Fate of Trifluralin in Soils and Plants

G. W. PROBST, TOMASZ GOLAB, R. J. HERBERG, F. J. HOLZER, S. J. PARKA, CORNELIUS VAN DER SCHANS, AND J. B. TEPE

Time-rate degradation studies on trifluralin in field soil show a decrease to a concentration of 10 to 15% within a half to 1 year. Experiments with carbon-labeled trifluralin reveal different degradative pathways in soil. Aerobic degradation proceeds through a dealkylation step followed by progressive reduction, whereas anaerobic degradation occurs with a preliminary reduction prior to dealkylation. Extensive residue analyses of tolerant crops indicate that trifluralin is not readily absorbed from soil. However, carrots absorb small quantities as determined by residue analysis and radioactive tracer studies. ^{14}C-labeled trifluralin was primarily distributed in the peel rather than the pulp. The major portion of incorporated radioactivity was identified as unchanged trifluralin. The main metabolite found was the dealkylated product.

Trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) is a selective pre-emergence, soil-incorporated herbicide which controls a wide variety of grasses and broadleaf weeds. Its herbicidal properties were discovered by investigators at Lilly Research Laboratories (*1*, *7*) and it is marketed under the trade name Treflan for use on many agronomic and horticultural crops. Tolerant crops include cotton, soybeans, snapbeans, lima beans, safflower, carrots, and several transplanted crops such as tomatoes and peppers.

Pure trifluralin crystallizes in yellow-orange prisms and melts at 48.5–49° C. It is readily soluble in organic solvents such as methanol, acetone, xylene, and chloroform. The solubility in water is less than 1 p.p.m. at 27° C. The vapor pressure is 1.99×10^{-4} mm. of mercury at 29.5° C., and the boiling point is 96–97° C. at 0.18 mm. of mercury. Trifluralin is susceptible to decomposition by ultraviolet radiation.

With discovery of pre-emergence herbicidal properties, investigations were undertaken to determine persistence and mode of degradation in soil (*6*) as well as the metabolic fate in plants (*3*).

An analytical residue procedure (*8*) for the assay of trifluralin in plant tissue and soil employs methanol extraction, removal of interfering substances on a Florisil column, and measurement by gas chromatography using an electron-affinity detector. Some types of material require the use of thin-layer chromatography prior to gas-chromatographic measurement. A modification permits determination of degradation products. Table I lists the model compounds of interest in these studies.

Trifluralin Persistence in the Field

Radioactive trifluralin in field plots was studied for 2 years to determine persistence, leaching, and decomposition. Soybeans were planted in a field plot of Miami silt loam soil in which trifluralin labeled with carbon-14 in the trifluoromethyl group (*5*) was incorporated into the top 2 inches of the soil at a rate of 0.75 pound per acre. Soil samples in the 0- to 3- and 3- to 6-inch layers were taken periodically and extracted exhaustively with methanol and the radioactivity in each extract was determined. Most of the radioactivity in the extracts was identified as trifluralin by thin-layer chromatography.

The results are shown in Figure 1. During the initial growing season, the level of radioactivity in the extract after 29 days was reduced to 39% of the original. The amount of extractable radioactivity continued to decrease, reached approximately 20% after 43 days, then sharply leveled off, and gradually decreased throughout the remainder of the 2-year period. Although the extractable radioactivity persistently remained at approximately 15%, the amount of trifluralin, identified by thin-layer chromatography, diminished continuously to less than 10% of original radioactivity.

After the first year of the study, the radioactivity present in the soil was determined by both direct combustion and extraction with methanol. Approximately 25% of the original radioactivity determined by direct combustion remained in the soil. This was more than that obtained by methanol extraction, indicating that a considerable portion of radioactivity was not recoverable by methanol extraction. In view of previous extraction studies, this extra radioactivity, obtained by combustion, is viewed as extensively degraded products of trifluralin.

No evidence was found that the radioactivity migrated laterally or in depth in the soil plot. Thin-layer chromatography of soil extracts indicated the presence of trace amounts of compound 2 (Table I). Other studies employing chemical assays and crab grass bioassays demonstrated that trifluralin does not leach from soil and is continuously degraded during the growing season. Less than 50 parts per billion of trifluralin were found in most soils about 160 days after application. Repeated application of trifluralin at recommended rates in soil revealed no build-up with time.

Trifluralin Persistence in the Growth Room

To study persistence under controlled conditions, trifluralin labeled with carbon-14 in the trifluoromethyl position was incorporated at 0.75 pound per acre in

Greenfield Laboratories, Eli Lilly and Co., Greenfield, Ind.

