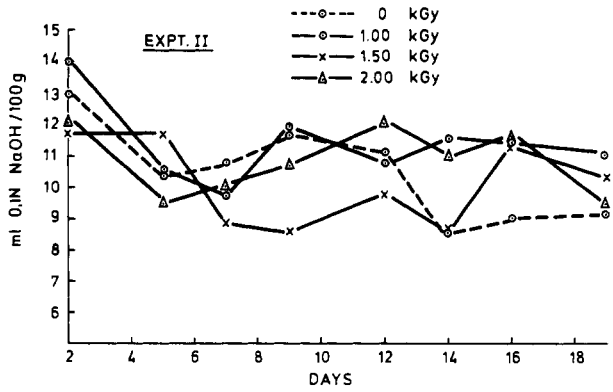

Figure 17. Titratable acidity: Hortus Gold papayas.

correlated with irradiation treatments.

CONCLUSION

The concentrations of ascorbic acid, carotenes, sugars, and titratable acidity have been monitored in two mango and two papaya cultivars as the fruits ripened from mature green to the edible-ripe stage, both with and without irradiation to doses up to 2.0 kGy.

Ascorbic acid and titratable acidity contents changed only slightly during ripening of the fruits and natural variation was greater than any change which may have been brought about by radiolysis. Virtually no difference in total sugar content could be observed between the irradiated and nonirradiated fruits in the five experiments using the revised analytical technique. In the earlier determinations random fluctuations were observed. In most of the experiments, irradiation produced an apparent *increase* in carotene content, but this was overshadowed by the much larger increase which occurred as a result of ripening.

There is, therefore, no evidence to support the theory that irradiation of these fruits causes any significant change in their biosynthesis of nutrients during ripening. In fact, any radiation-induced changes which do occur are too small to be detected against the background of natural variation of the various constituents, and by the changes in the content of these constituents produced during the physiological changes which take place in these fruits during ripening.

ACKNOWLEDGMENT

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Fate of [^{14}C]Trifluralin in Soil

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The degradation of trifluralin was investigated in field soil over a 3-year period. A 2-month laboratory investigation was also performed using field soil flooded with water. Twenty-eight transformation products were isolated and identified in these studies. None of the isolated transformation products exceeded 3% of the initially applied trifluralin. After 3 years, less than 1.5% of the applied trifluralin could be detected in soil, 4% was distributed among numerous transformation products, and 38% remained as soil-bound residues. α,α,α-Trifluorotoluene-3,4,5-triamine, a degradation product of trifluralin, appeared to be a key compound in the formation of soil-bound residues.

Trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) is a preemergence herbicide used for control of a wide variety of grass and broadleaf weeds in many agronomic and horticultural crops (Alder et al., 1960; Soper et al., 1961). The fate of trifluralin has been investigated in soil and plants (Probst et al., 1967; Golab et al., 1967) and in artificial rumen fluid and ruminant animals (Golab et al., 1969). Probst et al. (1975) and Helling (1976) have reviewed the results of numerous investigations on the fate of trifluralin under various physical and biological conditions.

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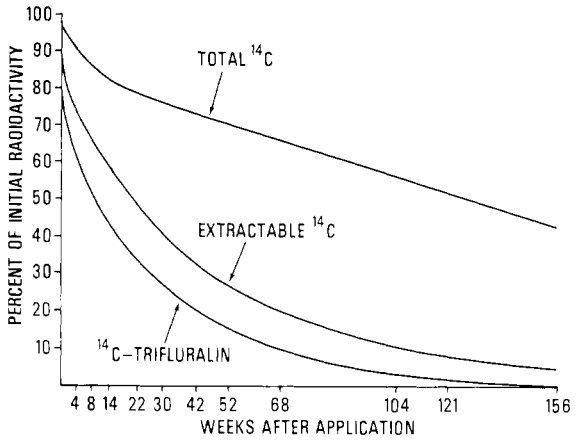


Figure 1. The rate of dissipation of [^{14}C]trifluralin in field soil.

The purpose of the current study was to investigate the degradation of trifluralin in soil under natural conditions over a 3-year period, to characterize further the transformation products formed under both field conditions and laboratory conditions, and to obtain information on the nature of the nonextractable soil-bound products.

MATERIALS AND METHODS

[^{14}C]Trifluralin. All [^{14}C]trifluralin was prepared in the Lilly Research Laboratories. Mixed labeled [^{14}C]-trifluralin (15% ring and 85% CF_3) with specific activities of 1.0 and 3.0 μCi/mg and uniformly ring-labeled [^{14}C]-trifluralin with specific activities of 2.5 and 8.5 μCi/mg were used in these experiments. Analyses indicated the trifluralin content of all samples to be greater than 99%. Ring-labeled α,α,α-trifluoro[^{14}C]toluene-3,4,5-triamine (TR-9) and 2,2′-azoxybis(α,α,α-trifluoro-6-nitro-*N*-propyl-*p*-toluidine) (TR-28) were similarly prepared.

Application. [^{14}C]Trifluralin was applied to six field plots at Greenfield, Ind., at rates ranging from 0.84 kg/ha to 6.72 kg/ha by incorporation into the top 7.5 cm of soil. The application rate of 0.84 kg/ha corresponded to the recommended rate for the soil texture used in these studies. The method of incorporation was the same as described for isopropalin by Golab and Althaus (1975). The soil was loam, as described by the United States Testing Company, Memphis, Tenn. Each plot consisted of a 0.65-m^2 area obtained within a 60-cm section of 91-cm diameter galvanized pipe which extended 40 cm below and 20 cm above the soil surface. All plots were fertilized with 6-24-24 fertilizer at a rate of 560 kg/ha prior to incorporation of the herbicide. Soybeans [*Glycine max* (L.) var. *Corsoy*] were planted after incorporation of the herbicide.

Flooded Soil. Two kilograms of soil were removed from an untreated field plot area and mixed with [^{14}C]trifluralin, sp act. 7.55 μCi/mg, at a concentration of 1.5 ppm. The soil was placed in a 4-L tin-plated container and covered with 5 cm of water. The system was maintained at ambient laboratory temperature.

Sampling and Extraction Procedures. Soil samples were removed from plots immediately after the application of [^{14}C]trifluralin and periodically thereafter, as indicated in Figure 1. Samples were collected at depths of 0–7.5 cm and 7.5–15 cm. Each sample was prepared by combining six subsamples obtained with a 2-cm diameter Hoffer tube at each sampling time from each experimental plot. Additional 0–38-cm samples were removed 12, 16, 24, and 36 months after application and divided into 2.5- or 7.5-cm segments for leaching analyses. Trifluralin and its extractable degradation products were extracted from 100–200-g soil samples with two 300–600-mL portions of methanol, followed by an equal amount of 50% aqueous methanol using an Omni-Mixer. The combined methanolic extracts were concentrated using a Rinco vacuum evaporator, and the aqueous phase was partitioned with chloroform and/or ethyl acetate.

Analytical Procedures. Soil samples were analyzed for total radioactivity and for trifluralin and its degradation products. Total radioactivity was determined by combustion analyses as described previously by Golab et al. (1970). Aliquots of the organic and aqueous phases were subjected to radioactive counting by liquid scintillation counting (LSC). The amount of soil-bound radioactivity was determined by combusting a portion of the extracted soil samples and counting the collected $^{14}CO_2$. Qualitative and quantitative analyses of organic extracts were performed by thin-layer chromatography (TLC), gas–liquid chromatography (GLC), and by radiochemical methods which included LSC and radioautography of thin-layer chromatograms (TLC-RA) alone and in conjunction with LSC. Where possible, confirmation of isolated degradation products was accomplished by mass spectrometry (MS) or a combination of gas chromatography–mass spectrometry (GC–MS). In some instances, high-pressure liquid chromatography (LC) was used for separation and purification of the isolated samples prior to MS.

Identification of the degradation products was established by comparison with the appropriate model compounds. Authentic model compounds were synthesized in the Lilly Research Laboratories and structures were verified by use of NMR, IR, MS, and elemental analyses. Two compounds, TR-43 and TR-44 (Table I), were supplied by Dr. J. O. Nelson, University of Maryland, College Park, Md.

Crude organic extracts were examined by TLC-RA and, when necessary, the extracts were purified and the radioactive degradation products crudely separated by gravity adsorption column chromatography (CC) or by small-bore column chromatography (SBCC) pressured with nitrogen. Silica gel or Florisil were used as adsorbents for both columns. Methodology for radiochemical and chromatographic analyses was essentially the same as that described previously (Golab et al. 1970, 1975; Golab and Althaus, 1975).

