Table III. Distribution of Radioactivity in the Solvent Extracted Soils as Determined by Treatment with Boiling Aqueous Triethanolamine

time, days	% of applied ^{14}C in bound form, by combustion	% of bound ^{14}C associated with[a]		
		fulvic substances	humic substances	total
1	8	<2	<2	<2
28	26	3	6	9
56	36	5	14	19
108	35	5	12	17
154	37	12	18	30

[a] Average of duplicate analyses, expressed as percent of applied [^{14}C]diclofop-methyl.

humic fractions was less than that determined by direct oxidative combustion of the soils prior to alkaline extraction (Table III). This implied that not all of the radioactivity was being extracted by the hot triethanolamine, and that a portion was remaining with the alkali insoluble humin fraction of the soil.

Chromatographic analysis of the ether extracts derived from the fulvic acid moiety confirmed diclofop-acid to be the only identifiable ^{14}C-containing products, as reported for the previous laboratory studies (Smith, 1977). The amounts of [^{14}C]diclofop-acid released from the soil at the end of the growing season seemed to be slightly greater than those observed from earlier field studies (Smith, 1979).

The identity of the bound radioactivity has not been established. It has been inferred that a portion of this ^{14}C may be attributed to diclofop-acid which, with time becomes irreversibly bound, or complexed, to soil components and from which it can only be released by treatment with hot triethanolamine (Smith, 1977, 1979). The earlier studies have also shown that the recoveries of diclofop-acid from fortified soils using hot triethanolamine is low, a fact attributable to adsorption of diclofop-acid to precipitated humic substances during workup (Smith, 1979). Thus, the radioactivity associated with the humic fraction could include contributions from the bound diclofop-acid, as well as from incorporation of small radioactive fragments (resulting from fission of the herbicide ring systems) into soil organic matter.

The main interest regarding bound pesticide residues is whether they will affect the growth of, or be taken up into, future crops (Kaufman et al., 1976; Helling and Krivonak, 1978). Experiments are therefore in progress to determine the phytotoxic significance, if any, of bound residues originating from diclofop-methyl applications.

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Metabolism and Selectivity of Fluchloralin in Soybean Roots

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Fluchloralin [*N*-(2-chloroethyl)-2,6-dinitro-*N*-propyl-4-(trifluoromethyl)aniline] is metabolized rapidly by soybean [*Glycine max* (L.) Merr.] roots to several chloroform-soluble and water-soluble metabolites and high levels of methanol-insoluble residue. No single metabolite represented more than 4% of the total ^{14}C in the roots. The posttreatment solution contained fluchloralin and all chloroform-soluble and possibly all water-soluble metabolites found in the root tissues. The metabolites in the posttreatment solution were formed by root tissues and not by chemical, photochemical, or microbial degradation. Four chloroform-soluble metabolites were isolated from the posttreatment solution and characterized by mass spectrometry. Soybean roots metabolized fluchloralin at a higher rate than corn (*Zea mays* L.) roots. This correlated with the greater resistance displayed by soybean roots (relative to corn roots) to fluchloralin injury.

Fluchloralin [*N*-(2-chloroethyl)-2,6-dinitro-*N*-propyl-4-(trifluoromethyl)aniline] is a relatively new substituted dinitroaniline herbicide and is similar structurally to trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine), a major dinitroaniline herbicide. The fate of these compounds in plants is important because of the increased use of dinitroanilines to control annual grasses and broadleaf weeds in major crops.

Dinitroaniline herbicides undergo several biotransformation reactions including N-dealkylation, nitro reduction, and cyclization when exposed to ultraviolet light (Leitis and Crosby, 1974; Newsom and Woods, 1973; Nilles and Zabik, 1974; Plimmer and Klingebiel, 1974), incorporated in the soil (Golab et al., 1970, 1975; Golab and Althaus,

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1975; Kearney et al., 1974, 1976; Probst et al., 1967; Smith et al., 1973), or incubated with microorganisms (Laanio et al., 1973; Williams and Feil, 1971). In soil, fluchloralin was dealkylated to *N*-(2-chloroethyl)-, *N*-propyl-, and unsubstituted α,α,α-trifluoro-2,6-dinitro-*p*-toluidine and cyclized to 2-ethyl-7-nitro-5-(trifluoromethyl)benzimidazole and 5-nitro-7-(trifluoromethyl)quinoxaline (Kearney et al., 1976). With the exception of the *N*-(2-chloroethyl) derivative, all of the above compounds were identified as photodegradation products of fluchloralin (Nilles and Zabik, 1974).

Little is known about the metabolic fate of the dinitroaniline herbicides in plants. Small amounts of the *N*-propyl- and *N*-propyl-2-amino derivatives and 4-(dipropylamino)-3,5-dinitrobenzoic acid were isolated from carrot (*Daucus carota* L.) roots grown in [$^{14}CF_3$]trifluralin-treated soil (Golab et al., 1967). Unchanged trifluralin constituted 84% of the radioactivity in the carrot roots. In contrast, trifluralin was metabolized extensively in peanut (*Arachis hypogaea* L.) and sweet potato [*Ipomoea batatas* (L.) Lam.] roots treated in nutrient solution (Biswas and Hamilton, 1969). Two N-dealkylated and reduced derivatives and α,α,α-trifluoro-2,6-dinitro-*p*-cresol were identified tentatively as minor metabolites of benefin (*N*-butyl-*N*-ethyl-α,α,α-trifluoro-2,6-dinitro-*p*-toluidine) in peanut and alfalfa (*Medicago sativa* L.) plants (Golab et al., 1970). Over 90% of the radioactivity in the above crops was unidentified polar products. Profluralin (*N*-cyclopropylmethyl-α,α,α-trifluoro-2,6-dinitro-*N*-propyl-*p*-toluidine) was metabolized rapidly to numerous nonpolar and polar metabolites in peanut (Wright et al., 1975) and soybean (Sumner et al., 1976). Several metabolites, including 2-methyl-4-nitro-6-(trifluoromethyl)-1*H*-benzimidazole were isolated from an incubation medium containing excised soybean leaves and ^{14}C-ring-labeled profluralin (Sumner et al., 1976).

The objectives of this study were to determine the extent of fluchloralin metabolism in soybean roots and to isolate and characterize structurally some of the metabolites. In addition, the metabolism of fluchloralin in resistant soybean was compared to susceptible corn to determine if differential metabolism is a factor in fluchloralin selectivity.

