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^{14}C-Residues of Trifluralin in a Soil and Their Uptake by Carrots

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Trifluralin (μ,μ,μ-trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine) is a selective pre-emergence, soil incorporated herbicide used for control of a wide variety of grass and broadleaf weeds in many agronomic and horticultural crops. A number of workers have investigated persistence, degradation and movement of trifluralin in soil and plants (Duseja and Holmes 1978; Golab et al. 1979; Helling 1976). When root crops such as carrot onions and turnips were grown in soil treated with trifluralin, most of the herbicide residues were reported to be present on their surface (Golab et al. 1967; Probst et al. 1967).

In Turkey the use of trifluralin in the cultivation of carrots is about 3240 kg a.i. (Anonymous 1996). The tolerance limits for the herbicide in carrot reported to be 1.0 ppm (Sitting 1980; Anonymous 1989).

The purpose of the study reported here was to investigate the persistence and degradation of ^{14}C-trifluralin in a soil under outdoor conditions. The uptake of the herbicide from the treated soil by carrots was also studied. A number of degradation products of trifluralin in the soil and carrots were identified and the nature of the nonextractable (bound) residues was determined.

MATERIALS AND METHODS

Trifluralin-Ring-UL-^{14}C (μ,μ,μ-trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine⇒TP–1) 500 μCi (19.1 mCi/m*M)* was supplied by Sigma Chemical Co.(via International Atomic Energy Agency, Vienna). The radiochemical purity of the herbicide was 90.15%. Reference standard of pure trifluralin (purity 99.5%) was a gift from Dow Elanco. The metabolites μ,μ,μ-trifluoro-2,6-dinitro-/V-propyl-*p*-toluidine (TR-2); μ,μ,μ-trifluoro-2,6-dinitro-*p*-toluidine (TR-3);μ,μ,μ-trifluoro-5-nitro-N^4, $N^4$6-dipropyltoluene-3,4-triamine (TR-4);and 4-(dipropylamino)-3,5-dinitrobenzoic acid (TR-21) were supplied by Biosciences Research Lab, U.S. Dept of Agriculture, ARS, Fargo, USA.

The soil used in this study was a sandy loam with a pH of 7.5 and 3.7% organic matter. Particle size analysis indicated 17.8% clay, 13.4% silt and 68.8% sand. The soil was air-dried, screened and passed through a 20-mesh sieve.

Carrots (Nandor F_1 variety) were grown in boxes as described later at the Turkish Atomic Energy Authority's Experimental Farm, Ankara, Turkey. The experiments were carried out under the outdoor conditions in boxes measuring 60x60x60 cm, constructed from galvanized-steel. The base of the boxes contained holes to permit the drainage of excess water (Kohli et al. 1973). The boxes (pots) were covered inside with polyethylene

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sheets. The bottom 25 mm of the box was packed with stone chips of near 25 mm diameter, and the stones were covered with a 25 mm layer of well-rotted turf. The two boxes were filled with sand-soil-manure mixture (5:3:3), and were placed in the pits such that the upper surface of the soil in boxes was at the level of the surrounding ground. ^{14}C-Trifluralin, applied at the recommended rates of 0.84 kg/ha, was incorporated into the top 7.5 cm of the soil. The specific activities for the 1st and 2nd pot were 3.385 µCi/mg and 3.828 µCi/mg. Carrots were sown on April 28, 1994. The fertilizer $(NH_4)_2SO_4$ was applied before and after emergence of carrots at the rate of 75 g/pot. The total rainfall during the growing season was 52.6 mm with a maximum of 18.4 mm and a minimum 0.0 mm. The mean air temperature from May to August was 20.5°C.

Soil samples were removed from the two pots immediately after application of ^{14}C-trifluralin and periodically thereafter one month interval until harvesting time. Nine soil samples from each pot were collected, combined, mixed and then two combined samples were taken for combustion. Samples were collected at depths of 0-7.5 cm, 7.5-15 cm, and 15-22.5 cm. The carrots were harvested on August 31,1994 from the two pots. The top and roots were separated, the roots were washed with cold water to remove any adhering soil particules whereas the tops were discarded.

All samples were analyzed or processed in duplicate and average values are reported.

