Metabolism of Carbon-14 Trifluralin in Carrots

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A surface residue of trifluralin (α,α,α-trifluoro-2, 6-dinitro-*N*,*N*-di-*n*-propyl-*p*-toluidine) was found in carrots grown in trifluralin-treated soil. To determine the total residue concentration and the nature of the metabolic products of the herbicide, carrots were grown in greenhouse soil treated with 0.75 pound per acre of trifluralin labeled with carbon-14 in the trifluoromethyl group. Unaltered trifluralin was the major source of radioactivity. The major conversion product was α,α,α - trifluoro - 2,6 - dinitro - *N* - (*n* - propyl)-*p*-toluidine.

Trifluralin is a selective, pre-emergence herbicide whose properties were discovered by Alder, Wright, Soper, and Pieczarka (*1*, *9*) and marketed under the trade name Treflan by Elanco Products Co. This compound is useful in the pre-emergence control of a wide variety of annual grasses and broadleaf weeds. Some tolerant crops include cotton, soybeans, snap-beans, lima beans, safflower, carrots, and several transplanted crops such as tomatoes and peppers.

When root crops such as carrots, onions, and turnips are grown in soil containing trifluralin, trifluralin residues are found on the surface. Carrots were chosen as a convenient plant in which to study surface concentration, possible penetration of the herbicide, and the presence of metabolites.

Material and Methods

Labeled Trifluralin. Trifluralin labeled with carbon-14 in the trifluoromethyl group was prepared by Marshall and McMahon (*6*). The radiochemical purity as determined by thin-layer chromatography on silica gel G in a system of methylcyclohexane–benzene (1 to 1) was greater than 99%. Its specific radioactivity was 9.05 μc. per mg.

Soil-Sample Preparation. Labeled trifluralin (9.21 mg.) was dissolved in acetone. One third of this solution was added to each of three glass jars containing 300 grams of air-dried greenhouse soil (one part sand and one part silty clay loam). The jars were rotated on a mechanical roller for 30 minutes. The covers were removed from the jars until the odor of acetone was no longer detectable. The contents of each jar were then mixed with 2000 grams of air-dried greenhouse soil.

Planting and Growing Conditions. Three 2-gallon glazed crocks (8.5 inches in diameter, 8.5 inches deep) having a surface area of 0.39 square foot were filled three-fourths full with greenhouse soil. A 1-inch layer of the C^{14}-trifluralin–incorporated soil was added. Carrot seeds of the variety Chantenay were sown and covered with the remainder of the C^{14}-trifluralin–incorporated soil. The trifluralin concentration (1.33 μg. per gram of soil) was equivalent to a field application rate of 0.75 pound per acre in a 2-inch layer of soil. The plants were maintained in a growth room with 12-hour light (80° F.) and dark (70° F.) periods. After 110 days the plants were harvested, the root systems washed, and the tops separated from the roots. All parts were frozen until assayed.

Extraction Procedure. Past experience has shown that methanol is the most useful solvent in extracting trifluralin from plant tissues. Carrots, whole or separated into peel and one or more pulp layers, were extracted with methanol in various ways: blending one or more times with methanol in an Omni-Mixer, stirring with methanol, and Soxhlet extraction with methanol for 1 hour. The carrot tops were dried at room temperature, ground, and Soxhlet-extracted.

After extraction, the methanol was evaporated on a Rinco rotary evaporator. The residue was partitioned between water and *n*-hexane. Three successive hexane portions were used. After separation of the hexane layer, the aqueous layer was extracted with chloroform after adjustment, in turn, to pH 7, pH 1, and pH 9 (Figure 1).

Thin-Layer Chromatographic Procedures. The various extracts were examined by thin-layer chromatography (TLC). Glass plates (20 × 20 cm.), coated with a 250-micron layer of silica gel GF (Brinkman No. 7730) and activated at 105° C. for 1 hour, were employed (*12*, *13*). Preparative plates used to obtain larger quantities of material for further measurements were coated with a 500-micron layer of silica gel GF.