Miami silt loam soil to a depth of 2 inches in 2-gallon glazed crocks. Soybean seeds were planted in one group of containers. Soil samples were taken at periodic intervals and extracted with methanol, and the extract was counted for radioactivity.

The rate of trifluralin disappearance increased slightly in soil with soybean plants, compared to containers without plants (Figure 2). The rate of disappearance under controlled conditions in the growth room is reduced considerably compared to studies conducted in the field. Small amounts of radioactive carbon dioxide were produced from both soils and plants. This suggests a slow, but extensive, degradation of trifluralin.

To determine if microorganisms play an important role, Brookston silty clay loam was autoclaved at 15-pound pressure for 21 hours. Trifluralin was incorporated in both autoclaved and nonautoclaved soil at 8 p.p.m.w. The soil was adjusted to approximately 75% of field moisture capacity and kept in an incubator at 80° F. Samples were taken at monthly intervals. A bioassay using the inhibition of crab grass served as an index of trifluralin content. The results (Figure 3) indicate that trifluralin was degraded

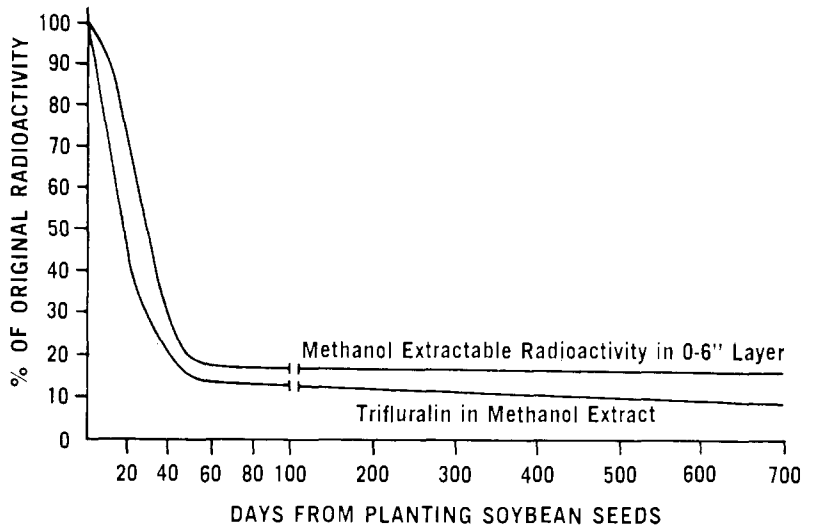
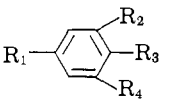


Figure 1. Persistence of ^{14}C-labeled trifluralin in field soil planted with soybeans

Table I. Model Compounds



Compound No.	Name	R_1	R_2	R_3	R_4
1	Trifluralin	CF_3	NO_2	$N(C_3H_7)_2$	NO_2
2	α,α,α-Trifluoro-2,6-dinitro-*N*-propyl-*p*-toluidine	CF_3	NO_2	NHC_3H_7	NO_2
3	α,α,α-Trifluoro-2,6-dinitro-*p*-toluidine	CF_3	NO_2	NH_2	NO_2
4	α,α,α-Trifluoro-5-nitrotoluene-3,4-diamine	CF_3	NO_2	NH_2	NH_2
5	α,α,α-Trifluoro-5-nitro-N^4-propyltoluene-3,4-diamine	CF_3	NO_2	NHC_3H_7	NH_2
7	α,α,α-Trifluoro-N^4,N^4-dipropyl-5-nitrotoluene-3,4-diamine	CF_3	NO_2	$N(C_3H_7)_2$	NH_2
9	α,α,α-Trifluoro-N^4,-N^4-dipropyltoluene-3,4,5-triamine	CF_3	NH_2	$N(C_3H_7)_2$	NH_2
12	α,α,α-Trifluoro-2,6-dinitro-*p*-cresol	CF_3	NO_2	OH	NO_2
13	α,α,α-Trifluoro-2-nitro-*p*-toluidine	CF_3	NO_2	NH_2	H
14	2-Nitro-*N*-propyl-*p*-toluidine	CF_3	NO_2	NHC_3H_7	H
15	4-(Dipropylamino)-3,5-dinitrobenzoic acid	COOH	NO_2	$N(C_3H_7)_2$	NO_2
26	α,α,α-Trifluorotoluene-3,4,5-triamine	CF_3	NH_2	NH_2	NH_2
31[a]	α,α,α-Trifluoro-N^4-propyltoluene-3,4,5-triamine	CF_3	NH_2	NHC_3H_7	NH_2

[a] Not available as a model compound.