MATERIALS AND METHODS

General Methods. Thin-layer chromatograms were developed to a 15-cm solvent front in one of the following solvent systems (v:v): (1) benzene/ethyl acetate (2:3), (2) cyclohexane/ethyl acetate (17:3), (3) ether/hexane (3:1), (4) benzene/ether (3:1), (5) hexane/acetone (5:1), (6) benzene/ethyl acetate/acetic acid (40:60:1), (7) ethyl acetate/xylene/acetic acid/water (25:1:2:2), (8) chloroform/methanol/water (65:25:3), and (9) chloroform/methanol/water/acetic acid (65:25:5:1). Radioactive zones on thin-layer chromatograms were detected with a radiochromatogram scanner and/or autoradiography. Fluchloralin and metabolites I and II also were detected by their yellow color.

The ^{14}C content of insoluble plant residues was quantitated by liquid scintillation counting after combustion to $^{14}CO_2$ in a Model 306 Packard Tri-Carb sample oxidizer. All other quantitative measurements of radioactivity were made directly by liquid scintillation spectrometry. The effluent from column chromatograms was monitored continuously with a radioactive flow monitor.

Electron impact mass spectra were obtained with a Varian MAT CH5 DF spectrometer at 70 eV. Samples that would gas chromatograph were introduced via a Varian Aerograph Series 200 gas chromatograph interfaced directly to the ion source (ambient temperature). This GC was equipped with a 3 m × 2 mm i.d. glass column packed with 3.5% OV-101 on 100–200 mesh Gas-Chrom Q. GLC conditions were as follows: helium carrier gas, 15 mL/min; column inlet temperature, 225 °C; and column temperature program, 2 min at 100 °C, 5 °C/min from 100 to 250 °C. Samples that would not gas chromatograph were inserted directly into the ion surce (temperature 250 °C) via a solid sample probe. Micro KBr pellet (1.5 mm) spectra were obtained with a grating IR spectrometer equipped with a beam condenser.

Radiochemical Purity. Uniformly ^{14}C-ring-labeled fluchloralin (sp act. 14 mCi/mmol) was purified by TLC in solvent 2. The radiochemical purity of the recovered [^{14}C]fluchloralin was at least 99% when analyzed by TLC in solvents 1 and 2.

Plant Material and Treatment. Soybean seeds (cv. Ada) were surface sterilized in 5% sodium hypochlorite and germinated between moist paper towels in the dark at 27 °C. Four days after germination the roots of 20–25 seedlings were inserted in a 300-mL foil-covered beaker containing 50 mL (555 000 dpm) of [^{14}C]fluchloralin solution (5 μM in 1% acetone). A total of 1.5 to 1.9 × 10^7 dpm of [^{14}C]fluchloralin was supplied to 600–700 seedlings in each experiment. The beakers were placed in a controlled-environment chamber with a 14-h photoperiod, 6.5 klux light intensity, 25 ± 2 to 23 ± 2 °C daytime temperature, and a 50 ± 5% relative humidity. The [^{14}C]-fluchloralin-treated seedlings were harvested after 48 h by rinsing the roots in distilled water, separating the seedlings into roots, hypocotyls, and cotyledons, and freezing for subsequent analysis.

Isolation and Purification of Metabolites from the Posttreatment Solution. Soybean seedlings absorbed 30–40% of the treatment solution over the 48-h treatment period. The remaining solution was designated as the posttreatment solution.

The purification of fluchloralin and its metabolites isolated from the posttreatment solution is outlined in Figure 1. All procedures were performed under low-intensity incandescent light to minimize photodegradation of fluchloralin and/or its metabolites. The posttreatment solution was partitioned four times with an equivalent volume of chloroform. The combined chloroform fraction was dried with anhydrous sodium sulfate, concentrated to dryness, redissolved in chloroform or methanol, and streaked across two 5 × 20 cm thin-layer plates. The plates were developed in solvent 1, scanned, and divided into zones A–E corresponding to peaks of radioactivity. The silica gel from each of the zones was eluted with ethyl acetate (zones A, B, and C) or absolute methanol (zones D and E). Further TLC separation of the eluted radioactivity from zones A to E gave the components as indicated in Figure 1. The major degradation products in zones A, B, C, and E were designated metabolites I, II, III, and IV, respectively. Each zone also contained one or more minor radioactive components.

The aqueous phase was concentrated under vacuum to 50 mL, absorbed to a 2.5 × 25 cm column of Amberlite XAD-2 and washed with distilled water. The water wash was discarded. Absorbed ^{14}C polar metabolites were eluted with methanol, concentrated to dryness, redissolved in 3 mL of water, and applied to a 1.5 × 50 cm column of DE-52 anion-exchange cellulose that had been previously equilibrated with 3.0 N acetic acid and washed with water to an effluent pH of 4.0–5.0. Two peaks eluted from the DE-52 column in one void volume with distilled water at a flow rate of 0.75 mL/min. The second peak was resolved