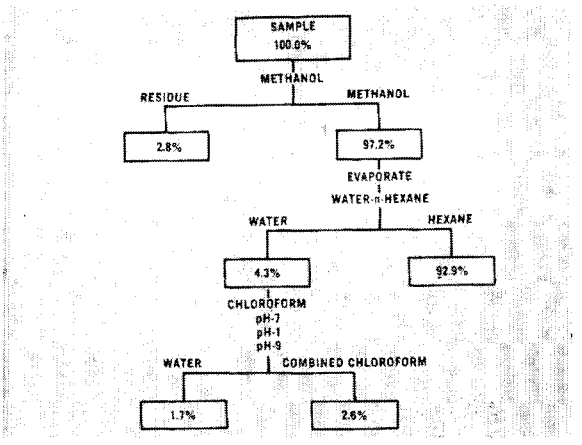

Figure 1. Extraction scheme employed for carrot root showing percentage distribution of radioactivity in each fraction

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Several one- and two-dimensional thin-layer chromatographic systems were developed to separate a series of model compounds chosen as possible metabolites of trifluralin. The simplest one-dimensional system, in which the plate was developed twice with carbon tetrachloride, separated metabolites and model compounds into groups. For purposes of group selection, the chromatoplate was sectioned into five zones (Figure 2). Model compounds were chromatographed on a separate lane and served to define the zones.

A more complete separation of the model compounds was accomplished with two-dimensional thin-layer chromatography employing the following solvent combinations: Solvent I, benzene–ethylene dichloride (1:1); Solvent II, *n*-hexane–methanol (97:3); and Solvent III, benzene–ethyl acetate–acetic acid (60:40:1, v./v.). With those solvent systems, more than 26 compounds chemically related to trifluralin were separated (*3*). The model compounds were co-chromatographed with the radioactive sample. The position of the known standards on the chromatogram was determined by their natural visible color or by their ultraviolet absorption.

Thin-Layer Radioautography. Radioautographs (*2*, *10*), employing 8 × 10 inch Kodak Blue Brand medical x-ray film, were prepared of representative TLC plates of various extracts. Exposure times ranged from a few to 119 days.

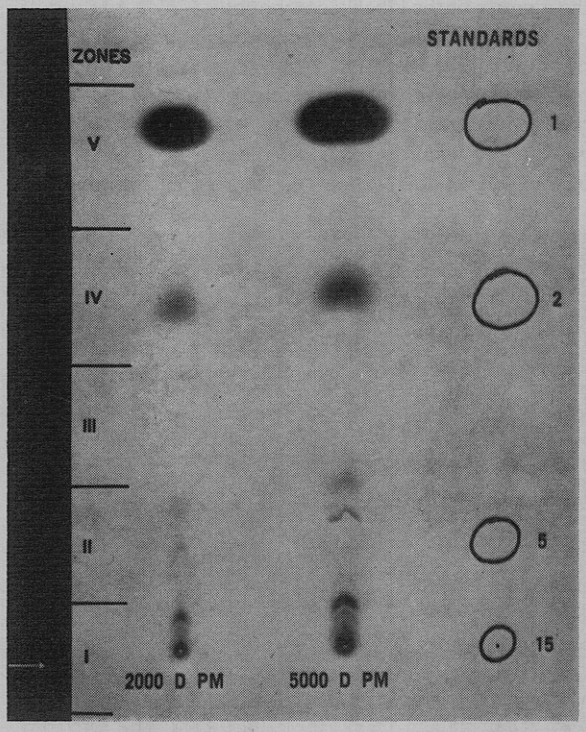

Figure 2. 118-day exposure radioautograph of thin-layer chromatoplate of the *n*-hexane extract, developed twice with carbon tetrachloride. Zones indicate the chromatoplate sections which provided material for liquid scintillation counting, gas chromatography, and reverse isotope dilution studies

The name, number, and zone position of the model compounds of interest in this study (from the 26 available) are: compound 1, zone V, trifluralin; compound 2, zone IV, α,α,α-trifluoro-2,6-dinitro-*N*-(n-propyl)-*p*-toluidine; compound 5, zone II, α,α,α-trifluoro-5-nitro-N^4-(*n*-propyl)-toluene-3,4-diamine; compound 15, zone I, 4-(di-*n*-propylamino)-3,5-dinitrobenzoic acid