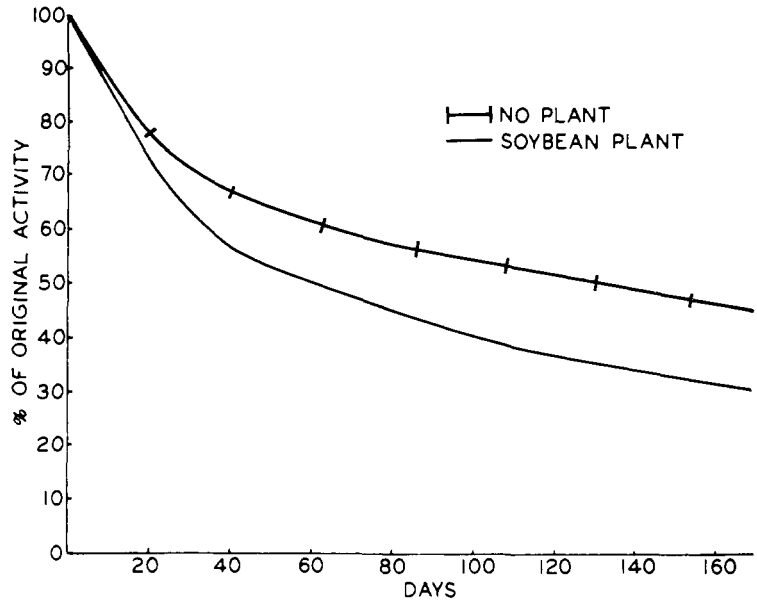


Figure 2. Persistence of ^{14}C-labeled trifluralin in soil under growth room conditions

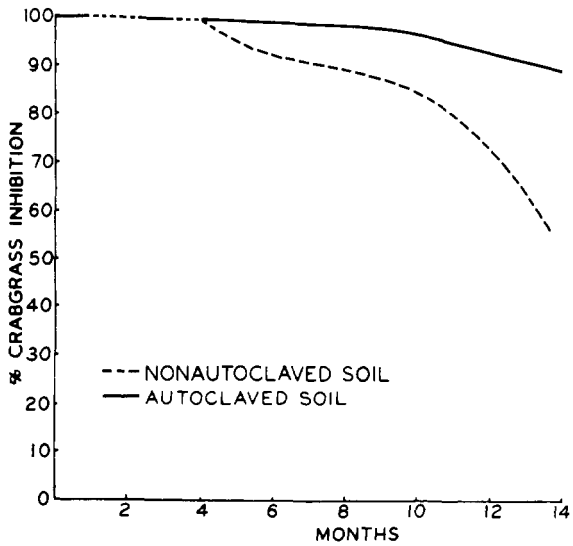


Figure 3. Persistence of trifluralin in autoclaved and nonautoclaved Brookston soil

more rapidly in nonautoclaved than in autoclaved soil.

Examination of many trifluralin-treated soils failed to show a buildup of specific microorganisms which could be credited with the degradation of trifluralin. Microorganisms may contribute to the eventual destruction of trifluralin to simple compounds such as carbon dioxide and water, but this cannot be considered the major pathway of trifluralin degradation.

Postulated Pathway of Aerobic Trifluralin Degradation

The term "aerobic" is defined as a soil condition of normal exposure to light, atmosphere, and moisture. Under this type of aerobic state, the results obtained from thin-layer chromatography, radioautography, and gas chromatography of soil sample extracts permit postulation of a pathway of trifluralin degradation as shown in Figure 4. Intermediates, corresponding to the model compounds, were detectable only in small amounts, suggesting rapid conversion to a mixture of polar products after the initial degradation step.

The material which remains at the origin on thin-layer chromatographic plates in the solvent systems described has been designated as polar products. This mixture could not be resolved into discrete identifiable substances in a variety of solvents. Hydrolysis failed to change its chromatographic behavior. However, strong reduction of the polar products with tin and hydrochloric acid yields a mixture containing compound 26, an aromatic triamine, as a major constituent. This suggests that polar products are formed by condensation of aromatic amines, which arise from the reduction of trifluralin nitro groups during its degradation.

