, Tissue Distribution, and Preliminary Characterization of Metabolites

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Male rats treated orally, or intraperitoneally, with 3.12 mg of *cis*-photochlordane/rat cumulatively excreted, respectively, about 86 and 88% of the dose in 3 weeks. The half-life of the compound was less than 1 day in orally treated and about 7 days in intraperitoneally treated rats. Highest concentration of the residues at the end of the 3 weeks was found in fat. Intraperitoneally treated rats showed higher residual radioactivity in all tissues. Analyses of the organic extracts of feces and urine from the treated rats showed at least 22 compounds in the former and 15 compounds in the latter. These metabolites were isolated, purified, and chromatographically characterized.

Chlordane has been a widely used insecticide for agricultural, industrial, and household purposes. The technical product is a mixture of several compounds (Cochrane and Greenhalgh, 1976) of which *cis*-chlordane is an important constituent. *cis*-Chlordane is more persistent in the environment (Sanborn et al., 1976) and more toxic to fish than other related components of technical chlordane (Podowski et al., 1979). Exposure of *cis*-chlordane to UV irradiation or sunlight (Benson et al., 1971) results in the formation of the photoisomer, *cis*-photochlordane, which has higher acute toxicity than the parent compound to several vertebrate species (Podowski et al., 1979). Whereas the fate of photoisomers of other cyclodienes has been studied in various organisms [photodieldrin in the rat (Dailey et al., 1970, 1972; Reddy and Khan, 1974), mouse, housefly, (Reddy and Khan, 1974), rabbit (Reddy and Khan, 1975, 1977a), and rhesus monkey (Nohynek et al., 1979); photoisodrin in mouse and houseflies (Reddy and

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