Soil samples (50-100 g) were shaken with 150-300 mL portions of methanol (x3) followed by an equal amount of 50% aqueous methanol (x2) using Orbital Shaker for 30 min. The combined filtrate was then concentrated to a small volume using Rotary evaporator and partitioned with ethylacetate. The organic phase was passed through a column of dried anhydrous sodium sulphate to remove any water (Golab et al. 1979; Lue et al. 1984).

The carrots were peeled and the pulp and peel were diced into small pieces. About 50-75 g portion of the pulp was extracted with methanol and 50% aqueous methanol as described earlier.

Combustion of soil and/or carrot samples were done in a Packard Sample Oxidizer, Model 306, to produce $^{14}C O_2$ The latter was absorbed in and admixed with an appropriate volume of Carbosorb E and Permaflour E. Liquids (extracts) were assayed in a Beckman Model LS 3801 Scintillation Spectrometer.

(i)Thin Layer Chromatography (TLC). TLC was performed with 20x20 cm precoated silica gel GF chromatoplates with 1.0 mm gel thickness. Plates were initially cleaned by developing in methanol. The metabolite TR-21 was methylated with diazomethane to obtain methylester. The five reference standards were then spotted on the plates and then developed in a solvent system comprising hexane:benzene(1:1). The developed plates were observed under UV light (λ=254 nm). Under these experimental conditions the following R_f values were obtained: TR-1 R_f 0.9; TR-2 R_f 0.79; TR-3 R_f 0.47; TR-4 R_f 0.75; and TR-21 (methylester) R_f 0.28. The concentrated carrot extracts were methylated with diazomethane and the volumes were adjusted to 1 mL. The extracts were then applied on plates at about 1 cm below the preabsorbent line and developed in hexane:benzene (1:1) solvent system in containers which were lined with paper and saturated prior to use. Polaroids photographs of the radioactive areas on the developed plates were taken with Beta Camera (Bethold, Model LB 292) and the plates were also scanned by TLC-scanner for ^{14}C (Bioscan System 2000, Autochanger 2000). The plates separated in three radioactive bands at R_f 0-0.14, 0.14-0.52, and 0.52-0.95. The material in these bands was scrapped off the plate and extracted with 20 mL acetone (x5). Each extracts were evaporated to a small volume and subjected for further clean up by column chromatography. (ii) Column Chromatography. Column chromatography was carried out with a glass column (20 x 1.5 cm) containing 4 g of florisil packed in

hexane:benzene (2:1). A mixed reference standard (1.6 mL) in hexane:benzene (2:1) was applied to the column and eluted with hexane:ethylether (99:1). Under these conditions recoveries for each reference standard were found as follows: TR-1, 89.4%; TR-2, 84.3%; TR-4, 69.5%; TR-21M, 87.5%. The carrot extracts from TLC plates as described earlier were brought to just dryness and dissolved in 1.6 mL mixture of hexane:benzene (2:1). The sample was applied to the column and eluted with 4 x 10 mL hexane:ethylether (99:1) solvent system. The eluates were combined and evaporated under air to just dryness, redissolved in 1.6 mL hexane:benzene (2:1), and finally analyzed by GC (Lue et al.1984; D'amato et al.1993). (iii) Gas Chromatography. The gas chromatograph was a Varian Model 3400 equipped with ^{63}Ni detector and a 15 m x 0.545 mm capillary column coated with Carbowax (1.0 mm) For the analysis of mixed standard the column was operated at 160°C and for TR-21M at 190°C. The nitrogen carrier flow rate, injector port temperature and detector temperature were 20 mL/min, 18O°C and 310°C, respectively. Under these experimental conditions the retention time for TR-1, TR-2, TR-3, TR-4 and methyl ester of TR-21 were 1.8, 3.1, 5.2, 9.9, and 8.8 min, respectively.

The identify of the compound was confirmed by comparing the GC retention times with those of authentic samples and by gas chromatography-mass spectrometry. A high resolution mass spectrometer, Model VGZAB-ZF, connected to a Varian GC Model 3710 was used. The mass spectra were recorded at 70 eV.