Factors of photosensitivity, variable moisture, oxygen content at different soil depths, and soil microflora all play a role under field conditions. Photosensitivity of trifluralin and associated compounds has been reported (*4*). The initial step in photodecomposition is the removal of one propyl group to form compound 2, also observed in field soil. This product serves as the intermediate for a dual pathway of decomposition. One pathway appears to be another dealkylation step forming compound 3, which then is reduced to compound 4. The alternative pathway involves a reduction of one nitro group of compound 2 to form compound 5, followed by a dealkylation to yield compound 4. Although a small amount of the trifluralin derivative with one nitro group reduced (compound 7) was detected, indicating an initial reduction, the main pathway of decomposition under

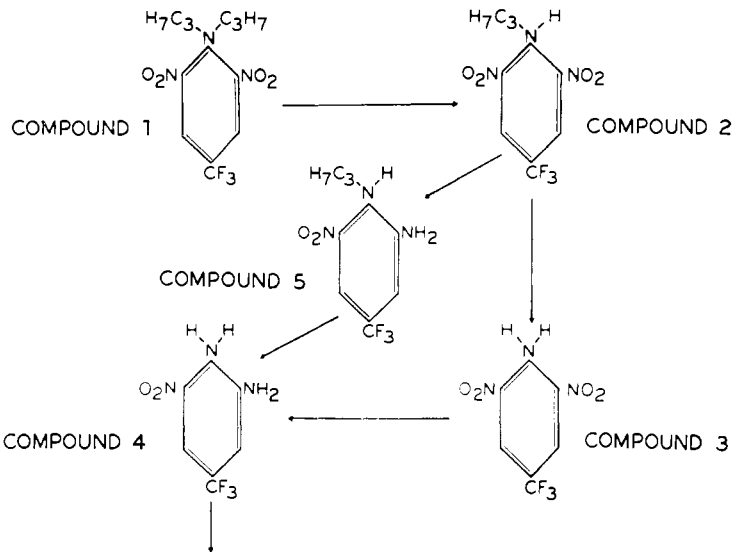


Figure 4. Postulated pathway of aerobic trifluralin degradation

the defined aerobic conditions appears to be dealkylation followed by reduction.

Anaerobic Degradation of Trifluralin

The degradation of herbicides under anaerobic conditions has not been studied extensively. However, under conditions of excessively high rainfall, coupled with low spots or poorly drained fields, a type of anaerobic state may exist for a short time.

The persistence of trifluralin under different moisture conditions was investigated by adding trifluralin to Brookston silty clay loam at 8 p.p.m.w. The treated soil was adjusted to moisture contents equivalent to 0, 50, 100, and 200% of field capacity. Soil samples were taken at weekly intervals and assayed by gas chromatography.

The loss of trifluralin at 200% of field capacity was very rapid, as indicated in Figure 5. In 10 days, 50% of the added trifluralin had disappeared; in 24 days, 84% had disappeared. Disappearance at 50 and 100% of field moisture capacity was considerably slower. In the air-dried soil (0% field capacity), some trifluralin was lost. This could possibly be attributed to volatility.

Anaerobic degradation was investigated further to determine if the disappearance of trifluralin could be associated with anaerobic microorganisms, and to determine effect of soil type and temperature. Brookston silty clay loam soil and Princeton fine sand were autoclaved for 14 hours at 15-pound pressure. Trifluralin was incorporated at 4 p.p.m.w. on autoclaved and nonautoclaved soil. The soils were adjusted to 200% of field capacity with sterile water and maintained at 38° and 76° F.

As shown in Figure 6, the disappearance of trifluralin in Brookston soil is retarded at 38° F. as compared with 76° F. At 38° F., the breakdown is more rapid in nonautoclaved than in autoclaved soil; at 76° F., the trifluralin was degraded within 7 days

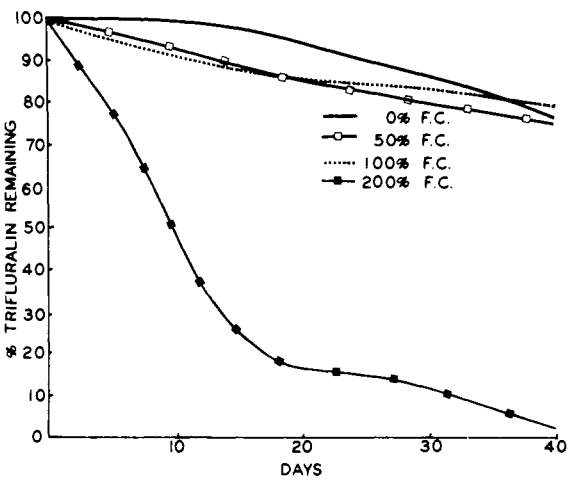


Figure 5. Trifluralin degradation in Brookston soil of differing field moisture capacity