Counting Procedure. All counting of radioactivity was done with a Packard Tri-Carb Series 3000 liquid-scintillation spectrometer. Whole or extracted tissues were burned in an electrically heated quartz combustion tube. Combustion products were absorbed in a mixture of 2-aminoethanol and methyl Cellosolve (30 to 70), diluted with toluene scintillator solution [toluene containing 0.5% 2,5-diphenyloxazole (PPO) and 0.01% 2,2′-*p*-phenylenebis(5-phenyloxazole) (POPOP)], and counted. Liquid extracts were dissolved in an appropriate scintillation solution and counted directly. Zones or spots from silica gel GF chromatoplates were scraped into scintillation vials and eluted with approximately 0.2 ml. of ethanol. Scintillation solution was added and samples were counted. Counting efficiencies were determined by internal standardization with toluene-1-C^{14} or by channels ratio standardization. Counting times ranged from 10 to 30 minutes depending on the level of radioactivity in the sample.

Gas Chromatographic Measurement. The separated zones on preparative TLC plates (500-micron absorbent layer) were removed, placed in a small glass column, and eluted with acetone. The solvent was removed and benzene was added. The resulting benzene solutions were analyzed by gas chromatography with a Jarrell-Ash, Model 28-730 instrument utilizing an electron capture detector (*11*).

Results and Discussion

A total of 10 carrots were examined, five as whole carrots, four separated into a $^1/_{16}$-inch peel and pulp, and one separated into a $^1/_{16}$-inch peel and eight pulp layers each approximately $^1/_{16}$-inch thick. The total radioactivity in the carrots, expressed as parts per million (p.p.m.) trifluralin, ranged from 0.49 to 0.86 p.p.m. with an average value of 0.65 p.p.m. The relative distribution of total radioactivity averaged 74.4% in the peel (range 66.0 to 84.2%) and 25.6% in the pulp (range 15.8 to 34.0%). The radioactivity level in the top, expressed as p.p.m. trifluralin, was 0.25 p.p.m. The distribution of radioactivity in the carrot divided into the nine layers was as follows: peel 68.8%; pulp layers (numbers from outside toward center) 1, 4.9%; 2, 6.7%; 3, 9.8%; 4, 5.7%; 5, 2.9%; 6, 0.7%; 7, 0.2%; 8, 0.1%. Two thirds of the radioactivity was found in the peel. Of the eight pulp layers, increased radioactivity was found in the third pulp layer, the junction of the phloem and xylem.

The average radioactivity in the various carrot root extracts is shown in Figure 1. The radioactivity was recovered effectively by methanol extraction. The majority of the methanol-extractable radioactivity proved to be hexane-soluble and constituted 92.9% of the total. By comparison, the hexane extract of leaves contained 49.0% of the total radioactivity.

The relative distribution of radioactivity in the hexane extract from carrot root in one-dimensional TLC system, as determined by liquid scintillation counting,

was as follows for zones I through V (Figure 2): 4.8, 1.4, 0.1, 4.7, and 89.0%. On the other hand, the hexane extract of leaves revealed the relative distribution of radioactivity in the corresponding zones to be 50.0, 7.9, 0.0, 1.7, and 40.3%, respectively.

The majority of the radioactivity found in the root extract migrates like trifluralin to zone V. Only 5% of the radioactivity is retained as polar origin material (zone I material). In the leaf extract, 40% of the radioactivity behaves like trifluralin, and 50% radioactivity exhibits properties of zone I polar products.