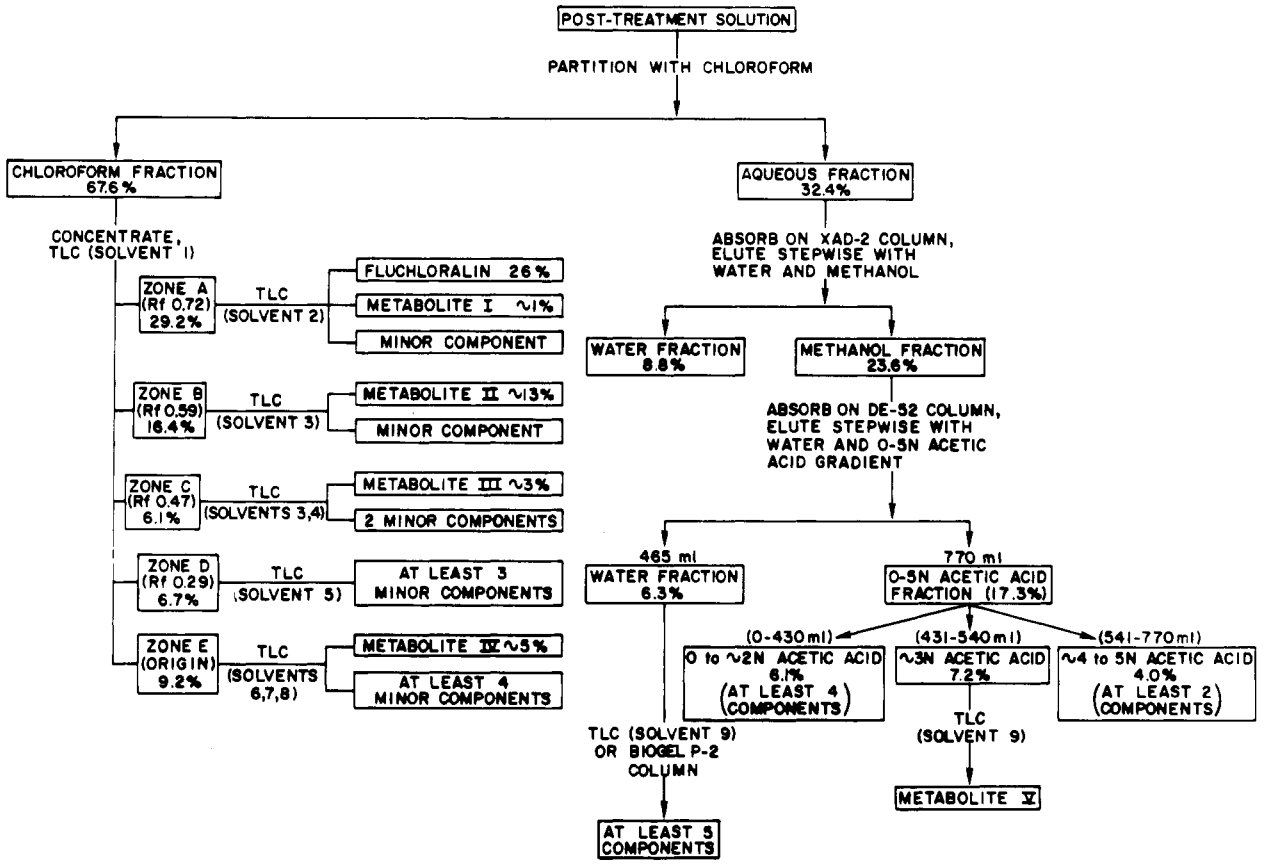

Figure 1. Scheme for the isolation of fluchloralin and metabolites I–V from the posttreatment solution after the treatment of soybean seedlings with [^{14}C]fluchloralin for a 48-h period.

into at least four radioactive components by TLC or column chromatography (1 × 55 cm Bio-Gel P-2 column). The DE-52 column was then eluted at 0.75 mL/min with a linear acetic acid gradient of 375 mL of 5.0 N acetic acid into 375 mL of water. One symmetrical peak eluting at approximately 3.0 N acetic acid (431–540 mL) was lyophilized, redissolved in 2 mL of water, purified by TLC in solvent 9, and designated as metabolite V. Recoveries of ^{14}C were generally better than 80% for the purification steps outlined in Figure 1.

Evaluation of Possible Nonplant Effects. Experiments were conducted to show that metabolites found in the posttreatment solution were due to root metabolism and not chemical, photochemical, or microbial degradation. Several experiments were conducted: (1) 50 mL of the [^{14}C]fluchloralin treatment solution without plants was placed in the dark for 48 h; (2) soybean seedlings treated with [^{14}C]fluchloralin as described were placed in the dark for 48 h; (3) soybean seedlings were grown in distilled water for 48 h, the plants were removed, and [^{14}C]fluchloralin was added to the remaining water (~35 mL) to give 5 μM fluchloralin in 1% acetone. This solution, excluding plants, was placed in the dark for an additional 48 h; (4) excised soybean roots were grown in the dark for 180 h in sterile liquid culture which contained 5 μM [^{14}C]fluchloralin. In all experiments to evaluate nonplant effects the posttreatment solution was partitioned with chloroform and the chloroform phase fractionated by TLC as described. The aqueous phase was discarded.

Extraction and Purification of Metabolites from Soybean Roots. Treated root tissue was frozen in liquid nitrogen, ground to a fine powder, and homogenized in an Omnimixer with a 5:1 (v/w) ratio of cold 80% methanol. The homogenate was filtered, and the remaining residue was extracted two additional times with 80% methanol. The methanol was removed from the combined filtrates under vacuum at 40 °C and the remaining aqueous solution was partitioned four times with an equivalent volume of diethyl ether. Both the ether and aqueous phases were fractionated into their numerous ^{14}C components as described (Figure 1).

Hypocotyl and cotyledon tissues were lyophilized, ground in a Wiley Mill, and combusted for quantitation of ^{14}C as described previously.

Characterization of Metabolites. Fluchloralin and its metabolites were characterized by spectral and/or chemical means. Fluchloralin, metabolite I, and metabolite III were subjected to GC–MS. Metabolite II was analyzed by MS (probe sample) and IR. Underivatized metabolite IV was subjected to both GC–MS and MS (probe sample). Fluchloralin and metabolite I were also subjected to the Bratton-Marshall diazotization and coupling reaction with and without previous treatment with 0.5% aqueous $SnCl_2$ (Shimabukuro et al., 1973). Metabolite IV was acetylated with acetic anhydride and fused $ZnCl_2$ for 3 h at 0 °C (Wolfrom and Thompson, 1963). The acetylated product was extracted with benzene (2 × 60 mL) and analyzed by GC–MS. Metabolite V (1 μg) was hydrolyzed under nitrogen with dilute acid (0.1 N HCl at 50 °C for 12 h) or strong acid (1.0 N HCl at 100 °C for 12 h). The acid hydrolysates were partitioned with ethyl ether (4 × 3 mL). The ether extracts were evaporated to dryness, redissolved in a small volume of methanol, and chromatographed by TLC in solvent 8.

Fluchloralin Selectivity. The effect of fluchloralin on the growth of soybean and corn (W64A × OH43) roots was measured with a petri dish bioassay as described by Parker (1966). Each petri dish contained five soybean or corn seedlings. The experiment was a completely random design and each treatment (one petri dish) was replicated

Table I. Distribution of ^{14}C in Soybean Seedlings Treated with ^{14}C-Ring-Labeled Fluchloralin

	% of total ^{14}C recov.[a]
posttreatment solution	23.6 ± 2.1
seedlings	76.4 ± 2.1
roots	(97.4 ± 0.2)[b]
hypocotyls	(2.0 ± 0.1)[b]
cotyledons	(0.6 ± 0.1)[b]

[a] Results are averages of four experiments (600-700 plants per experiment). Average recovery was 85.9 ± 2% based on administered dose. [b] Percent distribution of ^{14}C in the soybean seedlings.

Table II. Distribution of ^{14}C in Soybean Roots Treated with ^{14}C-Ring-Labeled Fluchloralin[a]

fraction	% of total ^{14}C
ether-soluble	31.3
(fluchloralin)	(13)
(metabolite I)	(~1)
(metabolite II)	(~2)
(metabolite III)	(~3)
(metabolite IV)	(~3)
(at least 11 minor ^{14}C components)	(~9)
aqueous	35.1
(metabolite V)	(4)
(at least 11 ^{14}C components)	(~31)
insoluble residue	33.6

[a] Average of two experiments.

three times. The experiment was conducted twice, and data from the two experiments were combined for statistical analysis.