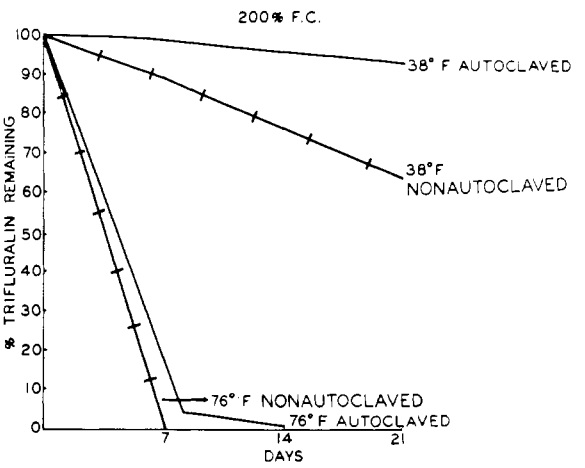


Figure 6. Anaerobic degradation of trifluralin in autoclaved and nonautoclaved soil as a function of temperature

in both autoclaved and nonautoclaved soil. Similar results were obtained with Princeton soil under identical conditions. It was concluded that the rate of breakdown is not influenced markedly by soil type. Trifluralin degradation is temperature-dependent and proceeds more rapidly in nonautoclaved soil.

This rapid degradation of trifluralin in soil supersaturated with water provides a unique system for monitoring the degradation process. An experiment was initiated employing carbon-14 ring-labeled trifluralin incorporated in Brookston silty clay loam at 4 p.p.m.w. The soil was placed in a container, water was added to approximately 1 inch above the surface, and it was incubated at 76° F. in a growth room. Samples were withdrawn at intervals for 14 days.

A small aliquot of each soil sample was burned to determine total radioactivity. The major portion of the sample was analyzed by thin-layer and gas chromatography to determine the distribution of trifluralin and possible degradation products. Wet soil samples were extracted with methanol and partitioned into methylene chloride by the addition of salt. The methylene chloride was evaporated to dryness. The resulting residue was transferred into *n*-hexane. A hexane solution of the residue was chromatographed on a Florisil column which was eluted progressively with hexane, benzene, and benzene-methanol. The hexane eluate contained only trifluralin. The benzene and the benzene-methanol eluates contained trifluralin degradation products.

Figure 7 reveals the degradation of trifluralin and the sequential formation of decomposition products in soil supersaturated with water. The study indicates the rapid formation of a major reduction product, compound 7. The maximum amount of this derivative appears during the fifth and sixth day, then gradually declines. With the progressive disappearance of compound 7, a concomitant rise in compound 9 and extractable polar products occurs. This suggests that compound 7 is the source of both types of decomposition products by different routes. Although the amount of compound 26 steadily increases with time, its formation cannot be considered a major pathway. On the other hand, compound 7 appears to be the prime intermediate in the formation of both the extractable polar products and nonextractable products. These results indicate that the conversion of trifluralin to compound 7 is the rate-limiting reaction. Conversion of compound 7 to polar products is obviously rapid and constitutes the major route of decomposition.

The amount of nonextractable products, determined by combustion of the residual methanol-extracted soil, is expressed as per cent of original radioactivity and constitutes 55% of the total. At 14 days, 80% of the original radioactivity is present as extractable polar products and nonextractable products; less than 20% is present as identifiable compounds.

To visualize trifluralin degradation, radioautographs of thin-layer chromatographic plates were prepared from all extracts and Florisil column eluates. The radioactive samples and the model compounds were cochromatographed in two dimensions with two- (*2*) and three-solvent systems. The chromatographic behavior of the model compounds in the three-solvent system is shown in Figure 8. The position of the model compounds on the chromatoplate, located by visible color or by ultraviolet absorption, was matched with the corresponding radioautographic x-ray film. The coincidence of radioactive spots with the position of the model compounds served as a criterion of identification.