Figure 2 is a radioautograph of a one-dimensional thin-layer chromatoplate showing the behavior of a typical carrot root hexane extract. The behavior of the hexane extract in the two-dimensional TLC system employing solvents I and II, determined by radioautograph, is shown in Figure 3. The figures provide evidence for compound 1, compound 2, traces of 5, and origin material. Figure 4 shows a radioautograph of the hexane extract chromatographed in two dimensions with solvents III, I, and II. The radioactive spots correspond to model compounds 1, 2, 5, and 15, and an unidentified material between 5 and 15. Thin-layer chromatography indicates the most prominent radioactive materials present in carrot roots are compound 1 (trifluralin) and compound 2 [α,α,α-trifluoro-2,6-dinitro-*N*-(*n*-propyl)-*p*-toluidine].

To verify the identity of these two prominent compounds, reverse isotope dilution technique was employed (*4*, *5*, *7*, *14*). To a portion of the zone V (one-dimensional TLC) material eluted from a preparative plate of the *n*-hexane extract, a weighed quantity of pure trifluralin was added. The material was recrystallized three times from *n*-pentane. The specific activities were determined after each recrystallization. Similarly, the zone IV material was combined with known α,α,α-trifluoro-2,6-dinitro-*N*-(*n*-propyl)-*p*-toluidine and recrystallized four times. Table I shows the specific activity values after each recrystallization. The specific activities of the zone V and IV materials vary little with successive recrystallizations and support the identity of the labeled compounds.

Table I. Reverse Isotope Dilution Identification

Recrystallization	Trifluralin, D.P.M-/Mg.	α,α,α-Trifluoro-2,6-dinitro-*N*-(*n*-propyl)-*p*-toluidine, D.P.M./Mg.
1st	6417	625
2nd	6254	650
3rd	6299	601
4th		600
Av.	6323 ±48.6	619 ±12.0
	± 0.77%	± 1.94%
Theory	6880	649
Purity	91.9%	95.3%

As a final means of confirmation, a small portion of the radioactive materials from zones V and IV of the preparative plate were examined by gas chromatography. Figure 5 shows the gas chromatographic behavior of trifluralin, α,α,α-trifluoro-2, 6-dinitro-*N*-(*n*-propyl)-*p*-toluidine, zone V, and zone IV material.

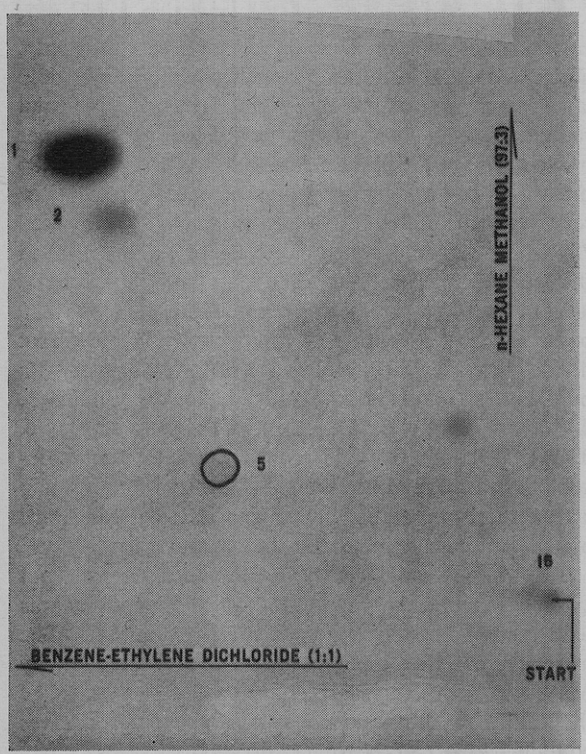

Figure 3. 118-day exposure radioautograph of two-dimensional thin-layer chromatoplate of the *n*-hexane extract, developed with solvent systems I and II