The metabolism of [^{14}C]fluchloralin in soybean was compared with that in corn. The roots of 4-day-old corn and soybean seedlings were treated as described previously. Two beakers each of corn and soybean seedlings were treated and one beaker of each species was harvested after 24 and 48 h. The ^{14}C in the seedling roots was extracted and fractionated into fluchloralin, ether-soluble metabolites, water-soluble metabolites, and methanol-insoluble residue as described previously. The experiment was conducted twice and the results expressed as an average of the two experiments.

RESULTS

Metabolism of Fluchloralin in Soybean Roots. Most of the fluchloralin in the treatment solution was absorbed by the roots of the 4-day-old soybean seedlings and metabolized rapidly. The distribution of ^{14}C in soybean seedlings is shown in Table I. Approximately 76% of the administered ^{14}C was present in the seedlings after 48 h. The fractionation of the ^{14}C remaining in the posttreatment solution showed only 26% as unchanged fluchloralin (Figure 1). This suggests rapid uptake and metabolism of fluchloralin by the roots and secretion or release of fluchloralin and/or its metabolites into the treatment solution. The distribution of ^{14}C within the seedlings showed limited acropetal translocation of fluchloralin and/or its metabolites. This agreed with the results of several trifluralin translocation studies (Ashton and Crafts, 1973). The seedling roots developed typical dinitroaniline injury symptoms (swelling of root tips and inhibition of new secondary root formation), but no visible symptoms of shoot injury appeared.

Fluchloralin was metabolized in soybean roots to at least 15 ether-soluble (nonpolar) and 12 water-soluble (polar) ^{14}C components. The terms nonpolar and polar were assigned arbitrarily to the organic and aqueous fractions, respectively. However, some of the ^{14}C components that partitioned into the organic phase were quite "polar" relative to the parent fluchloralin. Due to incomplete partitioning, some of the "polar" components in the ether-soluble phase and some of the water-soluble components may be the same. The distribution of ^{14}C in soybean roots is shown in Table II. The numerous ^{14}C components present in relatively low amounts made precise quantitation of each metabolite extremely difficult. None of the metabolites accounted for more than 4% of the total ^{14}C in the roots.

Components in Posttreatment Solution. The fractionation of ^{14}C in the posttreatment solution is shown in Figure 1. The values in Figure 1 are averages of two experiments. The treatment solution contained all the nonpolar and probably all the polar ^{14}C components detected in the roots (Table II). The relative concentration of metabolite II was greater in the posttreatment solution than in the roots (Table II). Peanut seedlings root-treated with [$^{14}CF_3$]profluralin also released metabolites of profluralin into the treatment solution (Wright et al., 1975).

R_f values in TLC solvent systems and the GLC retention times of fluchloralin and its metabolites are shown in Table III. Metabolite II was apparently unstable at high temperatures and would not GC. The identical retention times observed for metabolites III and IV are discussed later.

Evaluation of Nonplant Effects. The experiments demonstrated that the metabolites in the posttreatment solution were formed by the roots and not by chemical, photochemical, or microbial degradation. Fluchloralin degradation within 48 h in the dark was negligible when [^{14}C]fluchloralin was added to either distilled water or to water which had been exposed to soybean roots for 48 h. Fluchloralin metabolites were detected in the posttreatment solutions of soybean roots treated in the dark under sterile and nonsterile conditions. These metabolites were identical qualitatively by TLC to those isolated from the posttreatment solution of seedlings treated in the light under nonsterile conditions.

Characterization of Metabolites. The spectroscopic analyses were performed on the metabolites isolated from the posttreatment solution because of their higher degree of purity than the same compounds extracted from the

Table III. Thin-Layer and Gas-Liquid Chromatographic Comparison of Fluchloralin and Its Metabolites

	TLC							GLC,[a]
	R_f in solvent system							retention
compd	1	2	3	4	7	8	9	time, min
fluchloralin	0.72	0.64	0.89	0.95				20.0
metabolite I	0.72	0.42						18.8
metabolite II[b]	0.59	0.11	0.42	0.39				
metabolite III	0.47	0.0	0.49	0.37				22.5
metabolite IV	0.0	0.0			0.56	0.65		22.5
metabolite V						0.28	0.40	

[a] GLC parameters are found in the Materials and Methods section. [b] Metabolite IIB.

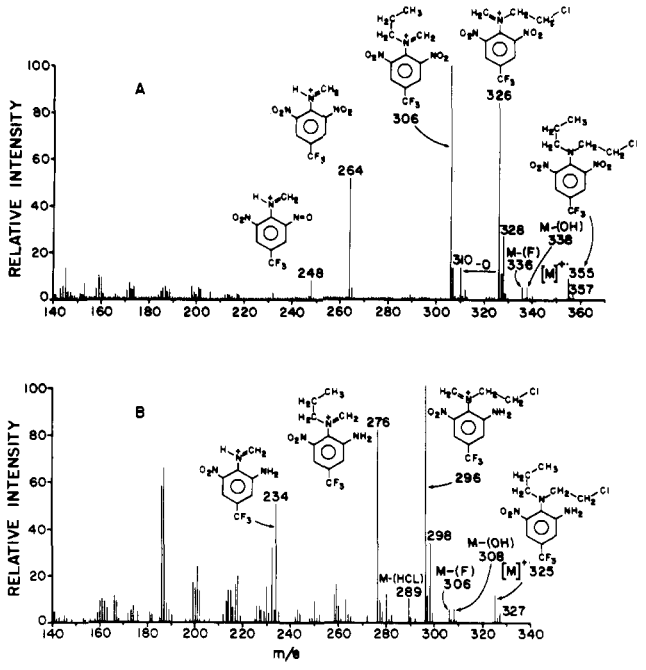

Figure 2. Mass spectra of fluchloralin (A) and metabolite I (B).

roots. Structural assignments to metabolites were made primarily on the evidence from mass spectrometry. Except for metabolite III, no authentic standards were available for chromatographic or spectroscopic comparison.

Fluchloralin. The mass spectrum of analytical grade fluchloralin is shown in Figure 2A. Fluchloralin isolated from soybean roots and the posttreatment solution gave a spectrum qualitatively identical to that of Figure 2A. This spectrum was characterized by a molecular ion at m/e 355 and ion fragments corresponding to the loss of OH and F. The presence of chlorine in the molecule is confirmed by the characteristic 3:1 isotopic ratios at m/e 355:357 and m/e 326:328. The intense ion fragments at m/e 326 (M $- C_2H_5$), 306 (M $- CH_2Cl$), and 264 (M $- C_4H_8Cl$) are consistent with the mass spectra of tertiary, alkylaryl amines (Budzikiewicz et al., 1967). The ions at m/e 310 and 248 most likely resulted from the loss of an oxygen atom from the even electron ions at m/e 326 and 264, respectively.