Thin-Layer Chromatography. Commercial silica gel plates 0.25 mm in thickness (60 F-254 or F-254 silica gel-kieselguhr fast running, E. Merck, Darmstadt) were used. Twelve solvent systems were utilized: (1) benzene–carbon tetrachloride (40:60), (2) benzene, (3) hexane–methanol (98:2), (4) hexane–methanol (97:3), (5) benzene–ethyl acetate (60:40), (6) benzene–1,2-dichloroethane (60:40), (7) benzene–1,2-dichloroethane (50:50), (8) benzene–ethyl acetate–acetic acid (60:40:1), (9) benzene–methanol (98:2), (10) benzene–methanol (90:10), (11) benzene–methanol (75:25), and (12) carbon tetrachloride. These solvent systems were used in one-dimensional and in two-dimensional chromatography. Typical combinations for two-dimensional chromatography were the following: 7 and 2, 3 and 1, 5 and 6, 9 and 8, 10 and 5, and 11 and 5.

Gas–Liquid Chromatography. A Hewlett-Packard F and M Scientific Model 402 high-efficiency gas chromatograph, equipped with electron affinity (EAD) ^{63}Ni and with flame ionization (FID) detectors, was used. Carrier gases and electrometer outputs were the same as those described by Golab et al. (1975) and Golab and Althaus (1975). The most commonly used columns were borosilicate, 3 mm i.d. × 180 cm, packed with 3 and 5% Carbowax 20M on Chromosorb W-HP, 100/200 mesh; and

1.5 and 5% XE 60 on the same support phase or on Solco 80/100. Column temperatures varied from ambient to 240 °C.

Gas Chromatography–Mass Spectrometry. A LKB gas chromatograph–mass spectrometer equipped with a Model 9000 mass marker was used. Column packing and conditions were the same as used for GLC analyses. Operational parameters were described previously by Golab and Althaus (1975).

Mass Spectrometry. All direct mass spectra were determined using a Varian-MAT Model 731 mass spectrometer. Electron impact spectra (EI-MS) were obtained by directly introducing the sample into the ion source. The ionizing energy was 70 eV. Accurate mass measurements were made using photoplates or peak matching. Field desorption spectra (FD-MS) were obtained using 10 μm emitters conditioned with benzonitrile by the method described by Varian-MAT.

High-Pressure Liquid Chromatography. A 2.12 mm i.d. × 62 cm liquid partition column (silicic acid for high resolution; Bio-Sil, 20 × 40 μm) was eluted with 0.05, 0.5, 1.0, and 2.0% isopropyl alcohol in heptane at a flow rate of 1 mL/min under nitrogen pressure of 300 to 600 psi. A Varian 254-nm UV detector was utilized. The technique of LC was used for final separation of the temperature-sensitive dimeric substances. The more polar phenolic and acidic substances were separated by the same technique using a 4 mm i.d. × 30 cm liquid partition column (Spherisorb silica, 10 μm) eluted with 20 and 25% isopropyl alcohol in heptane.

Derivative Formation. Methylation of aliquots of soil extracts was conducted using diazomethane in ether solution for derivatization of phenols and acids. The corresponding model compounds in Table I are designated by the letter M (methylated).

Compound TR-9 was further characterized by its derivatization to 4-(α,α,α-trifluoroacetamido)-2,6-bis(trifluoromethyl)benzimidazole using trifluoroacetic anhydride and triethylamine in methylene chloride. Such a derivatization was performed and utilized in adsorption–desorption investigations with TR-9 using GLC–EAD. Retention time for this derivitive of TR-9 on a 1% OV-17 column 3 mm i.d. × 180 cm at 190 °C was 1.2 min.

Experiments with Compound [^{14}C]TR-28 [2,2′-Azoxybis(α,α,α-trifluoro-6-nitro-*N*-propyl-*p*-toluidine)]. 1. *Greenhouse Experiment.* Ring-labeled [^{14}C]TR-28, sp act. 4.64 μCi/mg, was mixed with 2 kg of field soil at a rate of 1 ppm. One kilogram of soil was placed in a tin-plated container and covered with 5 cm of water, and a second kilogram was maintained in a similar metal container at 60% field moisture capacity. Both soils were sampled periodically and analyzed for [^{14}C]TR-28 and its degradation products.

2. *Field Experiment.* [^{14}C]TR-28, sp act. 4.64 μCi/mg, was incorporated in the top 7.5 cm of field plots similar to those used for the [^{14}C]trifluralin experiments. Application rates of 0.112 and 0.224 kg/ha were used. Soybeans were planted in both plots and analyzed for total radioactivity at 3, 8, and 16 weeks. Soil samples were removed periodically and analyzed.

3. *Photolysis of Compound [^{14}C]TR-28.* A 0.426 mM solution of compound [^{14}C]TR-28 in methanol was irradiated with a medium-pressure mercury Hanovia UV lamp (Cat. No. 679-A-36) covered with a corex glass filter. Nitrogen was passed through the solution during irradiation. The reaction was followed by TLC analysis and quantitated by radiochemical procedures. Compound TR-28 was not completely dissolved at the beginning of the photolysis but was completely solubilized after 3 h. The photolysis of compound TR-28 was also performed on silica gel TLC plates exposed to combined UV–fluorescent light.

Soil-Bound Degradation Products. 1. *Fractionation of Soil-Bound Radioactivity.* Soil obtained 1, 2, and 3 years after application of [^{14}C]trifluralin, and after extraction with methanol and aqueous methanol, was extracted with 0.5 N NaOH for 24 h in a shaker at room temperature. A 20-g sample was shaken with 100 mL of 0.5 N NaOH in a centrifuge tube and the soil suspension was centrifuged at 2000 rpm for 30 min. The supernatant was decanted and the soil washed twice with 50-mL portions of 0.5 N NaOH and recentrifuged. The soil was finally washed with three 50-mL portions of water, centrifuged, air-dried, and submitted for combustion analysis. The combined supernatants and water washes were analyzed for radioactivity by LSC. Portions of this supernatant were adjusted to pH 1, 7, and 13 and extracted with chloroform and/or ethyl acetate. The organic extracts were analyzed radiochemically. Another portion of the supernatant was acidified with concentrated HCl to pH 1 and the precipitated humic acid centrifuged. The remaining supernate contained the fulvic acid and was decanted. The precipitated humic acid was redissolved in 0.1 N NaOH. Both humic and fulvic acid fractions were analyzed for radioactivity by LSC.

A similar fractionation was performed on soil samples previously extracted with methanol and aqueous methanol using DOWEX A-1 chelating resin as the extractant rather than 0.5 N NaOH. Twenty milliequivalents of Na^+ and 200 mL of water were used for each 10 g of extracted soil which contained 440 mg of organic matter. Radiochemical analyses were performed in the same manner as with the soil samples extracted with 0.5 N NaOH.

2. *Comparative Adsorption–Desorption of Trifluralin and Some of Its Derivatives on Various Adsorbents.* The binding of trifluralin and certain selected derivatives to soil constituents, especially to humic acid, was investigated. Three adsorbents were used in these investigations: sea sand (weak adsorbent); sandy loam soil (medium adsorbent) which contained 61.2% sand, 21.2% silt, 17.6% clay, and 4.4% organic matter, and a mixture (strong adsorbent) of 12.5% commercial humic acid (Aldrich Chemical Co., Cat. No. 1675-2, mp 300) and 87.5% sea sand. A methanolic solution of the selected compounds was mixed for 30 min with 200 g of each of the three adsorbents in a glass container on a rotary mixer. The compounds were incorporated at a concentration ranging from 5 to 50 ppm. The compounds were extracted with methanol and with aqueous methanol in the manner described for extraction of field soil samples treated with trifluralin. Analysis of each compound was by gas chromatography using flame ionization detection (GC-FID). Six recoveries were made for each compound in each adsorbent. A duplicate sample was analyzed 30 min after mixing with dry adsorbent. Other duplicate samples were analyzed from moist adsorbent 30 min and 3 days after mixing. The latter two samples were moistened with 20 mL of water for each 200 g of adsorbent immediately after incorporation. Sample storage was at ambient laboratory temperature.