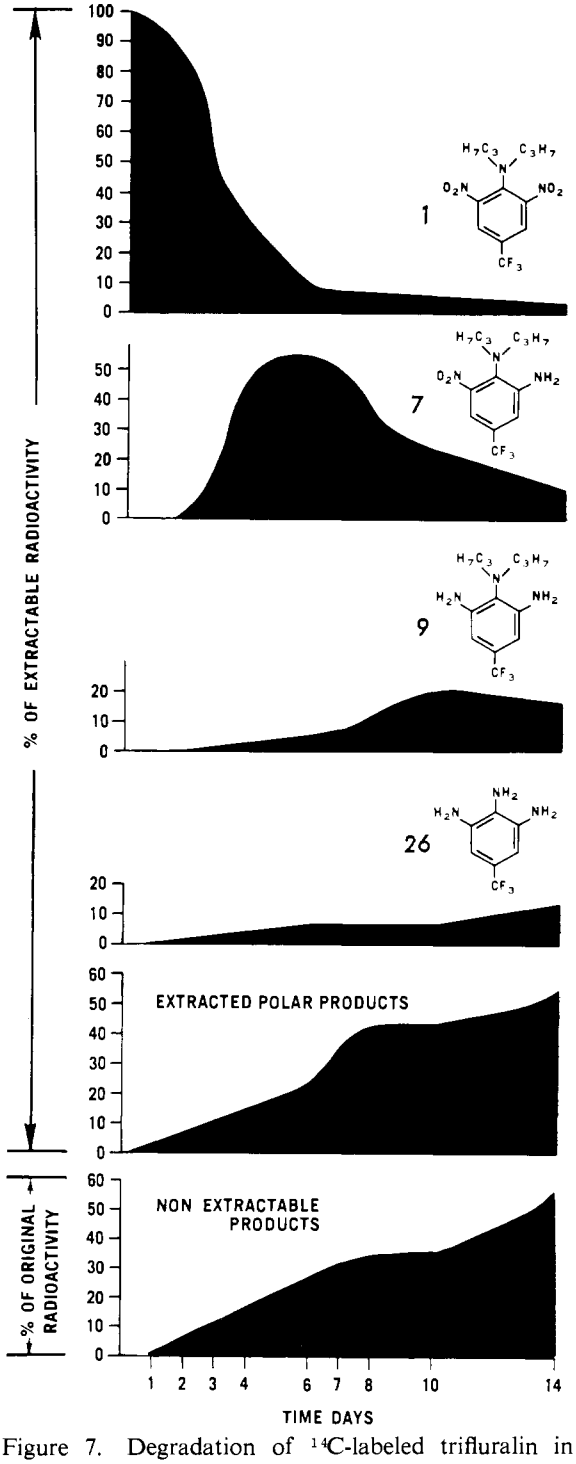


Figure 7. Degradation of ^{14}C-labeled trifluralin in soil supersaturated with water

Figures 9 and 10 are radioautographs of the benzene

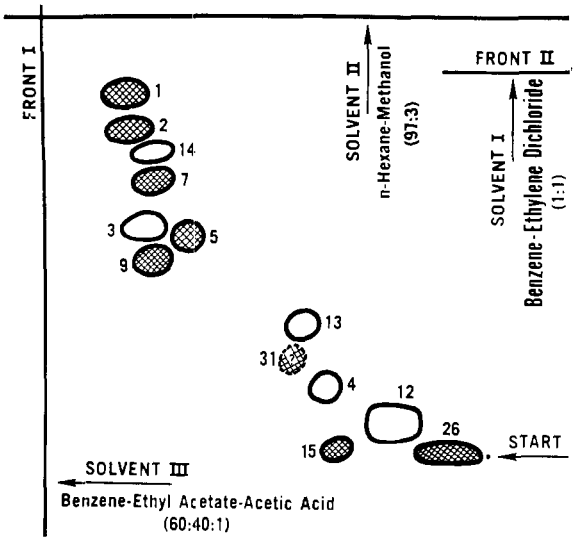


Figure 8. Thin-layer chromatography of model compounds on silica gel GF in three-solvent system

Cross-hatched zones, labeled model compounds of interest

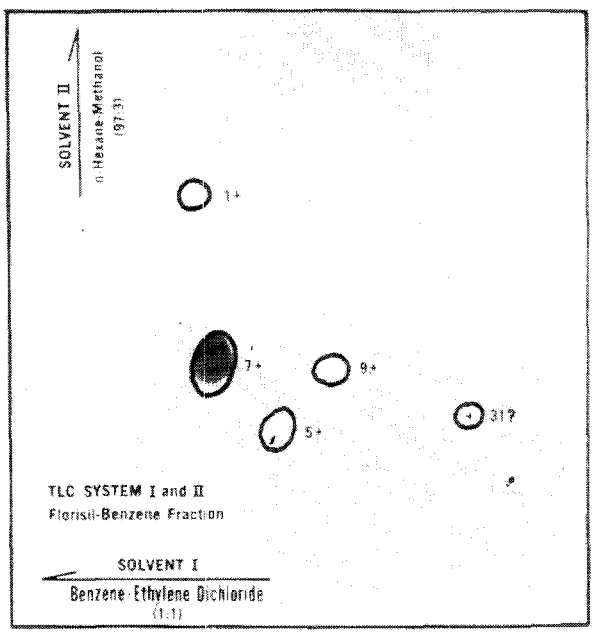


Figure 9. A 62-day exposure radioautograph of trifluralin degradation after 8 days in soil supersaturated with water