Metabolite I. The mass spectrum of metabolite I (Figure 2B) resembled that of the parent fluchloralin (Figure 2A). The molecular ion at m/e 325 was 30 mass units less than that of fluchloralin. The major ion fragments at m/e 296, 276, and 234 were also 30 mass units less than their analogous ions at m/e 326, 306, and 264, respectively, in the fluchloralin spectrum. These analogous ions were formed by the loss of the same fragments from their respective parent ions. Thus, alkyl side chains attached to the aniline nitrogen were still intact in metabolite I. The loss of OH and F confirms the presence of nitro and CF_3 groups. The only plausible structure which is consistent with this spectrum (Figure 2B) is the monoreduced derivative, 2-amino-6-nitro-*N*-(2-chloroethyl)-*N*-propyl-α,α,α-trifluoro-*p*-toluidine.

Metabolite I was positive to the Bratton-Marshall diazotization and coupling reaction. This confirmed the presence of a free aromatic amino group on the molecule. Fluchloralin was positive to the Bratton-Marshall reaction only after reduction with $SnCl_2$.

Metabolite II. The major ^{14}C component isolated from the chloroform phase of the posttreatment solution in each of six experiments was a yellow compound designated metabolite II (Figure 1). Although fractionation proce-

Table IV. Thin-Layer Chromatographic Comparison of Metabolites IIA, IIB, and IIC

compound	R_f in solvent system[a] 1	3	4
metabolite IIA	0.65	0.45	0.42
metabolite IIB	0.59	0.42	0.32
metabolite IIC	0.47	0.30	0.13

[a] TLC solvent systems are found in the Materials and Methods section.

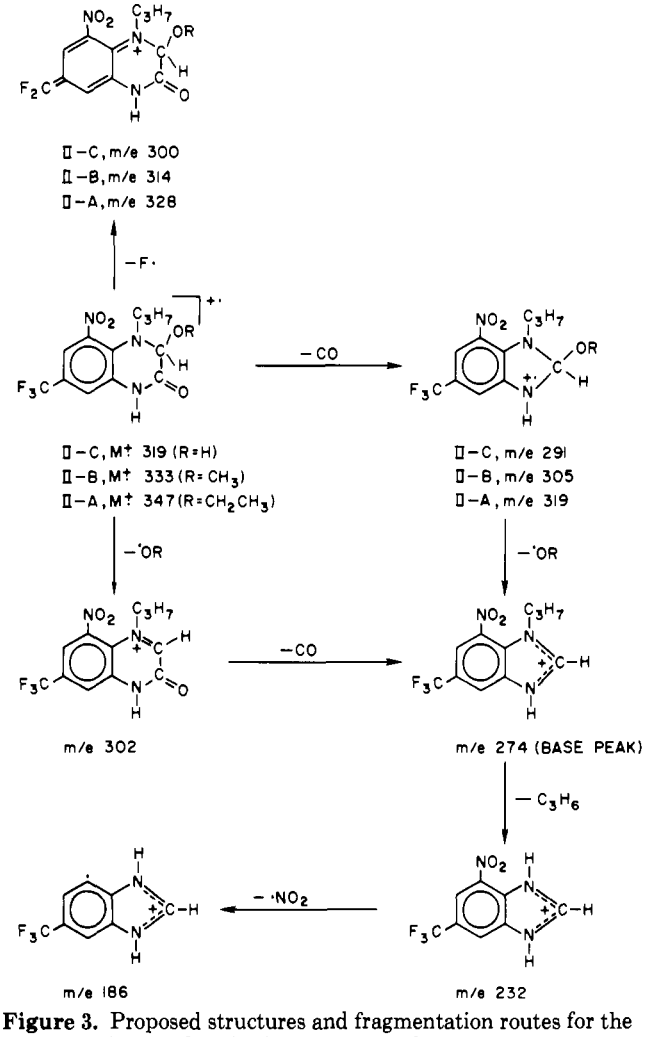

Figure 3. Proposed structures and fragmentation routes for the molecular ions and major ion fragments from the mass spectra of metabolites IIA, IIB, and IIC.

dures in each experiment were essentially identical, three distinct structurally related compounds (IIA, IIB, and IIC) were isolated. Only one of the three compounds was detected in each experiment; however, all may have been present and only the most prevalent one detected. The distribution of metabolites IIA and IIC in other experiments (both in the roots and the posttreatment solution) was nearly identical with that of metabolite IIB (Figure 1 and Table II). Metabolites IIA and IIB had similar R_f values on TLC in solvents 1, 3, and 4, while IIC was more polar (Table IV). All three metabolites had nearly identical infrared spectra with intense absorptions at 1700 cm^{-1}.

The mass spectrum of each compound had a base peak of m/e 274 and intense peaks at m/e 232 and 186 (Figure 3). Precise mass measurements of the above ions from metabolite IIB agreed to within 4 ppm of empirical formulae $C_{11}H_{11}N_3O_2F_3$, $C_8H_5N_3O_2F_3$, and $C_8H_5N_2F_3$, re-

spectively. This fragmentation was not characteristic of the spectra for the nitro-substituted *N,N*-dialkylanilines (Figure 2). Our interpretation is that the *N*-propyl side chain remained intact in metabolite IIB and was lost as C_3H_6 from the base peak (*m/e* 274) to yield *m/e* 232 which in turn lost an intact NO_2 group to yield *m/e* 186 with the retention of two nitrogens. The measured precise mass of the molecular ion of metabolite IIB (M· + 333) was within 2 ppm of the empirical formula $C_{13}H_{14}N_3O_4F_3$. This information coupled with an (M - 19) loss from the molecular ion (Figure 3) indicated that an aromatic CF_3-substituted ring was present and it was assumed that the nitrogen substituents occupied their original positions from fluchloralin. Therefore, the intense fragment ions (*m/e* 274, 232, and 186) of metabolite(s) II were assigned benzimidazole structures (Figure 3).

Mass spectra also indicated that metabolites IIA, IIB, and IIC contained no chlorine, differed in mass by CH_2 groups, and lost F, CO, and OR from their molecular ions (Figure 3). Simple homology (i.e., benzodiazine heterocycles bridged by C_2, C_3, or C_4 groups to give metabolites IIC, IIB, or IIA, respectively) would require unusual eliminations from the heterocyclic ring to yield such similar mass spectra. An alternative is to regard metabolite IIC (M· + 319) as a hemiacetal-like compound with a free hydroxy group, and IIB (M· + 333) and IIA (M· + 347) as its methoxy and ethoxy derivatives, respectively (Figure 3). Losses of *m/e* 45, 31, and 17 (the latter one of very low intensity) from the molecular ions of metabolites IIA, IIB, and IIC to yield a common ion at *m/e* 302 support this assignment (Figure 3). Metabolite IIC also shows a unique loss of 29 in addition to 28 to yield *m/e* 290 which suggests that IIC is not a simple homologue of IIB and IIC. All of these properties are compatible with the dihydroquinoxalinones proposed for the molecular ions in Figure 3.

