Bull. Environ. Contam. Toxicol. (1997) 59:750-756

Bulletin of Environmental Contamination and Toxicology

^{14}C-Residues of Trifluralin in Soil and Melon

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Received: 23 May 1997/Accepted: 2 September 1997

Trifluralin (μ,μ,μ-trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine), the most prominent member of a series of selective dinitroaniline herbicide family, was first marketed in 1960 for use on cotton. Registration has today expanded to more than fifty crops. This compound is useful in the pre-emergence control of a wide variety of annual grasses and broadleaf weeds in many agronomic and horticultural crops, including members of Cucurbitaceae (Anonymous 1992; Worthing and Hance 1991). The fate of trifluralin and other related herbicides in soil, plant, and animals has been investigated (Probst et al. 1967; Helling 1976; Duseja and Holmes 1978; Golab et al. 1979; Erkoç and Menzer 1985). 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). Previous studies have demonstrated that most of the residue was found in the peel part of carrot (Tiryaki et al. 1996).

In Turkey, about 12367 kg active ingredient of trifluralin are used in the cultivation of vegetables (Anonymous 1996). A maximum residue limit of 0.05 ppm has been established for tnfluralin in Cucurbitaceae (Sitting 1980).

Trifluralin markedly increases their resistance to vascular wilts caused by *Fusarium oxysporium* f.sp. *melonis* in the melon cultivation area (Grinstein et al. 1984). *Fusarium* wilt is most important disease in the melon plantation in Turkey (Karahan et al. 1981; Yildiz 1977).

The purpose of the study reported here was to investigate the persistence of ^{14}C-trifluralin residues in a soil and their uptake by melon. A number of metabolites of trifluralin in the soil and melon plants were identified. The quantity of the bound residues was also determined.

MATERIALS AND METHODS

Radiolabelled trifluralin, 100 μCi, was supplied by Dow Elanco (Environmental Chemistry Lab., Greenfield, Indian USA) and Sigma Chemical Co.(via Turkish Atomic Energy Agency, Ankara Turkey).The radiochemical purity of the herbicide was 98 %. Reference standard of trifluralin (purity 99.5%) was a gift from Dow Elanco. The metabolites μ,μ,μ-trifluoro-2,6-dinitro-*N*-propyl-*p*-toluidine (TR-2); μ,μ,μ-trifluoro-2,6-dinitro-*p* -toluidine (TR-3);μ,μ,μ-trifluoro-N^4, N^{4}dipropyltoluene-3,4-triamine (TR-7); and 4-(dipropylamino)-3,5-dinitrobenzoic acid (TR-21) were supplied by Biosciences Research Lab, U.S. Dept of Agric, ARS, Fargo, USA.

The soil used in this study was a sandy loam with a pH of 8.1 and 1.87 % organic matter. Particle size analysis indicated 35.3 % clay, 17.9 % silt and 46.7 % sand. The soil was air-dried, screened and passed through a 20-mesh sieve.

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Melon (Yuva variety) were grown at the Turkish Atomic Energy Authority, Ankara Nuclear Research and Training Centre's Experimental Farm, Ankara, Turkey. The experiments were carried out under the outdoor conditions in two galvanized-steel boxes which have been described in earlier publications (Tiryaki et al 1996 and 1997). Boxes were filled with sand-soil-manure mixture (12.6 kg/pot), and ^{14}C-trifluralin was applied at the recommended rates of 0.84 kg a.i./ha. The herbicide was incorporated to a depth of 7.5 cm, immediately after application. The specific activities for the first year and second year experiment were 0.401 µCi/mg and 1.141 µCi/mg, respectively. After the herbicide application, melon seeds were sown on May 14, 1991 and June 5, 1992. The total rainfall during the growing season was 110.7 mm with a maximum of 16.0 mm and a minimum 0.0 mm, same values for 1992 experiment were 69.3 mm, 15.3 mm and 0.0 mm, respectively. The mean air temperature from May to October 1991 was 18.3%, and from June to October 1992 was 18.8%.

Soil samples from 0-7.5 cm. deep were taken after herbicide application and periodically thereafter at one month intervals until harvesting time at depths of 0-7.5 cm, and 7.5-15 cm. The melon were harvested on October 23, 1991 and October 2, 1992 from the two pots. The fruits were sampled as a skin, flesh and seed. The cortex and stele of crown tissues were also sampled. Root samples were separated as hairy root and tap root. Remaining root part was divided into two parts as the upper half and the lower half of the main root. Leaves and stems were also sampled. Each branch was divided into three equal parts, then each part was separated into leave and stem. Leaves and stems in each part gathered from all branches were then analyzed separately.