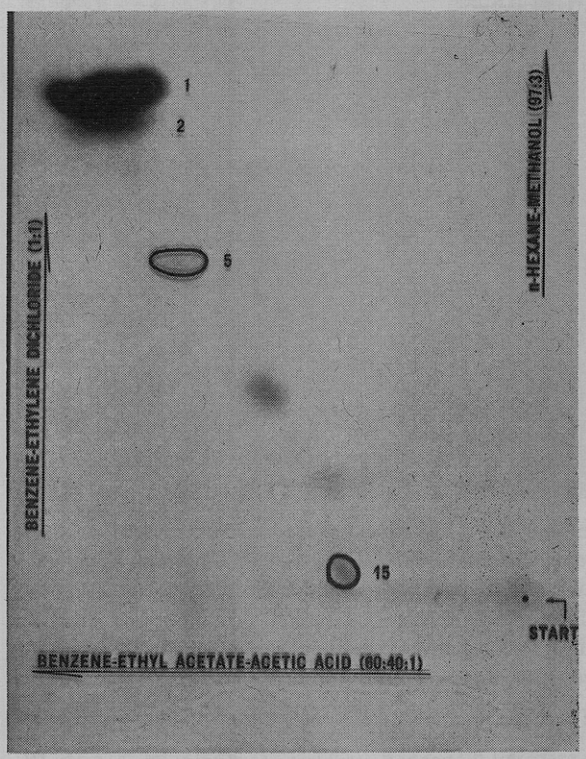

Figure 4. 119-day exposure radioautograph of two-dimensional thin-layer chromatoplate of the *n*-hexane extract developed with solvent systems III, I, and II

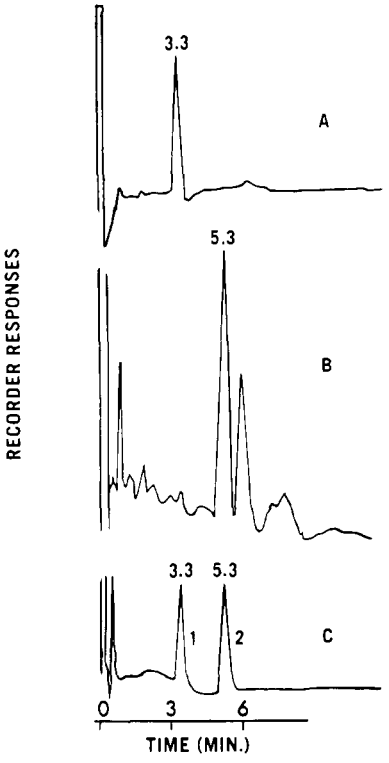

Figure 5. Gas chromatographic analysis of zones IV and V obtained from one-dimensional preparative thin-layer chromatoplate, compared with two model compounds

A. Substance eluted from zone V
B. Substance eluted from zone IV
C. Model compounds
   1. Trifluralin
   2. α,α,α-Trifluoro-2,6-dinitro-*N*-(*n*-propyl)-*p*-toluidine

Retention times of the zone V and IV materials are in agreement with those of the proposed model compounds. The extraneous peak observed in Figure 5 *B* is frequently encountered in one-dimensional TLC of plant extracts in the zone IV region. Although not shown in the figure, this peak appeared in the control extract as well.

The chloroform extracts and extracted aqueous phase were also examined in the TLC system. The radioactivity, in both extracts, was located primarily at the origin.

Trifluralin and one conversion product, compound 2, have been identified in the carrot root. Thin-layer chromatographic results indicated the presence of compounds 5 and 15, but identification has not been verified. Although identification by other criteria has not been established, compound 5 is assumed to be a minor metabolite in carrot. This judgment is supported by the appearance of compound 5 in trifluralin metabolic studies in soil (*8*). Radioautographic experiments in this investigation (Figure 4) indicated the presence of compound 15. However, this compound has not been observed previously in other trifluralin metabolic studies. Other experiments reveal that $C^{14}O_2$ is released from soil treated with trifluralin labeled with C^{14} in the trifluoromethyl position and also from plants grown in treated soils. This suggests the possibility that trifluralin is degraded to the acid form and subsequently is decarboxylated to yield $C^{14}O_2$. Therefore, the presence of compound 15 cannot be disregarded.

Compounds 2 and 5 have been found in soil treated with trifluralin (*8*). It has not been established that their presence in carrot root is a result of direct incorporation from the soil or of biological degradation in carrot tissue.

The heterogeneous unidentified mixture referred to as polar products probably constitutes the ultimate pathway of the fate of trifluralin degradation.

Acknowledgment

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