Attempts at preparation of 1,4-dihydro-1-methyl-2-hydroxyquinoxalin-3-one as a model compound by reduction of 1,4-dihydro-1-methylquinoxaline-2,3-dione with lithium aluminum hydride failed. Presumably, reduction occurred at the 3 position to yield the 3-hydroxy-2-one intermediate which dehydrated to form 1-methylquinoxalin-2-one. The stability of IIC favors the 2-hydroxy-3-one structure.

The formation of these compounds can be explained if one assumes that the plant produces a conjugate (possibly a glycoside) of the dihydroquinoxalinone. Methanolysis and hydrolysis of the conjugate explains the formation of IIB and IIC, respectively. The formation of IIA presumably is via the ethanol present as an inhibitor in the chloroform used in extraction. Compound IIA was only isolated when the chloroform extracts were dried, concentrated, and allowed to stand for 3 days, thus giving maximum opportunity for reaction with ethanol. Other work-up procedures involved addition of methanol immediately after the concentration step. Further support of these structures comes from the observation that IIA and IIB slowly decomposed to IIC (based on comparative TLC).

Metabolite III. The mass spectrum of metabolite III is shown in Figure 4. This spectrum was identical qualitatively with that of authentic 1-(2-chloroethyl)-2-ethyl-7-nitro-5-(trifluoromethyl)benzimidazole, while the authentic isomeric 2-chloromethyl-7-nitro-1-propyl-5-(trifluoromethyl)benzimidazole lacked the *m/e* 213, 244, and 286 fragments and had ions at *m/e* 199, 233, 235, 264, 266, 302, and 321 (Feil and Wien, 1978). Precise mass determinations of the ion fragments at *m/e* 244 (M -

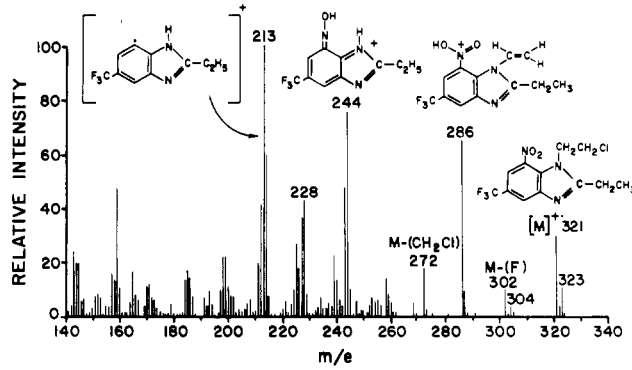

Figure 4. Mass spectrum of metabolite III.

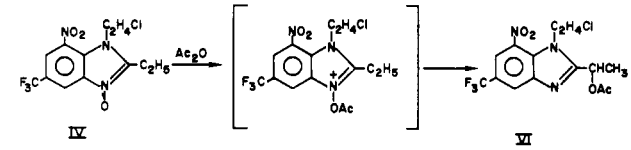

Figure 5. Proposed acetylation of metabolite IV.

C_2H_2ClO) and 213 (M - $C_2H_3ClNO_2$) agreed to within 4 ppm with the empirical formulae for the structures proposed here. The ion fragments at *m/e* 286 (M - Cl) and 244 were most likely the result of peri interactions between the 2-chloroethyl group at the 1 position and the nitro group at the 7 position on the benzimidazole ring (Budzikiewicz et al., 1967; Ramana and Vairamani, 1977; Tomer et al., 1973).

The mass spectrum of authentic 2-ethyl-7-nitro-1-propyl-5-(trifluoromethyl)benzimidazole (M· + 301) also showed fragment ions at *m/e* 213 (base peak), 214, and 244. The intensities of the latter two ions were nearly equal to that shown in Figure 4 (Kearney et al., 1976). Precise mass measurements of the ions at *m/e* 244 and 213 from the above compound also agreed to within 4 ppm with the empirical formulae proposed for the same ions in Figure 4.

Metabolite IV. Metabolite IV was relatively polar by TLC analysis and had a GLC retention time identical to that of metabolite III (Table III). Its mass spectrum by GC–MS was qualitatively identical with that of metabolite III (M· + 321, Figure 4).

Direct introduction of metabolite IV into the mass spectrometer by solid sample probe, however, produced a different base peak (*m/e* 302) and molecular ion (*m/e* 337). The precise mass measurement for the latter agreed to within 2 ppm with the empirical formula $C_{12}H_{11}N_3O_3F_3Cl$, one more oxygen atom than in metabolite III. Metabolite IV was tentatively identified as 1-(2-chloroethyl)-2-ethyl-7-nitro-5-(trifluoromethyl)benzimidazole 3-oxide based primarily on its loss of an oxygen atom to give an intense *m/e* 321 fragment ion. The M - 16 ion is characteristic of aromatic *N*-oxides (Budzikiewicz et al., 1967). An intense M - 16 ion was reported for authentic 2-ethyl-7-nitro-5-(trifluoromethyl)benzimidazole 3-oxide introduced directly into the mass spectrometer and it also decomposed thermally to the corresponding benzimidazole when it was subjected to GC–MS (Leitis and Crosby, 1974).

The reaction of metabolite IV with acetic anhydride also supported the *N*-oxide structure. The product was subjected to GC–MS and the observed molecular ion, *m/e* 379, was consistent with the side-chain acetoxylated derivative (VI) shown in Figure 5. The reaction probably involved an unstable acetylated intermediate which underwent a rearrangement involving an α-substituent on the ring. For example, 1,2-dimethylbenzimidazole 3-oxide formed 2-

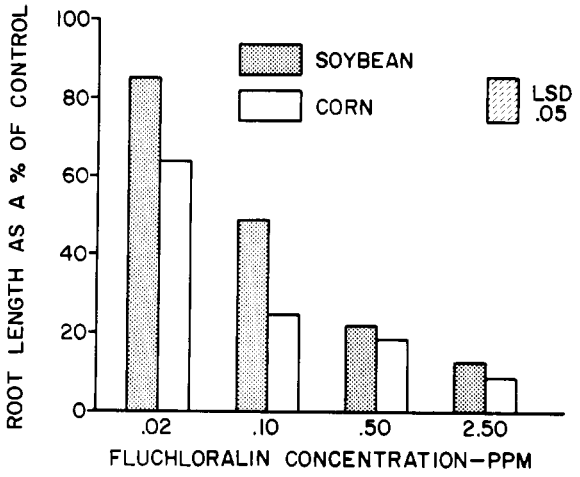

Figure 6. Effect of fluchloralin on the growth of soybean and corn seedling roots.