3. *Adsorption–Desorption Study of α,α,α-Trifluoro-[^{14}C]toluene-3,4,5-triamine (TR-9) on Various Adsorbents.* Compound [^{14}C]TR-9, sp act. 4.4 μCi/mg, was mixed with four adsorbents in a manner similar to that described above. The following adsorbents were used: sea sand, sandy loam soil, a mixture of 12.5% commercial humic acid with 87.5% sea sand, and a mixture of 20% clay (Ben-

Table 1. Reference Compounds, GLC Retention Times (t_r)[a] LC Retention Volumes (v_r)[b] TLC hR_{TR} and hR_f Values[c]

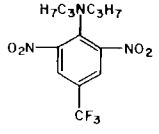
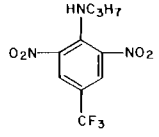
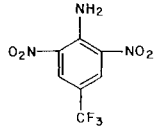
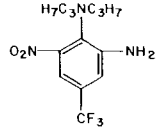
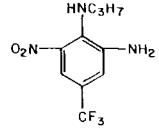
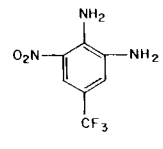
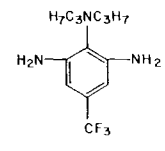
compd (mol wt)	structure	name	GLC t_r, min: 120 °C (100 °C)	GLC t_r, min: 140 °C (180 °C)	LC v_r, mL	hR_{TR} (hR_f) TLC solvent systems[d]: 1	2	4	5	6	10	12
TR-1 (335)		trifluralin α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine	2.9	1.3		(76	85	86	100 93	88	93	87)
TR-2 (293)		α,α,α-trifluoro-2,6-dinitro-*N*-propyl-*p*-toluidine	4.5	2.1		81 (61	95 81	88 76	99 92	98 86	100 94	67 58)
TR-3 (251)		α,α,α-trifluoro-2,6-dinitro-*p*-toluidine	3.3	1.5		34 (26	73 62	33 28	97 90	79 70	97 91)	
TR-4 (305)		α,α,α-trifluoro-5-nitro-N^4,N^4-dipropyltoluene-3,4-diamine	7.3	3.2		78 (59	91 78	53 46	96 89	96 85	96 90)	
TR-5 (263)		α,α,α-trifluoro-5-nitro-N^4-propyltoluene-3,4-diamine	7.6	3.4		32 (24	65 55	26 22	91 84	79 70	91 85)	
TR-6 (221)		α,α,α-trifluoro-5-nitrotoluene-3,4-diamine		12.6 (1.4)		3 (2	11 9	1 1	50 46	23 20	38 35)	
TR-7 (275)		α,α,α-trifluoro-N^4,N^4-dipropyltoluene-3,4,5-triamine	5.8	2.5		28 (22	55 47	47 40	96 89	67 59	95 88)	

Table I (*Continued*)

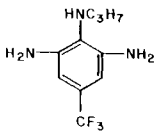
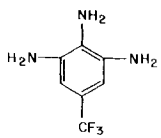
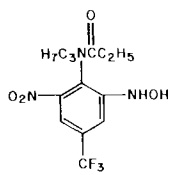
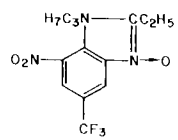
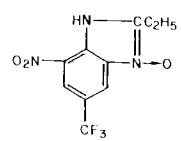
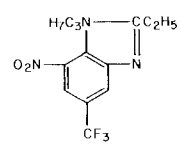
compd (mol wt)	structure	name	GLC t_r, min: 120 °C (100 °C)	GLC t_r, min: 140 °C (180 °C)	LC v_r, mL	hR_{TR} (hR_f) TLC solvent systems[d]: 1	2	4	5	6	10	12
TR-8 (233)		α,α,α-trifluoro-N^4-propyltoluene-3,4,5-triamine	9.9	3.9		0 (0	0 0	0 0	11 10	0 0	32 29)	
TR-9 (191)		α,α,α-trifluoro-toluene-3,4,5-triamine	10.5	5.2		0 (0	0 0	0 0	14 13	1 1	21 19)	
TR-10 (335)		α,α,α-trifluoro-2′-hydroxyamino-6′-nitro-*N*-propyl-*p*-propionotoluidide	2.5?			0 (0	0 0	0 0	48 45	0 0	36 34)	
TR-11 (317)		2-ethyl-7-nitro-1-propyl-5-(trifluoromethyl)benzimidazole 3-oxide	2.5			0 (0	0 0	0 0	0 0	0 0	22 20)	
TR-12 (275)		2-ethyl-7-nitro-5-(trifluoromethyl)benzimidazole 3-oxide		5.4		0 (0	0 0	0 0	4 4	0 0	20 18)	
TR-13 (301)		2-ethyl-7-nitro-1-propyl-5-(trifluoromethyl)-benzimidazole	6.7	3.0		7 (5	12 10	34 28	71 66	19 17	88 82)	

Table I (*Continued*)

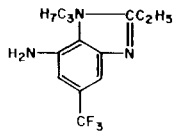
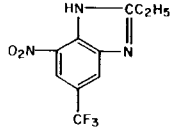
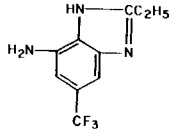
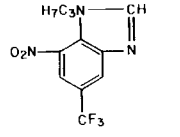
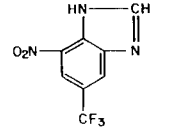
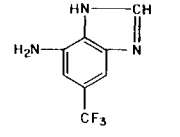
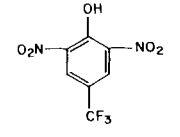
compd (mol wt)	structure	name	GLC t_r, min: 120 °C (100 °C)	GLC t_r, min: 140 °C (180 °C)	LC v_r, mL	hR$_{TR}$ (hR$_f$) TLC solvent systems[d]: 1	2	4	5	6	10	12
TR-14 (271)		7-amino-2-ethyl-1-propyl-5-(trifluoromethyl)-benzimidazole		26.8 (2.6)		0 (0	0 0	0 0	31 28	1 1	48 45)	
TR-15 (259)		2-ethyl-7-nitro-5-(trifluoromethyl)-benzimidazole	13.9	6.5		0 (0	1 1	0 0	51 47	1 1	57 54)	
TR-16 (229)		7-amino-2-ethyl-5-(trifluoromethyl)benzimidazole		35.0 (3.6)		0 (0	0 0	0 0	7 6	0 0	26 24)	
TR-17 (273)		7-nitro-1-propyl-5-(trifluoromethyl)benzimidazole	5.8			0 (0	4 3	12 11	56 52	7 6	77 72)	
TR-18 (231)		7-nitro-5-(trifluoromethyl)-benzimidazole	4.6			0 (0	0 0	1 1	24 22	0 0	30 27)	
TR-19 (201)		7-amino-5-(trifluoromethyl)-benzimidazole		(3.0)		0 (0	0 0	0 0	5 4	0 0	15 13)	
TR-20 (252)		α,α,α-trifluoro-2,6-dinitro-*p*-cresol				1 (1	3 3	0 0	22 20	2 2	13 12)	

Table I (*Continued*)

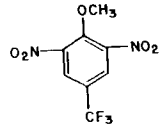
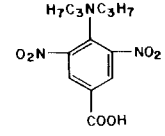
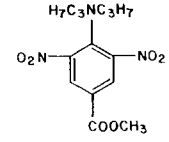
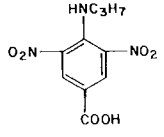
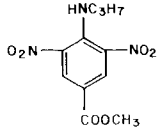
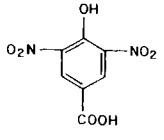
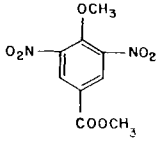
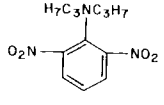
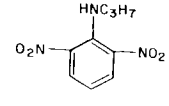
compd (mol wt)	structure	name	GLC t_r, min: 120 °C (100 °C)	GLC t_r, min: 140 °C (180 °C)	LC v_r, mL	hR_{TR} (hR_f) TLC solvent systems[d]: 1	2	4	5	6	10	12
TR-20M (266)		2,6-dinitro-4-(trifluoromethyl)-anisole	(3.9)			71 (58	92 82	69 63	99 92	93 84	97 91)	
TR-21 (311)		4-(dipropylamino)-3,5-dinitrobenzoic acid				0 (0	0 0	1 1	6 5	0 0	11 10)	
TR-21M (325)		methyl 4-(dipropylamino)-3,5-dinitrobenzoate	14.0	6.8		26 (21	58 52	90 83	98 92	68 61	98 92)	
TR-22 (269)		3,5-dinitro-4-(propylamino)-benzoic acid				0 (0	0 0	1 1	6 5	0 0	10 9)	
TR-22M (283)		methyl 4-(propylamino)-3,5-dinitrobenzoate		21.4		14 (11	39 34	62 54	98 92	52 46	97 92)	
TR-23 (228)		4-hydroxy-3,5-dinitrobenzoic acid				0 (0	0 0	0 0	0 0	0 0	0[e] 0)	
TR-23M (256)		methyl 3,5-dinitro-*p*-anisoate		5.7		13 (10	39 34	43 38	95 89	54 48	96 91)	
TR-24 (267)		2,6-dinitro-*N,N*-dipropylaniline		3.4		79 (61	94 82	74 65	98 91	95 84	100 94	67 58)
TR-25 (225)		2,6-dinitro-*N*-propylaniline		3.9		54 (42	83 73	75 65	98 91	85 75	99 93	40 35)

Table I (*Continued*)