Prepared from two-dimensional thin-layer chromatoplate of benzene fraction obtained from Florisil column

and benzene-methanol fractions from a Florisil column obtained from the soil sample taken on the eighth day of the experiment. They serve as selected examples of metabolite identification by radioautographic methods. The radioautograph in Figure 9, a two-dimensional chromatoplate developed with two solvents, reveals the prominence of compounds 7 and 9. Compound 5 and an unknown, designated as compound 31, are detectable. Although compound 31 is not now avail-

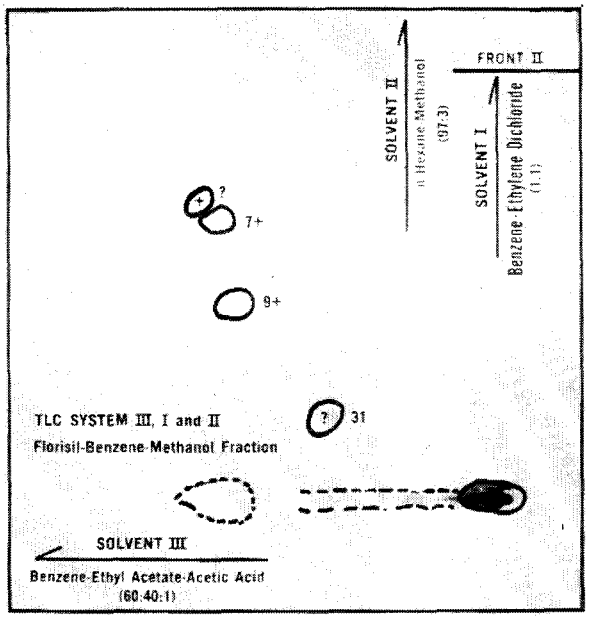


Figure 10. A 61-day exposure radioautograph of trifluralin degradation after 8 days in soil supersaturated with water

Prepared from thin-layer chromatoplate developed with three-solvent systems of benzene-methanol fraction obtained from Florisil column

able in the model compound series, the chromatographic behavior of the unknown suggests a compound of this nature. By contrast, the radioautograph in Figure 10 indicates compound 31 and substances on or near the origin as the major source of radioactivity. Compound 26 remains near the origin in the three-solvent system (Figure 8). The presence of compound 26 in the benzene-methanol eluate of the Florisil column was confirmed by radioautographic analysis of one-dimensional chromatoplates developed with ethyl acetate. The small amount of radioactivity associated with the chromatographic position of the model compounds supports the identification of these recognizable intermediates.

Postulated Pathway of Anaerobic Trifluralin Degradation

When soil is supersaturated with water, trifluralin begins to degrade immediately by the aerobic pathway until the oxygen in the system is depleted. Thin-layer chromatography of soil extracts 12 hours after supersaturation with water revealed the presence of small quantities of compound 2. Thereafter, the major degradation product is compound 7. Thin-layer chromatography and gas chromatography indicate that the primary reduction product can proceed to compound 9 or progress through a minor pathway of dealkylation prior to further reduction of the molecule. Either pathway eventually results in the formation of compound 26 as a final recognizable metabolite of the reduction and dealkylation processes (Figure 11). Polar products, extractable and nonextractable, appear