All samples were analyzed in duplicate. There were two pots each year indicating two replications. Therefore overall means for two years are reported throughout this paper. The residues, in ppm, are based on fresh weight of the plant samples. With the soil samples, the residues are based on oven dry weight.

Harvesting time soil and plant samples were extracted with methanol using Soxhlet extraction apparatus (L'annunziata 1979). The amount of samples which placed in paper thimble were about 40-50 g and 5-25 g soil and plant samples, respectively. The extracts then concentrated to a small volume using Rotary evaporator. The extracts were counted to determine extractable ^{14}C-residues in 1550 Tri-Carb Liquid Scintillation Analyzer.

Combustion of soil and/or melon samples were done in a Harvey Biological Oxidizer, OX-600. Combustion products were trapped in external traps which contain trapping solution. $^{14}CO_2$ released by combustion were analyzed liquid scintillation counting.

Thin Layer Chromatography(TLC) was can-ted out with 20x20 cm and 5x20 cm precoated silica gel F_{254} (Merck) chromatoplates, 0.25 mm thick. The five reference standards were then spotted on the plates and then developed in a solvent system comprising hexanelbenzene (35:65). The developed plates were observed under UV light (λ=254 nm). Under these conditions, the following R_f values were obtained: TR-1 R_f 0.88; TR-2 R_f 0.76; TR-3 R_f 0.42; TR-7 R_f 0.24; and TR-21 R_f 0.10 (in benzene-ethylacetate, 35:65, solvent system). The extracts were then applied on plates at about 1 cm below the preabsorbent line and developed in hexane:benzene and benzene-ethylacetate solvent systems in containers which were lined with paper and saturated prior to use. The material in these spots was scrapped off the plate to scintillation vial and added Insta-Gel scintillation cocktail for counting. Column chromatography was performed with a glass column (10x1.5 cm) containing 4 g of florisil. Column was conditioned with 5 mL methanol by passing through column. Extracts and/or reference standard (1 mL) were applied to the column and eluted with hexane:benzene (2:1). The eluates were evaporated under air to just dryness, redissolved in 1 mL methanol and

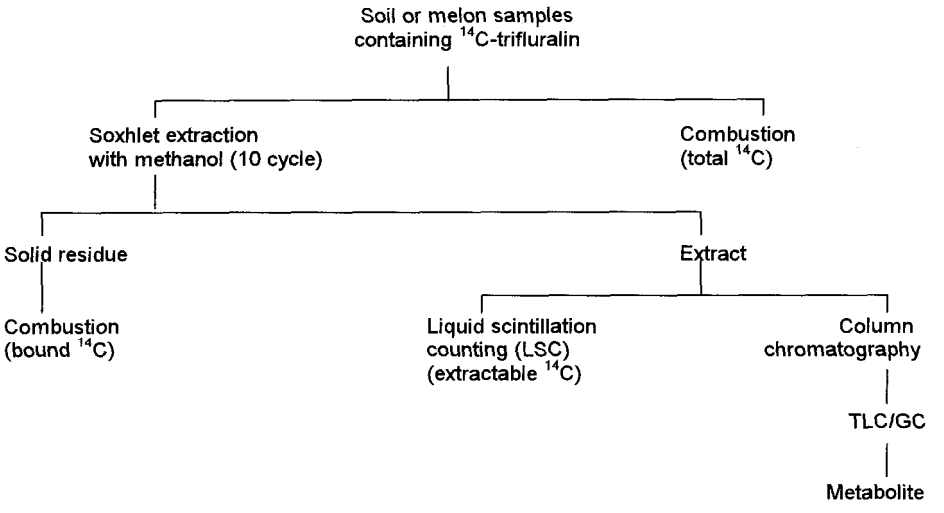

Figure 1. Scheme for analysis of ^{14}C-residues in soil and melon samples.

assayed in a 1550 Tri-Carb Liquid Scintillation Analyzer to determine extractable ^{14}C-residues. The gas chromatograph was a Varian Model 3700 equipped ECD detector and a 1.82 m x 2 mm glass column coated with OV-17. The operating conditions were as follows: column, detector, and injector port temperature were 150°C, 260°C, and 250°C, respectively. The nitrogen carrier gas flow rate was 18 mL/min. Under the GC conditions described TR-1 and TR-2 showed peaks at 3.2, and 3.6 min, respectively.