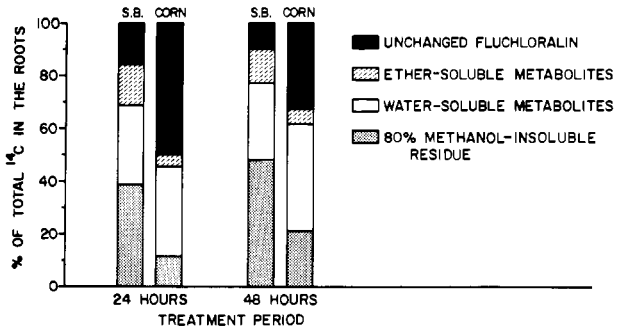

Figure 7. Metabolism of [^{14}C]fluchloralin by soybean (S.B.) and corn seedling roots. Four-day-old soybean and corn seedlings (15 seedlings per beaker) treated with 50 mL of 1% acetone solution of [^{14}C]fluchloralin (770 000 dpm, 1 μM).

acetoxymethyl-1-methylbenzimidazole when reacted with acetic anhydride (Katritsky and Lagowski, 1971).

In a recent trifluralin photodegradation study, however, Leitis and Crosby (1974) demonstrated that mass spectrometry alone cannot distinguish between a benzimidazole *N*-oxide and its probable precursor, a dihydroxy benzimidazoline. Thus, the available data do not distinguish between the benzimidazole *N*-oxide and 1-(2-chloroethyl)-2,3-dihydroxy-2-ethyl-7-nitro-5-(trifluoromethyl)-benzimidazoline as possible structures for metabolite IV. Both types of compounds have been reported in dinitroaniline photodegradation (Leitis and Crosby, 1974) and soil metabolism studies (Golab and Althaus, 1975). In each case a dihydroxy benzimidazoline was suspected of being a precursor of the benzimidazole *N*-oxide.

Metabolite V. Metabolite V was the only water-soluble metabolite isolated in sufficient quantity from the post-treatment solution for partial characterization. However, the quantity available was not sufficient for extensive purification and mass spectral analysis.

Metabolite V was acidic as demonstrated by its retention on a DE-52 anion-exchange column (Figure 1) and appeared to be a conjugate. At least 80% hydrolysis of metabolite V occurred under weak or strong acid conditions. The R_f value of metabolite V was 0.28 in solvent 8 (Table III). The hydrolyzed product has an R_f of 0.87 in the same solvent. The hydrolyzed product partitioned into ether but did not cochromatograph with fluchloralin or any of its chloroform-soluble metabolites in solvents 1, 2, or 3. The nature of the conjugate (metabolite V) was not determined positively but it may not be a simple glycoside. Metabolite V was not hydrolyzed by either β-D-glucosidase or hesperidinase (1 mg/mL in 0.5 mL of sodium acetate buffer, pH 5.3, overnight at 30 °C). However, a negative reaction to these two enzymes does not eliminate necessarily the substrate as a glycoside. At least 71% of the unhydrolyzed metabolite V partitioned into ethyl ether from an aqueous solution acidified to pH 3 with acetic acid.

Selectivity of Fluchloralin. The relative sensitivity of the primary roots of soybean and corn to varying concentrations of fluchloralin is shown in Figure 6. The seminal root of corn was significantly more sensitive to fluchloralin injury than the primary root of soybean at the lower concentrations (0.02 and 0.10 ppm) of fluchloralin. Root growth of both species was retarded severely and no significant differences in sensitivity were observed at the higher concentrations (0.50 and 2.50 ppm). The roots of both species displayed the typical dinitroaniline injury sympton (swelling of the root tips) especially at the higher concentrations.

Corn and soybean seedlings were tested for their ability to recover from fluchloralin injury by removing the seedlings from the petri dishes after a 48-h treatment period and placing them in moist paper towel rolls for an additional 48 h. Recovery was based on visible resumption of growth by the root tips. The soybean roots exposed to 0.02 ppm fluchloralin were indistinguishable visibly from the control roots after 48 h in the paper towel rolls but roots exposed to 0.10 ppm fluchloralin showed only partial recovery. Soybean roots exposed to 0.50 and 2.50 ppm fluchloralin and corn roots exposed to all four concentrations showed no recovery after 48 h in the paper rolls. However, corn shoots whose growth was retarded severely at 0.50 and 2.50 ppm fluchloralin showed limited recovery, indicating that corn shoots may be more resistant to fluchloralin injury than the roots.

The relative sensitivity of corn and soybean roots to fluchloralin injury was reflected in the relative rates at which the two species metabolized the herbicide (Figure 7). The roots of both species metabolized fluchloralin to ether and water-soluble metabolites and methanol-insoluble residue. However, unchanged fluchloralin in corn roots (50% of the total ^{14}C) was three times greater than in soybean roots (16% of the total ^{14}C) after 24 h. The same 3:1 concentration ratio existed after 48 h except the amounts of fluchloralin in the roots of both species (corn 32%, soybean 10%) were reduced by metabolism. The roots of both species absorbed nearly the same amount of ^{14}C from the treatment solution (corn, 65%; soybean, 64%) in 24 h. Most of this radioactivity (98%) remained in the roots of both species. The results show that fluchloralin is metabolized rapidly by a tolerant species like soybean and incorporated into methanol-insoluble residue.

DISCUSSION

The proposed metabolic fate of fluchloralin in soybean roots is summarized in Figure 8. None of the metabolites depicted represents more than 3% of the total radioactivity in the plant (Table II). This pattern of metabolism (trace quantities of numerous degradation products) is characteristic of the fate of many dinitroaniline herbicides in plants (Sumner et al., 1976; Wright et al., 1975), soil (Golab and Althaus, 1975; Golab et al., 1975; Kearney et al., 1976) and "in vitro" systems (Nelson et al., 1977). The reasons for this behavior appear to be the availability of several degradation pathways (including N-dealkylation, reduction, cyclization, natural product conjugation, and any combination thereof) to the highly reactive dinitroanilines and the lack of predominance of any one of these pathways over the others.