The SFE system (Suprex Model SFE-50, Suprex Corp., Pittsburg, PA) used consisted of a 250 mL syringe pump, a control module for the SFE system, an extraction oven, a 5 mL extraction vessel containing sample, and a four-port valve connected with the outlet restriction (fused silica tubing, 50 mm id) that was vented into the first of the three glass tubes containing 50 mL of methanol. The three glass tubes containing methanol were connected in series for collection of the released material. Extraction was carried out with the modified CO_2 using methanol (30% CH_3OH in CO_2) which was delivered by an HPLC pump (Vanan 2510). The flow rate of mobile phase was 2 mL/min, and the extraction was carried out at 18O°C and 375 atm for 2 h after initial equilibration of the SFE system for 5 min at 180°C and 150 atm.

RESULT AND DISCUSSION

The schematic diagram for the analysis of ^{14}C-trifluralin and its metabolites residues in soil and carrots is shown in Figure 1.

The amounts of total ^{14}C-residues recovered from soil over the growing period of carrots is shown in Figure 2a. The distribution of extractable and nonextractable (bound) ^{14}C-residues are also shown in Figs 2b and 2c. The data show that after four month of application, the combined three layers of soil (0-22.5 cm) contained 50.4%, 15%, and 23.8% of the initially applied radioactivity as total ^{14}C, extractable ^{14}C, and soil-bound ^{14}C-residues, respectively. The extractable ^{14}C-residues decreased with time. This in turn corresponded with an increase in bound ^{14}C-residues (Figs 2b and 2c). Golab et al.(1979) observed that after 3 years of application of ^{14}C-trifluralin, the 0-15 cm soil layer contained 43.5% of the initially applied radioactivity. Smith and Muir (1984) found after 45 weeks, radioactivity recovered from the ^{14}C-trifluralin treated plots was 77% of the applied, while nonextractable activity accounted for 10% of the initially applied.

The persistence and movement of ^{14}C-trifluralin residues in soil changed with the time. Thus, after one month about 97.5% of the applied radioactivity was still present in the combined three depths of the soil. However, four months later only about half of applied radioactivity was present. Duseja and Holmes (1978) found that after 25 and 120 days application (0.75 lb/acre application rate), 48.3% and 25.9% of applied radioactivity, respectively, was present in the combined soil sample from the three depths.

Soil samples, collected at the three depths from the pots treated with ^{14}C-trifluralin by

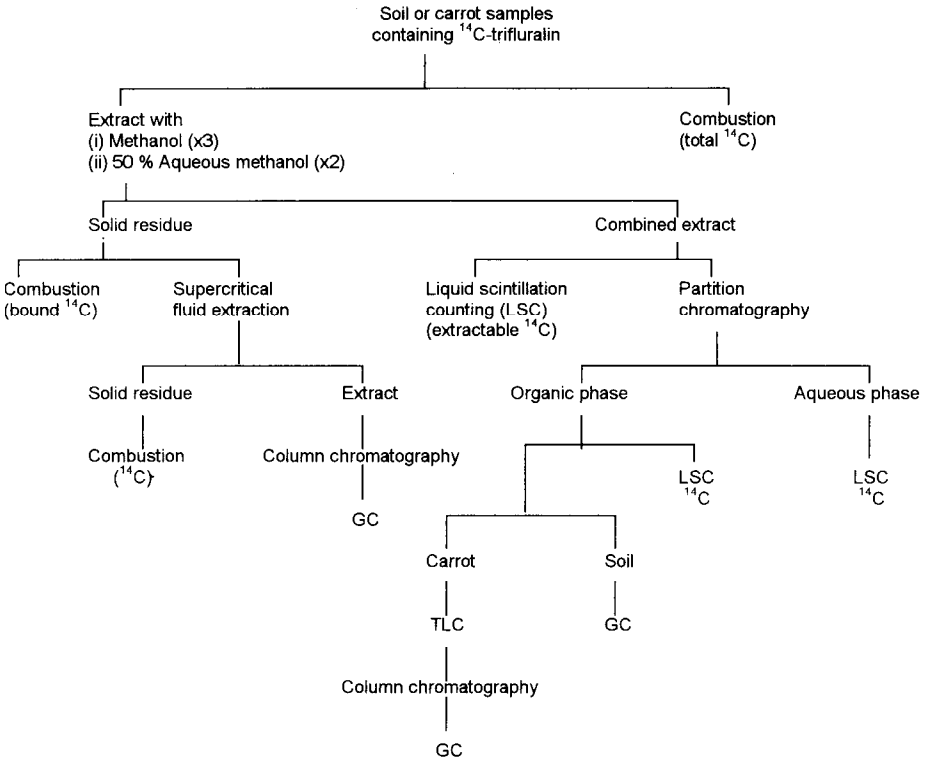

Figure 1. Schematic diagram for the analysis of ^{14}C-trifluralin residues in soil and carrots.