RESULTS AND DISCUSSION

The analytical procedure used for the extraction and determination of ^{14}C residues in soil and melon samples is given in Figure 1.

The amount of total ^{14}C-residues recovered from soil over a melon vegetation period is shown in Figure 2. Four month later, the radioactivity recovered from the combined two layers of soil (0-15 cm) accounted 68.9% of the initially applied as total ^{14}C-residues (Table 1). The extractable ^{14}C-residues were less than non-extractable (bound) ^{14}C-residues in the both (0-7.5 cm and 7.5-15 cm) soil layers(Table 2). 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 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 non-extractable ^{14}C-residues accounted for 10%.

The persistence and movement of ^{14}C-trifluralin residues in soil changed with the time. Thus, after one month about 82.3% of the applied radioactivity was still present in the combined two depths of the soil, while 4 months later only about 68.9% of applied radioactivity was present (Table 1). Duseja and Holmes (1978) reported that 25 and 120 days after application (at an application rate of 0.75 lb/acre), 48.3% and 25.9% of initially applied radioactivity, respectively, was present in the combined soil sample from the two depths.

Soil samples, collected from the pots at the two depths, were analyzed for the presence of the herbicide. The results (Figure 2 and Table 1) indicate a small amount of downward

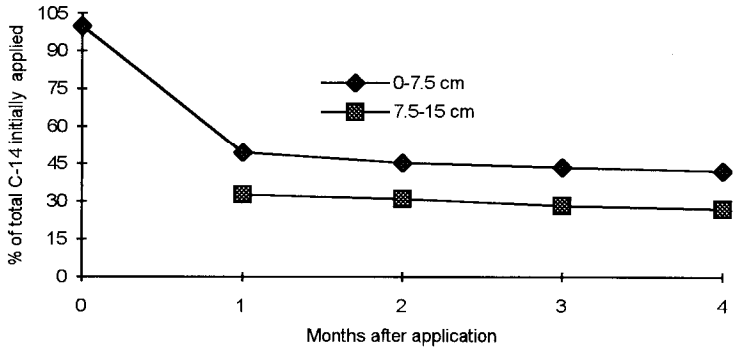

Figure 2. Total ^{14}C-residues in the soil (based on ^{14}C initially applied, 2.264 ppm=l00%)

Table 1. Persistence and movement of ^{14}C-trifluralin in the soil.[1]

Date of sampling (months)	Average residue found					
	0-7.5 cm		7.5-15 cm		Total	
	ppm	%[2]	ppm	%[2]	ppm	%[2]
Zero time	2.264	100.0	-	-	2.264	100.0
1	1.123	49.6	0.740	32.7	1.863	82.3
2	1.019	45.2	0.702	31.0	1.721	76.2
3	0.978	43.5	0.637	28.2	1.615	71.7
4	0.949	42.0	0.607	26.9	1.556	68.9

[1] Averages of two years
[2] Based on ^{14}C initially applied, 2.264 ppm = 100%

movement of the herbicide. Most of the applied radioactivity was located in the zone of incorporation. Thus, about 42.0-49.6% and 26.9-32.7% of the applied radioactivity remained in the 0-7.5 cm and 7.5-15 cm soil layers, respectively. Golab et al.(1979) observed that, after 12 and 24 months (at an application rate of 1.68 kg a.i./ha) 91% and 98.8% of the radioactivity were located in the 0-15 cm zone, and 76% and 95% in the zone of incorporation.

Table 2 shows the total, extractable and bound ^{14}C residues in the soil and melon plant. After 4 months, soil and most of the melon samples showed the presence of bound ^{14}C-residues in greater than extractable ^{14}C-residues.

Most of the residue was located in the root part of melon plant. The data (Table 2) show that the hairy roots and tap roots contained 1.073 ppm, and 0.901 ppm, respectively. Similar findings were explained by a number of researchers (Probst et al.1967; Golab et al.1967; Golab et al.1979). They reported that root crops grown in soil treated with trifluralin, had shown to contain herbicide residues on the surface. On the other hand, the cortex of crown and stele of crown contained 0.489 ppm and 0.238 ppm, respectively (Table 2).