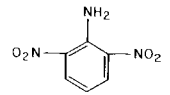
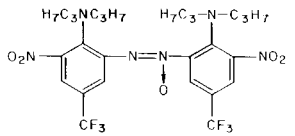
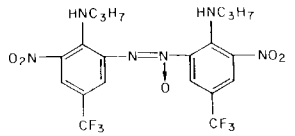
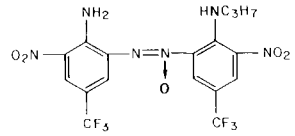
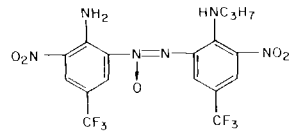
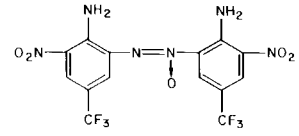
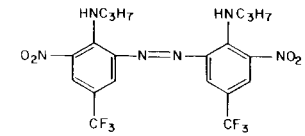
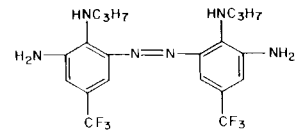
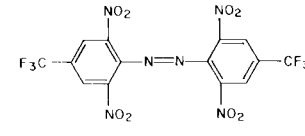
compd (mol wt)	structure	name	GLC t_r, min: 120 °C (100 °C)	GLC t_r, min: 140 °C (180 °C)	LC v_r, mL	hR$_{TR}$ (hR$_f$) TLC solvent systems[d]: 1	2	4	5	6	10	12
TR-26 (184)		2,6-dinitroaniline		2.4		29 (22	61 54	43 37	98 90	66 58	97 91)	
TR-27 (622)		2,2′-azoxybis(α,α,α-trifluoro-6-nitro-*N*,*N*-dipropyl-*p*-toluidine)			5	(76	89	87	102 94	90	94	82 71)
TR-28 (538)		2,2′-azoxybis(α,α,α-trifluoro-6-nitro-*N*-propyl-*p*-toluidine)			12	57 (44	91 80	78 63	100 93	95 83	100 93	27 23)
TR-29 (496)		*N*-propyl-2,2′-azoxybis(α,α,α-trifluoro-6-nitro-*p*-toluidine)			41	16 (12	59 51	26 22	100 93	81 72	98 91)	
TR-30 (496)		*N*′-propyl-2,2′-azoxybis(α,α,α-trifluoro-6-nitro-*p*-toluidine)			29	23 (17	72 62	38 33	100 93	85 74	98 92)	
TR-31 (454)		2,2′-azoxybis-(α,α,α-trifluoro-6-nitro-*p*-toluidine)			47	6 (5	39 34	6 5	99 92	61 53	97 90)	
TR-32 (552)		2,2′-azobis-(α,α,α-trifluoro-6-nitro-*N*-propyl-*p*-toluidine)			8	67 (52	92 81	84 73	100 94	95 84	100 93	37 32)
TR-33 (462)		3,3′-azobis-[N^2-propyl-5-(trifluoromethyl)-*o*-phenylenediamine]				1 (1	5 4	5 4	63 58	9 8	53 50)	
TR-34 (498)		α,α,α,α′,α′,α′-hexafluoro-2,2′,6,6′-tetranitro-*p*,*p*′-azotoluene				27 (20	88 75	0 0	100 92	88 76	98 90)	

Table I (*Continued*)

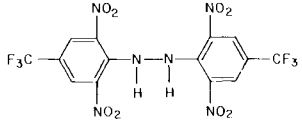
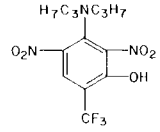
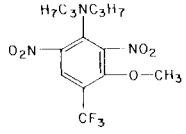
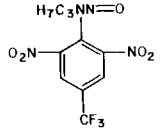
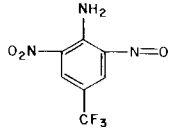
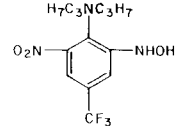
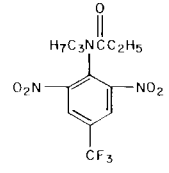
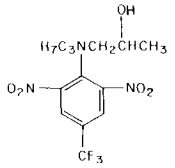
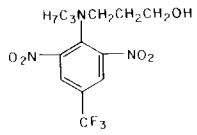
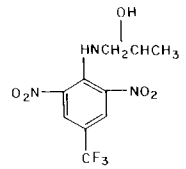
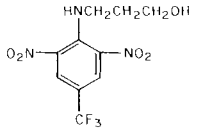
compd (mol wt)	structure	name	GLC t_r, min: 120 °C (100 °C)	GLC t_r, min: 140 °C (180 °C)	LC v_r, mL	hR_{TR} (hR_f) TLC solvent systems[d]: 1	2	4	5	6	10	12
TR-35 (500)		1,2-bis(α,α,α-trifluoro-2,6-dinitro-*p*-tolyl)hydrazine				4 (3	38 32	0 0	96 88	50 43	86 79)	
TR-36 (351)		α,α,α-trifluoro-4,6-dinitro-5-(dipropylamino)-*o*-cresol				2 (1	2 2	0 0	19 17	3 3	3 3)	
TR-36M (365)		2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl)-*m*-anisidine		1.7		(74	86	84	100 92	87	92)	
TR-37 (322)		α,α,α-trifluoro-2,6-dinitro-*N*-nitroso-*N*-propyl-*p*-toluidine		2.1		44 (32	82 70	39 32	99 90	86 74	97 89)	
TR-38 (235)		α,α,α-trifluoro-6-nitro-2-nitroso-*p*-toluidine	1.6			29 (22	59 51	35 28	98 89	70 60	96 88)	
TR-39 (321)		α,α,α-trifluoro-2-hydroxyamino-6-nitro-*N*,*N*-dipropyl-*p*-toluidine	8.1	2.6		30 (23	53 47	11 9	97 91	71 60	84 80)	
TR-40 (349)		α,α,α-trifluoro-2′,6′-dinitro-*N*-propyl-*p*-propionotoluidide		6.9		4 (3	19 17	21 19	98 91	23 21	96 89)	

Table I (*Continued*)

compd (mol wt)	structure	name	GLC t_r, min: 120 °C (100 °C)	GLC t_r, min: 140 °C (180 °C)	LC v_r, mL	hR_{TR} (hR_f) TLC solvent systems[d]: 1	2	4	5	6	10	12
TR-41 (351)		α,α,α-trifluoro-2,6-dinitro-*N*-(propan-2-ol)-*N*-propyl-*p*-toluidine		5.0		9 (7	19 17	24 20	96 89	28 25	92 85)	
TR-42 (351)		α,α,α-trifluoro-2,6-dinitro-*N*-(propan-3-ol)-*N*-propyl-*p*-toluidine		8.8		2 (2	6 5	4 3	83 78	10 9	65 60)	
TR-43 (309)		α,α,α-trifluoro-2,6-dinitro-*N*-(propan-2-ol)-*p*-toluidine		(2.2)		2 (2	7 6	3 2	91 85	12 10	69 64)	
TR-44 (309)		α,α,α-trifluoro-2,6-dinitro-*N*-(propan-3-ol)-*p*-toluidine		(3.1)		2 (1	4 3	2 2	79 74	7 6	64 58)	

[a] t_r given for 1.5% XE-60 column 3 mm i.d. × 180 cm at indicated column temperature. [b] LC conditions, see Methods. [c] $hR_{TR} = 100 \times R_f$ relative to trifluralin, $hR_f = 100 \times R_f$. [d] Solvent system 12 was used with fast running TLC plates, all other solvent systems were used with silica gel TLC plates (see Methods). Systems (1) benzene–carbon tetrachloride (40:60), (2) benzene, (4) hexane–methanol (97:3), (5) benzene–ethyl acetate (60:40), (6) benzene–1,2-dichloroethane (60:40), (10) benzene–methanol (90:10), (12) carbon tetrachloride. [e] TR-23 was mobile on TLC plate with solvent system 8 [benzene–ethyl acetate–acetic acid (60:40:1)] and 11 [benzene–methanol (75:25)].

Table II. Leaching of ^{14}C in Field Soil Treated with [^{14}C]Trifluralin Expressed as a Percent of Radioactivity in Total Soil Sample

	months (mo) after application and rates of application, kg/ha[a]				
depth, cm	12 (mo) 1.68 kg/ha	16 (mo) 1.68 kg/ha	24 (mo) 1.68 kg/ha	36 (mo) 1.68 kg/ha	36 (mo) 6.72 kg/ha
2.5	35	21	21	29	44
5.0	47	32	28	34	40
7.5	15	31	27	17	7
10.0	1	9	10	7	2
12.5	0.5	2	3	3	2
15.0	0.3	1	2	2	2
	(98.8)[b]	(96)	(91)	(92)	(97)
17.5	0.2	1	3	2	2
20.0	0.2	0.6	1.5	2	1
22.5	0.1	0.5	1	1	0.1
25.0	0.1	0.4	1	0.7	
27.5	0.2	0.3	1	0.5	
30.0	0.1	0.4	0.5	0.6	
32.5	0.1	0.3	0.5	0.5	
35.0	0.1	0.3	0.3	0.4	
38.0	0.1	0.2	0.2	0.3	

[a] 1.68 kg/ha corresponds to an application rate two times (twice the recommended rate) and 6.72 kg/ha corresponds to an application rate eight times that normally recommended for the soil type. [b] Cumulative amount of radioactivity from 0-15-cm depth.

tonite, Volclay KWK) and 80% sea sand. Methanol, aqueous methanol, 0.5 N NaOH, and Dowex A-1 chelating resin were used for extraction of radioactivity from these adsorbents. Radiochemical methods were used for the qualitative and quantitative analyses of the recovered radioactivity.