incorporation in the upper 7.5 cm soil layer, were analyzed for the presence of the herbicide and its degradation products. The results in Figure 2 and Table 1 indicate a small amount downward movement of the herbicide and/or its metabolites. Most of the applied radioactivity was located in the zone of incorporation. Thus, about 11-44% and 5.5-8.0% of the applied radioactivity remained in the 7.5-15 cm and 15-22.5 cm soil layers, respectively (Figure 2a). Golab et al.(1979) observed that, after 12 and 24 month (at the rate of 1.68 kg a.i/ha application) 91% and 98.8% of the radioactivity was located in the 0-15 cm zone and 76% and 95% of the activity in the zone of incorporation,

Examination of soil extracts by GC indicated the presence of TR-1 (trifluralin) and TR-2 (Table 1). Four month later, soil extracts showed the presence of TR-2 in greater concentration than TR-1. None of the metabolites found were present more than 3.5% of the initially applied ^{14}C-trifluralin at any time during the growing period. Similar observation have been reported earlier indicating that the metabolites formed were about 3% of the initial herbicide applied (Golab et al.1979). The data in Table 1 also show the presence of small amounts of the metabolites TR-3, TR-4, and TR-21M. Similar findings have been reported by Golab et al.(1967) and Probst et al.(1967).

Soil-bound ^{14}C-residues were considered to be those which were not extracted with methanol and 50% aqueous methanol as described earlier. The nature of these ^{14}C-residues was determined by subjecting the solvent extracted soil to supercritical fluid extraction (SFE). It was noted that even after SFE of the solvent extracted soil some of the ^{14}C-residues still remained nonextractable (Table 2). SFE released ^{14}C-residues between 12.5% and 66.6% of the total bound ^{14}C. The SFE extracts obtained were analyzed by GC. The amount of the extractable ^{14}C-residues by SFE are shown in