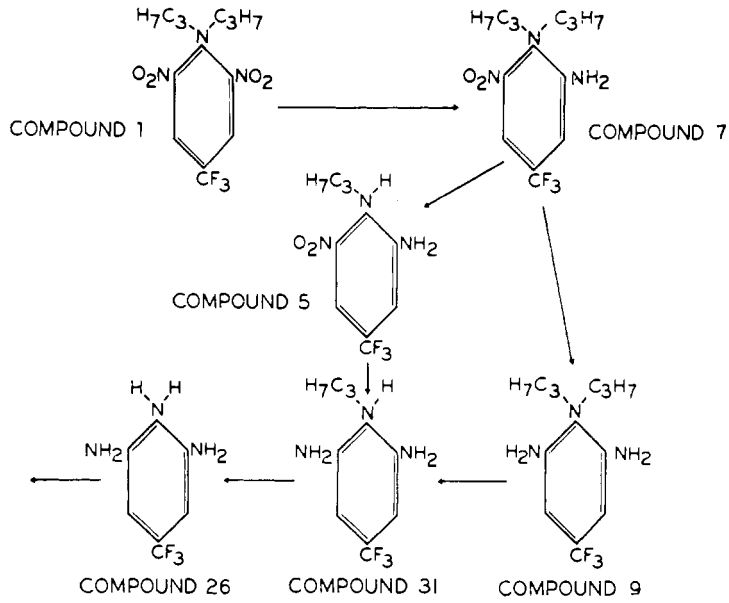


Figure 11. Postulated pathway of anaerobic trifluralin degradation

simultaneously with trifluralin degradation and account for most of the radioactivity observed in these investigations.

Metabolic Fate of Trifluralin in Plants

Residue analysis, with a sensitivity of 5 to 10 p.p.b., indicates that trifluralin or its degradation products are not incorporated in the leaves, seeds, or fruit of a wide variety of tolerant crops. Soybeans and cotton plants show no trifluralin residue, but roots from plants grown in soil exhibit a residue in the region of soil incorporation only. Root crops, such as onions and garlic, contain the trifluralin residue only in the outer shell. On the other hand, carrot roots incorporate trifluralin depending on age and size of the root as well as depth and rate of trifluralin incorporation in soil (*3*).

Soybeans and Cotton Grown in Soil Treated with ^{14}C-Trifluralin

Although analysis revealed no residue in soybeans or cotton plants, a study was undertaken to determine the distribution of residual radioactivity in plants grown in soil containing ^{14}C-trifluralin labeled in the *n*-propyl or the trifluoromethyl group. The radioactivity from both types of labeled trifluralin was distributed in lipids, glycosides, hydrolysis products, protein, and cellular fractions. With propyl-labeled material, approximately 25 to 30% of the original radioactivity was found in the cellular fraction. The radioactivity from ^{14}C-trifluoromethyl trifluralin resided principally in the glycoside fraction. Hydrolysis of the glycoside fraction and thin-layer chromatography of the hydrolytic products revealed no trifluralin or major degradation products in soybean plants or cotton seeds. Small amounts of radioactive carbon dioxide were liberated from soil and plants. The universal distribution of the radioactivity without definite identification of trifluralin or recognizable metabolites suggests nondescript incorporation. Radioactivity in cotton seed was equivalent to 25 p.p.b. calculated as trifluralin.

Metabolism of Trifluralin in Carrots

Since trifluralin residues occur on the outside layer, or peel, of root crops from soil incorporation, the amount of trifluralin and its degradation products in carrots was of interest.

Carrots grown in soil treated with trifluralin labeled with ^{14}C in the trifluoromethyl group were examined for trifluralin and its degradation products (*3*).

The average total radioactivity in seven individual carrots was found to be 0.65 p.p.m. calculated as trifluralin, with a range of 0.49 to 0.86 p.p.m. The distribution of radioactivity in the carrot was: 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, 8.01%. In addition to confirming the majority of the radioactivity in the peel, an increased quantity of radioactivity was found in the third pulp layer, the junction of the phloem and xylem. Ninety-seven per cent of the radioactivity in the carrots was extractable, with 93% remaining in hexane after water partitioning. Investigation by thin-layer chromatography, gas chromatography, and radioautography showed that most of the radioactivity in hexane was trifluralin (84%), with compound 2 representing approximately 4.3%. The identity of these compounds was verified by reverse isotope dilution (*9*) and gas chromatography.

Trace amounts of compounds 5 and 15 were identified by thin-layer chromatography, with one trace zone of

radioactivity not identified. Less than 5% of non-identified polar products were present.

Trifluralin in trace amounts (less than 50 p.p.b.), was the only identifiable product in the carrot greens.

Conclusions

This investigation reveals the presence of trifluralin and other degradation products in the carrot root. It is not possible to determine if this was the result of direct incorporation of these compounds existing in soil or biological conversion by carrot tissue. The presence of model compound 15, in which the trifluoromethyl group had been changed to a carboxyl group, suggests that this compound was not incorporated from soil, as it appears exclusively in the plant tissue. Extensive investigation under a variety of conditions provides no evidence of the existence of this compound in soil.

Although synthetic model compounds have provided a means of postulating metabolic pathways of trifluralin degradation, the importance of polar products cannot be disregarded. Formation of the unidentified polar product mixture appears to be the main pathway for the ultimate degradation of trifluralin into carbon dioxide in water.

Acknowledgment

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