RESULTS AND DISCUSSION

Rate of Dissipation of [^{14}C]Trifluralin from Field Plots. The rate of dissipation of [^{14}C]trifluralin and of total radioactivity from field soil over a 3-year period is shown in Figure 1. The distribution of radioactivity (0–15-cm soil layer) among trifluralin, extractable transformation products, and nonextractable soil-bound residues is also shown. Although the average rainfall at the location of these experiments was 100 cm/year, there were significant variations in climatic conditions each year during the experimental period. Consequently, the experimental results varied from year to year and simple averaging of the data was confusing. To obtain a more realistic picture of the rate of dissipation, the analytical data were fitted to an exponential equation: $y = e^{a-b(x)}$ or $\ln y = a - b(x)$, where y = calculated percent, a and b = constants calculated from observed values by a least-squares method, and x = elapsed time in weeks after initial application. The values were calculated from fitted equations and plotted in Figure 1.

The data in Figure 1 show that after 1 year 69% of the applied radioactivity was present in the 0–15-cm soil layer, 14% was present as trifluralin, 12% was present as numerous extractable degradation products (extractable ^{14}C minus [^{14}C]trifluralin), and 43% (total ^{14}C minus extractable ^{14}C) was present as soil-bound radioactive residues. After 3 years the 0–15-cm soil layer contained 43.5% of the applied radioactivity, 1.5% trifluralin, 4% extractable degradation products, and 38% soil-bound radioactive residues.

Leaching of [^{14}C]Trifluralin and Its Degradation Products in Field Soil. Soil samples, collected at depths up to 38 cm from field plots treated at 1.68 and 6.72 kg/ha with [^{14}C]trifluralin by incorporation in the upper 7.5-cm soil layer, were analyzed for total radioactivity. The results in Table II indicated no significant leaching of either trifluralin or its degradation products. Most of the radioactivity (91–98.8%) was located in the 0–15-cm zone and 76–95% of the radioactivity remained in the zone of incorporation. Only traces of the radioactivity were detected in the deeper zones of the soil.

Identification of Extractable Transformation Products. The degradation pattern of trifluralin as depicted in Figure 1 is characterized by a rapid initial dissipation due most probably to the volatility of the compound, by rapid formation of soil-bound transformation products, and by the formation of small quantities of extractable transformation products. The amount of extractable transformation products reached a maximum of 12% of the applied trifluralin between 8 and 52 weeks after application and declined to 4% after 3 years.

Examination of crude soil extracts by TLC-RA in various solvent systems indicated the formation of more than 30 transformation products of trifluralin in soil under field conditions. None of the products formed exceeded 3% of the applied [^{14}C]trifluralin at any time during the 3-year period. Twenty-eight products were isolated and identified in the extractable portion. The presence of four additional products was observed in trace amounts. Extraction of up to 10 kg of soil and use of numerous model compounds in comparative chromatographic investigations were the primary techniques used in identification of most of the degradation products observed on TLC-RA. Chemical structures, names, molecular weights, and chromatographic characteristics of 49 model compounds used in this study are given in Table I. The transformation products formed from trifluralin in soil under field conditions and estimated by radiochemical methods are given in Table III.

Sufficient amounts of trifluralin and seven transformation products were isolated for identification by direct comparison with model compounds using various chromatographic methods and for verification of their structures by mass spectrometry. The following products were verified: TR-1, TR-2, TR-13, TR-15, TR-17, TR-20, TR-28, TR-32 (see Table I). The mass spectra of these products and of the model compounds given in Table I are discussed and published elsewhere (Golab and Occolowitz, 1979).

An additional 21 transformation products were isolated from soil and their nature tentatively established by direct comparison with model compounds using various chromatographic methods. The products were: TR-3 to

Table III. Estimated Amounts of Transformation Products in Field Soil Samples Calculated as Percent of Applied [^{14}C]Trifluralin

transformation products[a]	elapsed time after application, months							
	0.5 mo	1 mo	2 mo	4 mo	12 mo	16 mo	24 mo	36 mo
compound								
TR-2	1.5	1.8	2.6	2.8	1.7	0.8	0.4	0.2
TR-3 & TR-5[b]	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.05
TR-4	Tr[c]	0.05	Tr	0.05	0.1	0.1	0.07	0.04
TR-10 & TR-11[d]	ND[e]	0.5	1.5	0.6	ND	ND	ND	ND
TR-13	0.5	1.3	1.2	1.5	2.0	1.6	0.8	0.5
TR-15	ND	0.5	1.0	0.7	1.5	1.1	1.0	0.6
TR-17	0.3	0.9	1.0	0.2	1.0	0.5	0.6	0.4
TR-20	ND	ND	0.7	0.5	1.0	0.5	0.1	0.1
TR-28	0.05	0.5	0.5	0.6	0.5	0.6	0.3	0.3
TR-29	ND	0.05	0.1	0.05	Tr	0.1	0.08	0.07
TR-32	*f*	*f*	*f*	*f*	0.1	0.1	0.05	0.04
total of above	2.5	5.8	8.8	7.2	8.1	5.5	3.5	2.3
other degr. product[g]	2.5	5.4	3.4	3.2	3.9	5.3	3.5	2.0
unaccounted RA	2.0	2.8	0.8	0.6	0.2	1.2	1.5	0.2
soil bound RA[h]	9.0	14.0	20.0	28.0	43.0	44.0	42.0	38.0
RA in humin[i]	*f*	*f*	*f*	*f*	11	*f*	11	12
RA in humic acid	*f*	*f*	*f*	*f*	15	*f*	14	11
RA in fulvic acid	*f*	*f*	*f*	*f*	17	*f*	17	15

[a] Estimated average amount in 15-cm top soil obtained from various plots by radiochemical analyses. [b] Initially compounds were not well separated. [c] Tr = trace amount, less than 0.01%. [d] Data for both compounds, constant transformation of TR-10 to TR-11 occurred on TLC plates. [e] ND = not detected. [f] Not analyzed for. [g] Represents radioactivity corresponding to 15 identified degradation products which could not be estimated singly. [h] Radioactivity not extracted with methanol and aqueous methanol. [i] Separation given in Materials and Methods.

TR-12, TR-14, TR-16, TR-18, TR-19, TR-21, TR-29, TR-31, TR-36, TR-39, TR-40, and TR-41. An insufficient amount of these compounds was obtained from soil for mass spectral identification.

A very small amount of certain identified transformation products was formed in soil under field conditions, but larger amounts of these products were formed in soil flooded with water. This applied to the following: TR-4, TR-7, TR-9, TR-14, TR-19, TR-21, TR-36, and TR-39. Traces of four additional products, TR-22, TR-23, TR-30, and TR-44, were observed on TLC-RA in some of the soil extracts, indicating their probable formation.

Potential products not detected were compounds TR-24, TR-25, TR-26, TR-27, TR-33, TR-34, TR-35, TR-37, TR-38, TR-42, and TR-43 (Table I).

The identified tranformation products of trifluralin can be divided into four categories: (1) products formed by oxidative dealkylation of the *N*-propyl groups, reduction of nitro groups, and a combination of both reactions (TR-2 to TR-9); (2) cyclization products in the form of benzimidazoles (TR-11 to TR-19); (3) dimeric condensation products in the form of azoxy and azo derivatives (TR-28 to TR-32); and (4) miscellaneous products which resulted from oxidation/hydroxylation and reduction reactions. Compounds of the latter group are represented by TR-10, TR-40, and TR-41, which are oxidized in the propyl group; TR-21, oxidized in the trifluoromethyl group; TR-20, in which the *N*-dipropyl group was replaced by hydroxyl; TR-10 and TR-39, in which the nitro group has been reduced to hydroxylamino; and TR-36 in which the ring was hydroxylated. An indication of the polarity of the identified degradation products can be found in Table I where TLC R_f values are given for seven solvent systems.

Of special interest was the detection of a series of benzimidazoles, TR-14, TR-16, TR-17, TR-18, and Tr-19, characterized by dealkylation and/or reduction of the remaining nitro group. The possible formation of dimeric products from trifluralin was reported by Probst et al. (1967) and by Golab et al. (1969) in their work on fate of trifluralin in soils, plants, and ruminant animals. Crosby and Leitis (1973) and Leitis and Crosby (1974) have reported the possible formation of dimeric products in their work on the photolytic degradation of trifluralin.