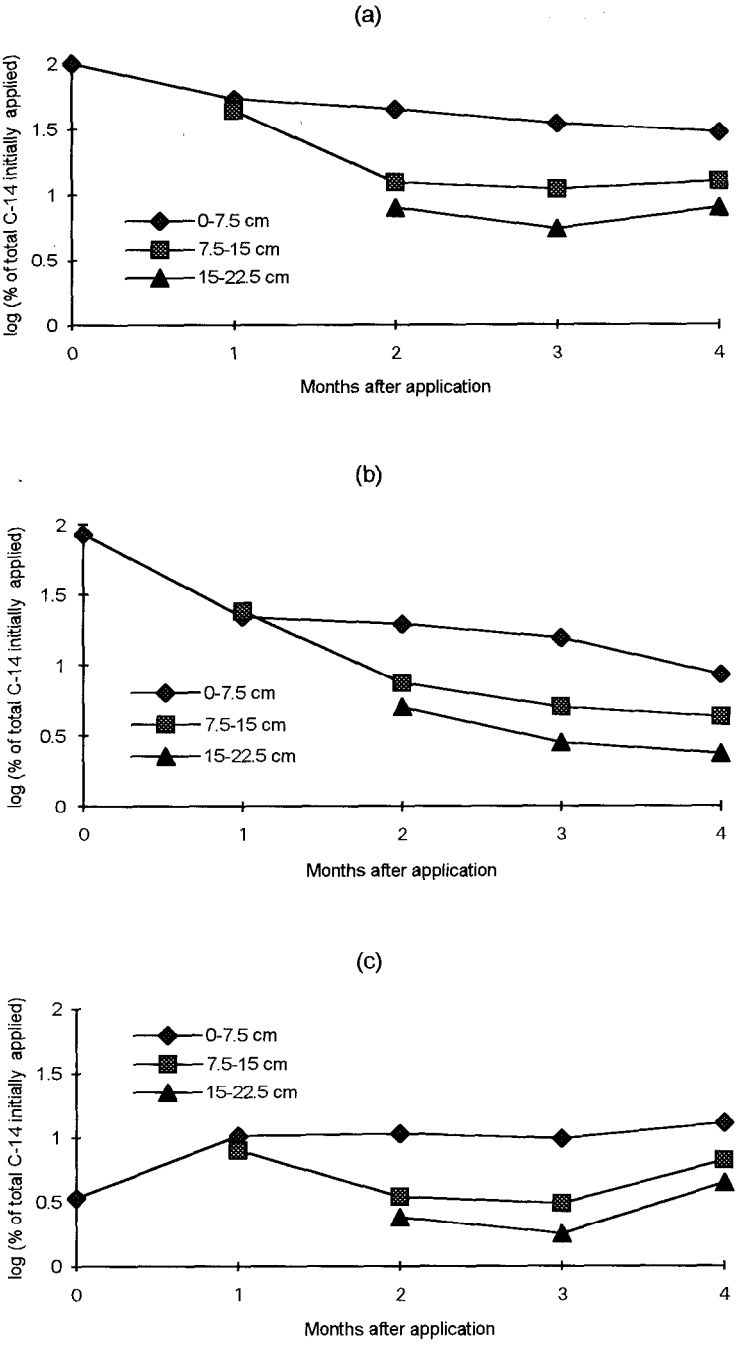

Figure 2. Total (a), extractable(b) and bound (c) ^{14}C -residues in the soil (based on ^{14}C initially applied, 2.687 ppm = 100 %).

Table 1. Identification of extractable ^{14}C-residues in the soil (Average of two pots).

Time of sampling (months)	Depth of soil (cm)	TR-1 ppm	TR-2 ppm	TR-3 ppm	TR-4 ppm	TR-21M ppm
Zero time	0-7.5	2.172	0.018	nd[1]	nd	nd
1	0-7.5	0.178	0.027	nd	0.001	nd
	7.5-15	0.163	0.052	nd	nd	0.013
2	0-7.5	0.351	0.007	nd	0.012	0.004
	7.5-15	0.119	0.012	0.029	0.003	nd
	15-22.5	0.071	0.017	0.033	0.014	nd
3	0-7.5	0.222	0.037	0.003	0.001	0.017
	7.5-15	0.061	0.030	0.004	nd	nd
	15-22.5	0.044	0.021	0.003	nd	0.002
4	0-7.5	0.040	0.092	nd	nd	nd
	7.5-15	0.030	0.039	0.009	nd	0.004
	15-22.5	0.004	0.035	0.007	nd	0.002

[1]not detected

Table 2. Bound ^{14}C-residues in soil extracted with supercritical fluid extraction (Average of two pots)

Time of sampling (months)	Depth of soil (cm)	^{14}C-bound in soil ppm	^{14}C-extracted by SFE %[1]	^{14}C-unextracted[2] by SFE %[1]
Zero time	0-7.5	0.091	57.6	41.2
1	0-7.5	0.277	58.6	43.5
	7.5-15	0.214	66.6	37.1
2	0-7.5	0.292	46.2	47.1
	7.5-15	0.093	39.8	54.7
	15-22.5	0.065	26.1	65.4
3	0-7.5	0.266	33.7	57.4
	7.5-15	0.084	19.6	72.0
	15-22.5	0.048	12.5	67.7
4	0-7.5	0.345	51.0	47.4
	7.5-15	0.176	38.3	71.0
	15-22.5	0.121	18.2	71.8

[1]based on total bound ^{14}C
[2]determined by combustion.

Table 3. Identification of supercritical fluid extractable ^{14}C-residues in the soil (Average of two pots)[1].

Time of sampling (months)	Depth of soil (cm)	SFE ^{14}C-residue ppm	TR-1 ppm	TR-2 ppm	TR-4 ppm	TR-21M ppm
Zero time	0-7.5	0.052	nd[2]	nd	nd	nd
1	0-7.5	0.162	0.031	0.001	0.013	nd
	7.5-15	0.142	0.022	nd	0.026	0.010
2	0-7.5	0.134	0.018	nd	0.007	0.001
	7.5-15	0.039	nd	nd	0.003	nd
	15-22.5	0.017	0.010	nd	0.007	nd
3	0-7.5	0.089	0.031	nd	0.014	0.009
	7.5-15	0.016	nd	nd	0.005	nd
	15-22.5	0.006	0.003	nd	0.003	nd
4	0-7.5	0.176	0.021	0.005	0.001	nd
	7.5-15	0.067	0.011	nd	0.008	nd
	15-22.5	0.022	0.009	nd	0.009	0.004

[1]TR-3 could not be eluted through the Florisil column with hexane:ethylether (99:1)solvent system
[2]not detected.