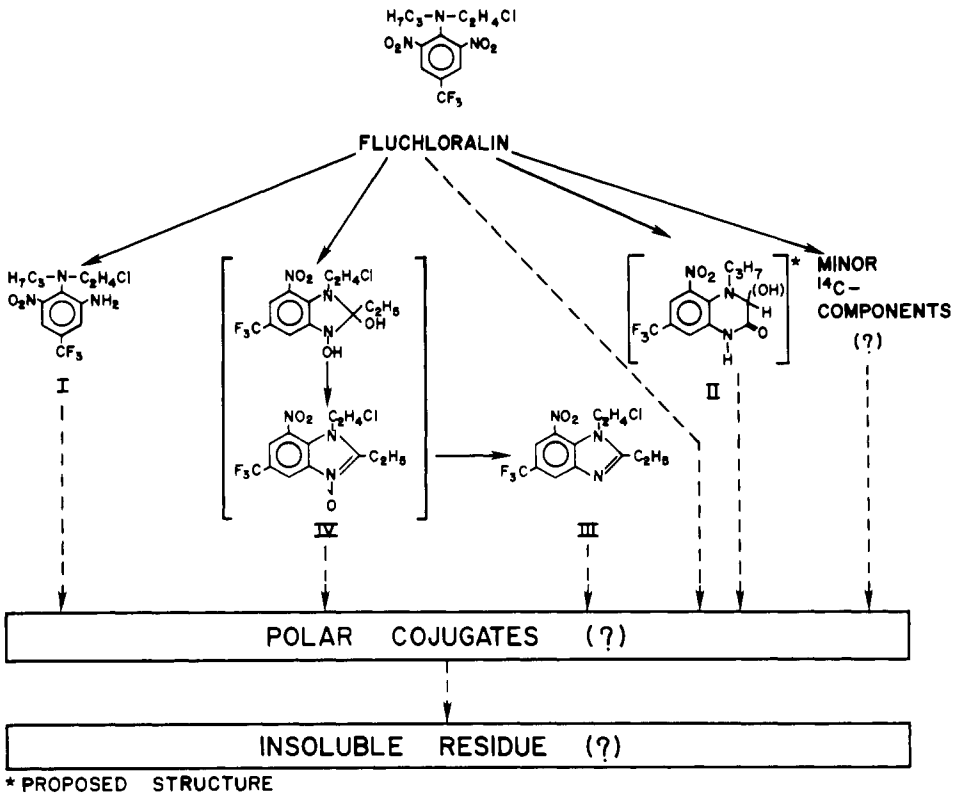

Figure 8. Proposed metabolism of fluchloralin in soybean roots. Metabolite II is a proposed structure. The structures in brackets for metabolite IV are also proposed structures based on mass spectrometry. Broken arrows indicate unknown reactions.

The characterization of the 2-amino derivative (I) demonstrates that dinitroaniline herbicides such as fluchloralin are subject to nitro reduction in plants. Earlier reports on the isolation of nitro-reduced metabolites of trifluralin (Golab et al., 1967; Probst et al., 1967) and benefin (Golab et al., 1970) in plants failed to differentiate between plant and soil metabolism.

Leitis and Crosby (1974) suggested that dihydroxy benzimidazolines and benzimidazole *N*-oxides may account for some of the "polar products" reported in plants treated with dinitroaniline herbicides (Golab et al., 1970; Probst et al., 1967). Their suggestion is based on the similarity of products formed between photodecomposition and plant metabolism of herbicides (Crosby, 1972). The characterization of metabolites III and IV gives direct evidence that plants metabolize fluchloralin (and possibly other dinitroaniline herbicides) by a cyclization pathway similar to the trifluralin cyclization pathway in photodegradation (Leitis and Crosby, 1974).

Metabolite II may be unique to fluchloralin since it presumably arises from a cyclization reaction between the C_2 of the 2-chloroethyl group and a nitro group with a concomitant loss of chlorine. Indeed, the unsaturated quinoxaline isolated from photolysis (Nilles and Zabik, 1974), soil (Kearney et al., 1976), and "in vitro" fluchloralin degradation studies (Nelson et al., 1977) appears to be unique to fluchloralin. It is conceivable that metabolite II may be a precursor of this unsaturated quinoxaline even though the latter was not detected in this study.

The comparative metabolism of fluchloralin between soybean and corn roots suggests that the basis for selectivity in the root tissue is the relative rate at which fluchloralin is metabolized and detoxified (soybean > corn). Within physiologically selective concentrations, the soybean root appears to detoxify fluchloralin at a rate sufficient to prevent irreversible injury while the corn root does not. However, there are other factors such as herbicide placement and growth characteristics of the seedlings which also contribute to the selectivity of fluchloralin between soybean and corn.

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A Comparison of the Influence of Thermal Processing and Broiling on Naturally Occurring and Spiked Residues of 1,1,1-Trichloro-2,2-bis(*p*-chlorophenyl)ethane and Its Metabolites in Ground Beef

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Raw and processed samples of ground beef containing naturally occurring residues of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) and its metabolites were compared with the same ground beef to which an ethanol–water solution of *p,p'*-DDT had been added. Two treatments, a 104 °C steritort process for 342 min and broiling patties for 4 min on each side, were found to cause different losses of pesticides. No difference existed between method of heating with respect to 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD) and 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (DDE) in nonspiked or spiked samples. Nonspiked samples decreased in DDT content ($P < 0.01$) on steritort processing and broiling. Spiked samples, however, lost DDT on steritorting ($P < 0.05$) but not during broiling. Total residues (DDE + DDD + DDT) also differed between spiked and nonspiked samples. Results indicated that research with pesticide residues should be conducted on naturally contaminated rather than artificially spiked matrices.

There exist numerous reports with respect to degradation of pesticide residues resulting from thermal processing and preparation of foods. Studies involving heat treatment of foods containing naturally occurring residues as well as foods to which pesticides have been added at residue levels (i.e., "spiked") have been reported.

Initial studies on the influence of heat treatment on pesticide residues were performed with artificially contaminated foods. Tressler (1947) and Britten and Fairing (1950) added various pesticides in solution to batches of fruits and vegetables for thermal processing. Losses after canning ranged from 20 to 95%. Farrow et al. (1966) studied the dehydrohalogenation of 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) to 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane (DDD) during processing of spinach. Retail cans of spinach were spiked with an acetone solution of *p,p'*-DDT. Recanning and processing revealed no *p,p'*-DDT but substantial amounts of *p,p'*-DDD were found, indicating a conversion of DDT to DDD.

In an effort to determine which food components were responsible for conversion of DDT to other compounds during thermal processing, Ralls and Cortes (1972) investigated the changes of DDT in aqueous solution during heating with vitamins, amino acids, peptides, casein, yeast nucleic acid, and diphosphopyridine nucleotide. Results demonstrated that pH was not a factor in DDT transformations but amino acids containing a sulfhydryl group promoted hydrogenolysis of DDT to DDD.

Stemp and Liska (1966) and McCaskey and Liska (1967) attributed the reduction of artificial residue contaminates in milk during processing to codistillation with water vapor during thermal processing. Langlois et al. (1964) determined the effects of processing and storage of dairy products on natural and artificial residues of DDT and lindane. Generally, the manufactured products contained the same amount of pesticides as raw milk when expressed

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