Another series of transformation products resulted from oxidation/hydroxylation in the propyl group at carbon-1 (TR-10 and TR-40), carbon-2 (TR-41), and possibly carbon-3 (TR-44). Identified products indicated hydroxylation of trifluralin (TR-1) and of the monodealkylated derivative of trifluralin (TR-2). Nelson et al. (1976) reported an aliphatic hydroxylation at carbon-2 and carbon-3 of the propyl group of trifluralin and of TR-2 by rat liver microsomes. Zulaliam et al. (1976) have also reported extensive oxidation and/or hydroxylation of the aliphatic groups of penoxalin [*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzamine] in the rat and goat. They observed hydroxylation in the methyl and propyl groups and further oxidation to the carboxylic acid.

Partially reduced compounds have been detected in which one nitro group was reduced to the hydroxylamine (TR-10 and TR-39). These products were expected as the intermediates in the formation of benzimidazoles and dimeric azoxy compounds.

Aromatic hydroxylation of trifluralin resulted in the formation of TR-36. Although this transformation product was formed in soil under both aerobic and anaerobic conditions, more was formed under anaerobic conditions. Aromatic hydroxylation of the trifluralin molecule indicates the possibility of a similar hydroxylation of its transformation products, including the triamine derivative (TR-9). Aromatic hydroxylation may make the ring susceptible to rupture, which would facilitate eventual mineralization.

Compound TR-10, α,α,α-trifluoro-2′-hydroxyamino-6′-nitro-*N*-propyl-*p*-propionotoluidide, was determined to be the precursor of TR-11, 2-ethyl-7-nitro-1-propyl-5-(trifluoromethyl)benzimidazole 3-oxide (Table I and Figure 2). Conversion of TR-10 to TR-11 occurred on thin-layer chromatoplates or in organic solvents and was evidenced by TLC, GLC, MS, and IR. The previously published structure of the precursor to TR-11, 2-ethyl-2,3-dihydroxy-7-nitro-1-propyl-5-(trifluoromethyl)benzimidazoline, by Golab and Amundson (1975) and by Probst et al. (1975), has been determined to be in error. The MS and IR evidence for the new structure of TR-10 is dis-

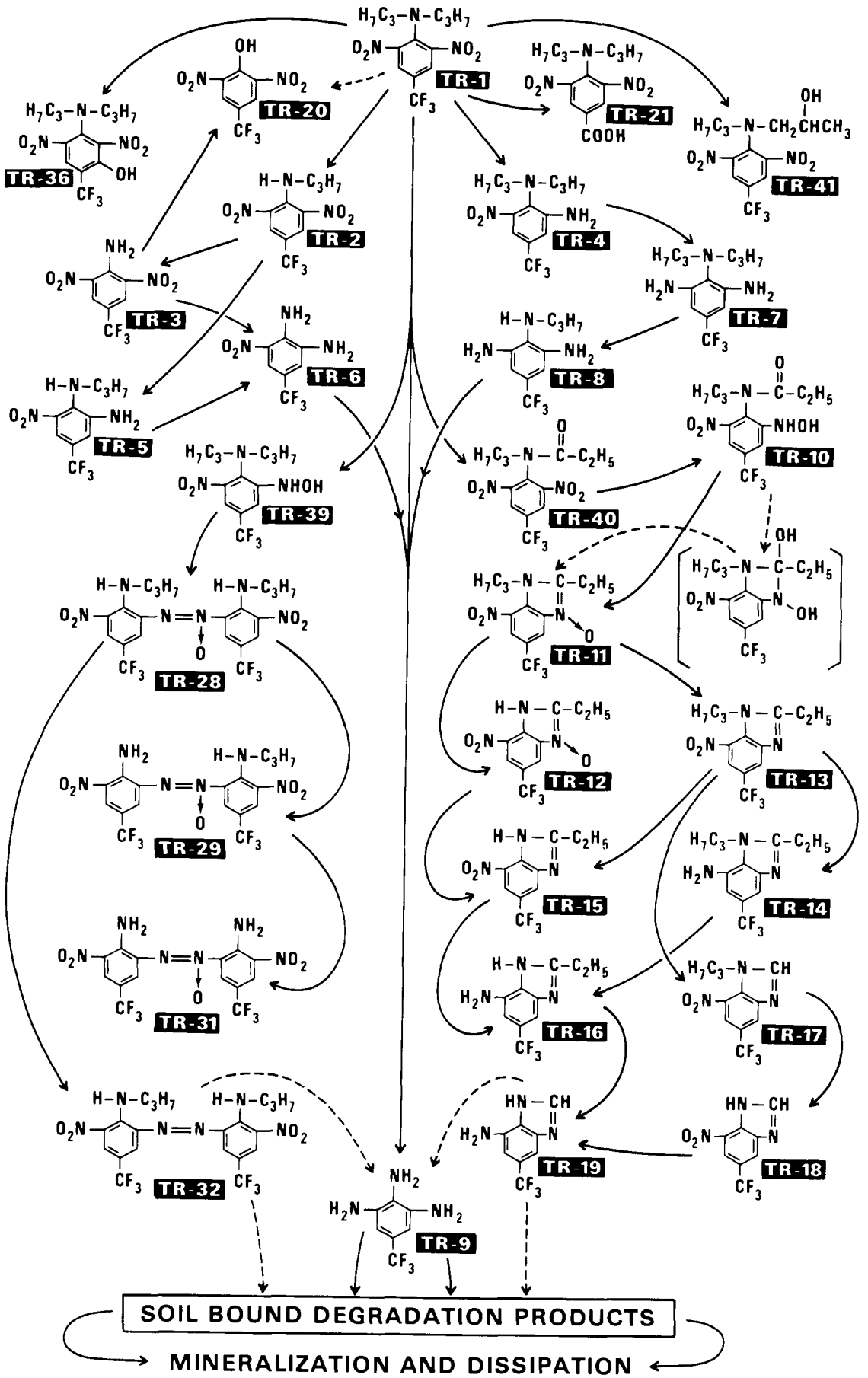


Figure 2. Postulated pathways of [^{14}C]trifluralin transformation in field soil. (→) Most likely route. (- - →) Possible alternate route.

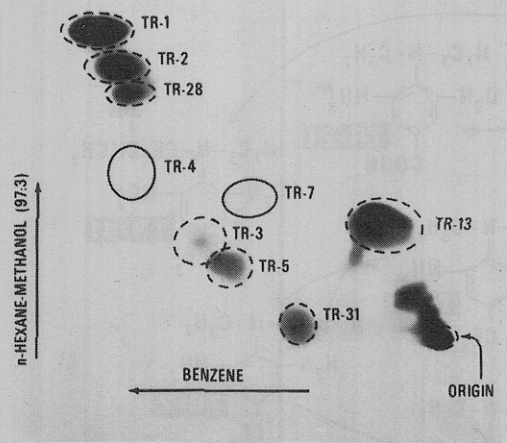


Figure 3. An 80-day exposure radioautograph of 16-month soil extract from plot treated with [^{14}C]trifluralin. Prepared from two-dimensional TLC plate of crude methanol extract and chromatographed with nine model compounds. Solid lines indicate model compound zones which contained a trace of radioactivity but which were not visible on TLC-RA.

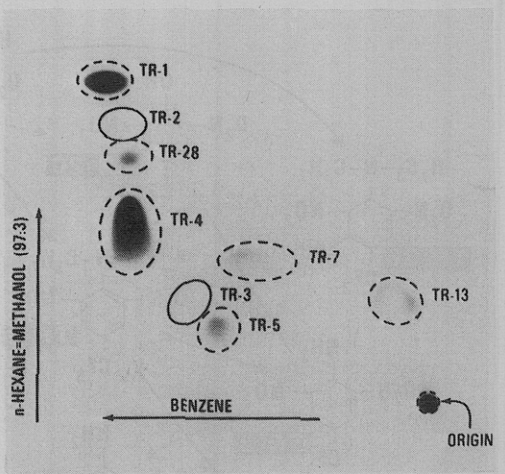


Figure 4. A 40-day exposure radioautograph of 2-week flooded soil extract. Prepared from two-dimensional TLC plate of crude methanol extract chromatographed with eight model compounds. Solid lines indicate model compound zones which contained a trace of radioactivity but which were not visible on TLC-RA.