Table 3. The SFE extracts obtained from the soil samples containing bound ^{14}C-residues showed the presence of mainly the parent compound TR-1 and metabolite TR-4. None of the metabolites was present in the bound form at zero time. Furthermore, some of the SFE extract also showed the presence of trace amounts of TR-2 and TR-21.

Most of the radioactivity was located in the peel part of carrot. The distribution of the total ^{14}C-residues in the tops, peel and pulp were 0.283 ppm, 0.689 ppm, and 0.035 ppm, respectively. Previous studies have demonstrated that most of the radioactivity was found in the third layer. It was observed that 84.5% ^{14}C was present in the phloem (sum of first, second and third pulp layer) and 10.7% ^{14}C in the xylem (Tiryaki et al. 1996). Similar results have been reported by Golab et al.(1967) and Probst et al.(1967).

The pulp of carrots is normally consumed as food produce. Therefore, further analysis was carried out for ^{14}C-residues in this material. The total ^{14}C, extractable ^{14}C, and bound ^{14}C-residues in the pulp of carrots were 0.035 ppm, 0.023 ppm and 0.009 ppm, respectively. It was observed that about 1.3% of initially applied radioactivity to soil was present in the carrot pulps. The methanol extracts of pulps, contained residues of TR-1 and TR-2 in the amount of 0.010 ppm and 0.004 ppm, respectively. Golab et al.(1967) reported the presence of the main compund (TR-1) and a major conversion product (TR-2) in the carrot pulp extracts, Trace amount of TR-5 and TR-21 were also found in carrots but identification could not been verified due to presence of very small amounts of these compounds.

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REFERENCES

Anonymous (1989) Herbicide handbook of the weed science society of America Sixth Edition. Herbicide Handbook Committee, Geneva, NewYork

Anonymous (1996) Ytlltk kayitlar, T.C.Tarim Bakanligi, Koruma Kontrol Genel Müdürlügü, Ankara, Turkey

D'amato A, Semeraro I, Bichi C (1993) Simultaneous determination of linuron and trifluralin residues in carrots and their pulp by liquid chromatography and gas chromatography. J AOAC lnternat 76: 657-662

Duseja DR, Holmes EE (1978) Field persistence and movement of trifluralin in two soil types. Soil Sci 125:41-48

Golab T, Herberg RJ, Parka SJ, Tepe JB (1967) Metabolism of carbon-14 trifluralin in carrots. J Agric Food Chem 15: 638-641

Golab T, Althaus WA, Wooten HL (1979) Fate of [^{14}C]trifluralin in soil. J Agric Food Chem 27: 163-179

Helling CS (1976) Dinitroanilines in soils. J Environ Qual 5:1-15.

Kohl J, Zarif S, Weisberger I, Klein W, Korte FJ (1973) Fate of aldrin-^{14}C in sugar beets and soil under outdoor conditions. J Agric Food Chem 21:855-857

Lue LP, Lewis CC, Melchor VE (1984) The effect of aldicarb and its persistence in carrots, soil and hydroponic solution. J Environ Sci Health 827 (3) 343-354

Probst GW, Golab T, Herberg RJ, Holzer FJ, Parka SJ, Cornelius Van der Schans, Tepe JB(1967) Fate of trifluralin in soil and plants. J Agric Food Chem 15: 592-599

Sitting (1980) Pesticide Manufacturing and Toxic Materials Control Encyclopedia, 754-755

Smith AE, Muir DCG (1984) Determination of extractable and nonextractable radioactivity from small field plots 45 and 95 weeks after treatment with [^{14}C]dicamba, (2,4-dichloro [^{14}C] phenoxy) acetic acid, [^{14}C]triallate, [^{14}C]trifluralin. J Agric Food Chem 32: 588-593

Tiryaki O, Gözek K, Yücel Ü, Ilim M (1996) The effect of food processing on ^{14}C-trifluralin residues in carrot. Toxicol Environ Chem 53:227-233