Table 2. Total, extractable and bound ^{14}C-residues in the soil and melon plant samples at harvest[1]

Sampling[2]	Total residue	Extractable residue		Bound residue	
	ppm	ppm	%[3]	ppm	%[3]
SOIL					
0-7.5 cm	0.951	0.328	34.5	0.514	54
7.5-15 cm	0.609	0.224	36.8	0.306	50.2
MELON PLANT					
Hairy root	1.073	0.611	57	0.5	46.6
Tap root	0.901	0.346	38.4	0.408	45.2
Cortex of tap root-lower half	0.444	0.179	40.3	0.265	59.7
Stele of tap root-lower half	0.186	0.075	40.2	0.118	63.4
Cortex of tap root-upper half	0.632	0.213	33.7	0.353	55.9
Stele of tap root-upper half	0.174	0.057	32.5	0.106	61
Cortex of crown	0.489	0.127	25.9	0.278	56.9
Stele of crown	0.238	0.077	32.4	0.147	61.9
Lower stems	0.149	0.059	39.4	0.068	45.5
Median stems	0.05	0.038	77.3	0.014	27.3
Upper stems	0.043	0.029	68.4	0.011	26.3
Lower leaves	0.138	0.061	44.3	0.066	47.5
Median leaves	0.063	0.027	42.9	0.032	50
Upper leaves	0.048	0.025	52.4	0.023	47.6
FRUIT					
Skin	0.007	0.005	66.7	*[4]	*
Flesh	0.002	*	*	*	*
Seed	0.016	0.007	42.9	0.009	57.1

[1] Averages of two years
[2] For details see "Materyals and Methods"
[3] Based on total residues
[4] Not detected

Total ^{14}C-residues in the skin, flesh and seed of melon fruit were 0.007 ppm, 0.002 ppm and 0.016 ppm, respectively (Table 2). These residue levels were under the maximum residue level set up by EPA (Environmental Protection Agency) as a 0.05 ppm for Cucurbitaceae (Sitting 1980).

TLC analysis of soil extracts indicated the presence of TR-1 (trifluralin) and TR-2 (Table 3). After 4 months, soil extracts contained more TR-2 concentration than TR-1. None of the metabolites found were present more than 0.124 ppm (5.5% of the initially applied ^{14}C-trifluralin) at four month later soil samples. Similar findings 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 3 also show the presence of small amounts of the metabolites TR-3, TR-7, and TR-21. Similar results have been explained by Golab et al(1967) and Probst et al.(1967). In the plant samples, which could examine by TLC, hairy root and tap root contained 0.249 ppm and 0.133 ppm residue as TR-1, and 0.104 ppm and 0.057 ppm as TR-2. The 0.020 ppm and 0.007 ppm residues were located in the cortex of crown as TR-1 and TR-2, respectively, and 0.011 ppm and 0.007 ppm in the stele of crown. Trace amounts of TR-3, TR-7 and TR-21 were also found in the plant samples (Table 3).

Table 3. Identification of extractable ^{14}C-residues in the soil and melon samples at harvest [1]

Sampling[2]	TR-1	TR-2	TR-3	TR-7	TR-21
	ppm	ppm	ppm	ppm	ppm
SOIL					
0-7.5 cm	0059	0.124	0.004	*[3]	*
7.5-15 cm	0.059	0.075	0.004	*	*
MELON PLANT					
Hairy root	0.249	0.104	0.003	0.003	*
Tap root	0.133	0.057	0.003	0.004	*
Cortex of tap root-lower half	0.070	0.032	*	0.003	0.004
Stele of tap root-lower half	0.034	0.004	*	*	*
Cortex of tap root-upper half	0.086	0.036	0.004	*	*
Stele of tap root-upper half	0.010	0.004	*	*	*
Cortex of crown	0.020	0.007	*	*	*
Stele of crown	0.011	0.007	*	*	*
Lower stems	0.010	0.007	*	*	*
Median stems	*	*	*	*	*
Upper stems	*	*	*	*	*
Lower leaves	0.011	0.004	*	*	*
Median leaves	*	*	*	*	*
Upper leaves	*	*	*	*	*
FRUIT					
Skin	*	*	*	*	*
Flesh	*	*	*	*	*
Seed	*	*	*	*	*

[1] Averages of two years
[2] For details see "Materyals and Methods"
[3] Not detected

Acknowledgment. The authors thank the Research Foundation of Ankara University for partial support of this research.

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