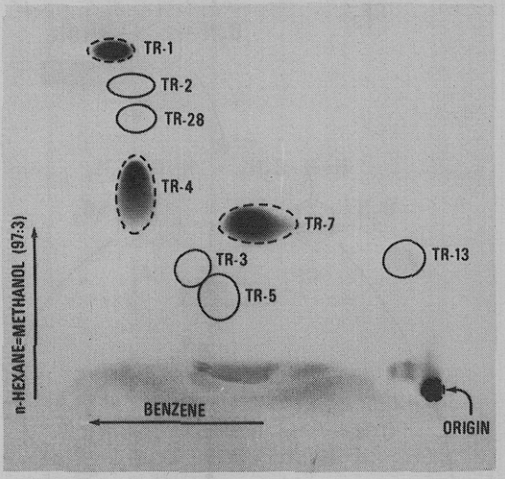


Figure 5. A 40-day exposure radioautograph of 4-week flooded soil extract. Preparation and description of TLC-RA was the same as shown in Figure 4.

cussed by Golab and Occolowitz (1979). The dihydroxybenzimidazoline is probably a reaction intermediate between compounds TR-10 and TR-11 (Figure 2). Reported similar chemical structures for dihydroxybenzimidazolines from isopropalin (Golab and Althaus, 1975) and from oryzalin (Golab et al., 1975) are also incorrect and have the open-ring structures of hydroxyaminopropionotoluidides.

Field soil treated with [^{14}C]trifluralin was flooded with water to create an anaerobic-like condition, and soil samples were analyzed periodically to determine the rate of trifluralin degradation and to identify the extractable transformation products formed. The major products formed in this system were compounds with the nitro group reduced, TR-4, TR-7, and TR-14. Other products were present in detectable amounts.

The difference in the nature of the transformation products formed under aerobic and anaerobic conditions is more quantitative than qualitative. Reduction also occurred under aerobic conditions and oxidative dealkylation under anaerobic conditions, e.g., TR-5, a monodealkylated monoreduced transformation product of trifluralin, was formed under both conditions. The major aerobic degradation product of trifluralin, compound TR-2, was present only in trace amounts in flooded soil. The major transformation products of trifluralin in flooded soil, compounds TR-4 and TR-7, were present only in trace amounts in aerobic soil.

The quantitative differences in the formation of transformation products of trifluralin under aerobic and anaerobic conditions are presented in Figures 3, 4, and 5. Figure 3 shows the thin-layer radioautogram of a crude extract of a soil sample removed from a field plot 16 months after application of [^{14}C]trifluralin. The following separated products were visible: TR-1 (trifluralin), TR-2 (monodealkylated), TR-28 and TR-31 (azoxy products), TR-3 (didealkylated), TR-5 (monoreduced, monodealkylated), and TR-13 (benzimidazole). The more polar transformation products were located close to the origin of the TLC. It should be noted that only traces of TR-4 and TR-7 (mono- and direduced products of trifluralin) were present on TLC-RA.

Figures 4 and 5 show thin-layer radioautograms of soil extracts obtained from [^{14}C]trifluralin-treated soil flooded with tap water for 2 and 4 weeks, respectively. Several observations can be made from these figures: (a) products of oxidative dealkylation, like compounds TR-2 and TR-3, were present in trace amounts; (b) the major transformation product in 2-week-old soil (Figure 4) was monoreduced trifluralin, TR-4; (c) the same soil extract showed appreciable amounts of several other compounds, such as the azoxy derivative (TR-28), the direduced product (TR-7), the monodealkylated–monoreduced product (TR-5), and the benzimidazole (TR-13); (d) after a period of 4 weeks (Figure 5), less of the parent compound (TR-1) was present; TR-5, TR-13, and TR-28 were further degraded and were present in very small amounts; and the main product, TR-4, was further reduced to TR-7, which was the main transformation product in the 4-week soil extract. Another major transformation product, TR-14, was located with other minor products at the origin on TLC-RA. These two figures show that the formation of transformation products is a dynamic process. A faster degradation of trifluralin (85% in 3 weeks and 95% in 8 weeks), a faster rate of conversion to soil-bound radioactive residues (25% in 3 weeks and 50% in 8 weeks), and a slow disappearance of total radioactivity (less than 20% in 8 weeks) were observed in the flooded samples. Also, more radioactive degradation products were extractable (20–25%) in the 8-week flooded soil sample than in soil samples obtained from the field plots. This extractable

radioactivity contained 23 transformation products which were identified by various chromatographic methods, and traces of six additional products were observed. The following products were identified: compounds TR-4 to TR-21, TR-28, TR-32, TR-36, TR-39, and TR-40. Traces of the following products were detected: compounds TR-2, TR-3, TR-22, TR-23, TR-31, and TR-35 (Table I).

Fate of TR-28 [2,2′-Azoxybis(α,α,α-trifluoro-6-nitro-*N*-propyl-*p*-toluidine)] in Soil. Since the dimeric compounds represented a new class of transformation products of trifluralin, the fate of one of these products was investigated in both soil and plants. The major representative of this class was an azoxy compound, TR-28, which was present at a level of 0.6% of the applied trifluralin 16 months after application (Table III). TR-28, labeled with ^{14}C in both rings, was investigated under greenhouse aerobic and anaerobic conditions and under field conditions.

In the greenhouse aerobic studies, 60% of the applied TR-28 was present 8 weeks after application, 15% was present as soil-bound radioactive residues, and 25% was present as extractable degradation products. The majority of the extractable products were more polar than TR-28.

Faster degradation of TR-28 occurred under anaerobic conditions. Three weeks after incorporation of [^{14}C]TR-28, approximately 23% of the applied radioactivity was soil bound, 22% remained as compound TR-28, and 17% was TR-32, a less polar reduction product of TR-28. The remainder of the radioactivity represented a mixture of polar degradation products formed from TR-28. After 8 weeks in flooded soil, 90% of the applied radioactivity remained in the soil. Of this, 75% was soil bound and 15% was extractable. The extractable radioactivity was divided among the originally applied TR-28 (5%), the less polar degradation product, TR-32 (5%), and the remaining 5% was a mixture of several more polar products which were not identified.

Results from field plots indicated that the compound was not phytotoxic and that it was further degraded. Soil samples obtained 3.5 months, 1 year, and 2 years after application contained 45, 40, and 25% of the applied [^{14}C]TR-28, respectively. After 2 years 10–20% of the applied radioactivity had dissipated, 30% was soil bound, and the remainder represented a mixture of degradation products. Of the remaining radioactivity, 90–95% was located in the top 15-cm soil layer, indicating that TR-28 and its transformation products did not leach. Application rates of 0.112 and 0.224 kg/ha of [^{14}C]TR-28 to field plots exceeded the concentration of TR-28 found in soil originally treated with trifluralin by a factor of approximately 20 and 40, respectively.

No significant amounts of radioactivity above control levels were found in soybean plants grown in the soil treated with [^{14}C]TR-28. The amount of radioactivity was determined by combustion of soybean plant samples obtained 3, 8, and 16 weeks after planting. Wheat seeded as a rotation crop in the plots after the soybeans were harvested, and harvested 1 year after application of [^{14}C]TR-28, did not contain significant amounts of radioactivity in mature straw and grain.

Essentially no [^{14}C]TR-28 remained after 10 h irradiation in methanol (see Methods). Compound [^{14}C]TR-28 exposed to combined UV and fluorescent lights on silica gel TLC plates was completely degraded after 2 weeks.

Soil-Bound Degradation Products. The previously postulated pathway of trifluralin transformation in soil (Golab and Amundson, 1975; Probst et al., 1975) was based on isolated and identified extractable degradation products formed in soil. These pathways, however, failed to identify the soil-bound residues. It was apparent from previous studies, and from this work, that trifluralin transformations in soil progressed by a variety of mechanisms, e.g., dealkylation (predominant under aerobic conditions), reduction (predominant under anaerobic conditions), oxidation, hydrolysis, cyclization, condensation, and combinations of these reactions resulting in the formation of more than 30 degradation products. The formation of phenolic (TR-20) and acidic (TR-21) products was probably followed by further reduction and/or dealkylation. The final identifiable degradation product was TR-9, a triamine derivative of trifluralin, which was detected in small amounts in soil flooded with water and in even smaller amounts in field soil plots. Testing has indicated that all products formed from trifluralin in soil are less phytotoxic than trifluralin (Lilly Research Laboratories, unpublished data).

During the transformation study of trifluralin, the majority of the radioactivity remained in soil as nonextractable or soil-bound material. Soil-bound radioactive residues were considered to be those not extractable with methanol and methanol–water. The rapid formation of soil-bound degradation products in field soil is shown in Figure 1 and in Table III. The great difficulty with the classical method of extraction of organic matter with 0.5 N NaOH and subsequent fractionation into humin, humic acid, and fulvic acid is that the method gives rise to products which are not definite chemical entities. Extraction of soil-bound radioactivity with Dowex A-1 chelating resin was less efficient than with 0.5 N NaOH.

Soil-bound radioactivity extracted by alkali could not be further partitioned into organic solvents in amounts sufficient to pursue identification. Therefore, an experiment was designed to find which degradation product of trifluralin might be associated with the "soil-bound residue" (see Methods). An adsorption–desorption study was performed with selected degradation products using three adsorbents: sand, soil, and humic acid (12.5%) mixed with sand. The results of this study are given in Table IV. All compounds under investigation could be recovered from sand and soil with the exception of the triamine derivative of trifluralin (TR-9). Recovery of TR-9 was obtained only by means of its immediate derivatization to 4-(α,α,α-trifluoroacetamido)-2,6-bis(trifluoromethyl)-benzimidazole which was determined by GLC using electron affinity detection. Recoveries of TR-9 were 15% from 1-h dry-sand samples, 13% from 1-h moist-sand samples, and less than 0.1% from 3-day moist-sand samples. Recoveries were 0.8% from 1-h dry-soil samples, less than 0.1% from 1-h moist-soil samples, and none from 3-day moist-soil samples. Trifluralin and many of its derivatives were recoverable from the humic acid–sand mixture. No recoveries from the humic acid–sand mixture were obtained when the compounds had both nitro groups reduced. This was observed with the substituted and nonsubstituted triamine derivatives of trifluralin, TR-7 and TR-9, and with the reduced benzimidazole derivatives, TR-14, TR-16, and TR-19. The substituted triamine derivative, TR-7, was partially recovered at the higher pH (Table IV) and the nonsubstituted triamine derivative, TR-9, was not recovered at any pH. The results suggested that TR-9 was either itself bound to the humic substances or that it is an intermediate to other compounds which are chemically bound or complexed with humic substances. It should be noted that reasonable recoveries of the nonsubstituted monoamine derivative TR-3 and the substituted and nonsubstituted diamine derivatives, TR-4,

Table IV. Adsorption-Desorption of Trifluralin and Selected Derivatives (Percent Recovery[a] from Various Adsorbents)

adsorbents[b] and recovery time[c]	compounds																
	TR1	TR2	TR3	TR4	TR5	TR6	TR7	TR7 (pH[f] 12)	TR9[d]	TR9 (pH 12)	TR14	TR15	TR16	TR18	TR19	TR20 (pH 1.5)	TR21 (pH 1.5)	TR28[e]
sea sand																		
dry, 1 h	92	100	97	100	88	100	84	*g*	15	*g*	90	*g*	98	*g*	*g*	82	86	*g*
moist, 1 h	82	94	87	43	95	100	100	*g*	13	*g*	*g*	*g*	*g*	64	78	*g*	*g*	*g*
moist, 3 days	99	85	100	31	74	86	94	*g*	Tr[h]	*g*	46	93	36	96	83	100	100	100
soil																		
dry, 1 h	99	93	98	100	75	99	92	*g*	0.8	*g*	84	*g*	62	*g*	*g*	58	55	*g*
moist, 1 h	96	92	99	99	100	100	84	*g*	Tr	*g*	*g*	*g*	*g*	84	84	*g*	*g*	*g*
moist, 3 days	99	89	89	100	64	57	60	57	ND[i]	ND	55	84	38	99	46	85	59	95
humic acid/sea sand																		
dry, 1 h	88	100	99	68	77	24	ND	*g*	ND	*g*	ND	*g*	ND	100	ND	100	65	*g*
moist, 1 h	99	95	100	96	62	61	ND	*g*	ND	*g*	ND	*g*	*g*	93	ND	*g*	*g*	*g*
moist, 3 days	98	99	93	99	13	28	ND	32	ND	ND	ND	84	ND	98	ND	91	76	67

[a] Average of two–three analyses. [b] Adsorbent (pH in water): sand (5.8), soil (8.0), humic acid–sand (4.8). [c] Elapsed time from incorporation to extraction. [d] TR9 was analyzed by GC-EC after its derivatization and by radiochemical methods using ^{14}C-labeled compound. [e] TR28 was ^{14}C labeled and analyzed by radiochemical methods. [f] Compounds were extracted at given pHs. TR20 and TR21 were analyzed by GLC after methylation. [g] Not analyzed. [h] Tr = trace. [i] ND = nondetectable.

TR-5, and TR-6, were obtained from all three adsorbents. Bartha (1971) and Hsu and Bartha (1974) reported that chloroanilines, the monoamine residues from propanil herbicide, were chemically bound and complexed with the humic substances in soil.

Compound [^{14}C]TR-9 demonstrated an interesting fate when mixed with various adsorbents (see Methods). The following observations were made from studies on the fate of [^{14}C]trifluralin in soil and from studies in which [^{14}C]TR-9 was mixed with various adsorbents: (1) Because all precursors of TR-9 were detected in field soil (TR-2 to TR-8), it is a logical conclusion that TR-9 is the next member in the transformation pathway (Figure 2). Indeed, traces of TR-9 were detected by TLC-RA in soil under aerobic field conditions and under anaerobic laboratory conditions. (2) Compound TR-9 and/or [^{14}C]TR-9 degraded quickly when mixed with soil and various adsorbents. However, TR-9 is stable in crystalline form. (3) When [^{14}C]TR-9 was mixed with sand or with a mixture of 20% clay in sand, degradation occurred rapidly. Approximately 90% of the radioactivity was recoverable from both adsorbents using methanol, followed by methanol–water extraction. Only 15% of the extracted radioactivity matched on TLC-RA with the position of the reference TR-9. The remaining extracted radioactivity showed more than 16 spots on TLC-RA, of which 10 were less polar and six more polar than TR-9. The results suggest that clay slightly enhanced the degradation of TR-9. (4) When [^{14}C]TR-9 was mixed with 12.5% humic acid in sand, approximately 14% of the radioactivity was recovered, but none of the recovered radioactivity could be identified as [^{14}C]TR-9. (5) After [^{14}C]TR-9 was mixed in soil, approximately 35% of the radioactivity was recovered in 1 h, 28% in 1 day, and 22% in 3-day samples, respectively. In the extractable portion, only 1% of the radioactivity from 1-h samples and 0.1% from 1-day samples matched on TLC-RA with the position of the reference TR-9. The radioactivity nonextractable with methanol followed by methanol–water was considered to be soil bound. Further extraction of this soil-bound radioactivity from 1-day soil samples with 0.5 N NaOH and with Dowex A-1 chelating resin removed an additional 36 and 23% of the originally applied radioactivity, respectively. Only 0.4% of the radioactivity could be extracted from either the NaOH or Dowex A-1 solutions with organic solvents and this radioactivity was not further analyzed.

One-day soil samples which contained incorporated [^{14}C]TR-9 were also extracted directly with 0.5 N NaOH and Dowex A-1 resin without prior extraction with methanol and methanol–water. The sodium hydroxide solution removed 35% and Dowex A-1, 29%, of the applied radioactivity. Only 3% of the initial radioactivity from the NaOH extract and 2% from the Dowex A-1 extract could be partitioned into organic solvents, and only 0.5 and 0.3% of the original radioactivity matched position with reference TR-9 and TLC-RA, respectively.

Soil samples containing [^{14}C]TR-9 were also extracted with 0.5 N HCl in a manner similar to the extraction with 0.5 N NaOH. Only 4% of the applied radioactivity was recovered from soil and only 0.4% of the radioactivity could be partitioned into an organic solvent.

CONCLUSIONS

The results obtained from degradation studies of trifluralin in soil under field and laboratory conditions indicate a rather complex transformation pathway for this herbicide (Figure 2). Each transformation product appeared to undergo further change as evidenced by the lack of accumulation of any one product and by the relatively

small amount of extractable products over the 3-year period.

It was apparent from these studies that the trifluoromethyl group of trifluralin remained intact through the various transformations of the compound, except for the formation of TR-21 and TR-22 in which the trifluoromethyl group was oxidized to the carboxylic acid. The final fate of the trifluoromethyl group is most probably mineralization to fluoride.

The isolation of only very small amounts of TR-3 vs. relatively larger amounts of TR-20 may indicate the relative ease of nucleophilic substitution of the unhindered didealkylated amino group of TR-3 by hydroxyl group.

Aromatic hydroxylation of trifluralin and its transformation products may facilitate rupture of the benzene ring leading to the eventual mineralization and dissipation of the trifluralin degradation products. This may explain the decrease of total and soil-bound radioactivity with time.

Investigation of the soil-bound radioactive residues indicated that all identified transformation products except TR-9 could be recovered from soil. Although it was not established if TR-9 and/or its transformation products were complexed with soil organic matter in the soil and with humic acid in the humic acid–sand mixtures, TR-9 appeared to be implicated in the soil-bound residues. It is possible that not only those transformation products which result from dealkylation and reduction of trifluralin degrade to TR-9 but that the degradation of benzimidazoles and azoxy compounds may also result in formation of TR-9. The transformation of trifluralin and its degradation products through TR-9 to soil-bound residues would explain the presence of only traces of TR-9 in field soil samples over the 3-year period.

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