Fate of Benefin in Soils, Plants, Artificial Rumen Fluid, and the Ruminant Animal

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Benefin, a selective, preemergence, soil-incorporated herbicide, decomposed rapidly in soil under defined anaerobic conditions, with approximately 5% detectable after 16 days; whereas, under defined aerobic conditions, 50% of the originally incorporated benefin was detectable after 120 days and only 12.1% after 352 days. Identifiable degradation products were the dealkylated, reduced, and oxidized derivatives of benefin, and an unidentified polar mixture. Soil-incorporated ^{14}C-labeled benefin was not readily absorbed into growing peanut plants and alfalfa. Most of the radioactivity absorbed by plants was not extractable. In the extractable portion, the identifiable degradation products were similar to those found in soil. Artificial rumen fluid destroyed benefin rapidly; only 0.1% of the original benefin remained after 12 hr incubation. Identifiable degradation products were reduced forms of benefin and nonidentifiable polar substances. Radioactivity from ^{14}C-labeled benefin orally administered to the lactating ruminant animal was excreted in the urine (10.8%) and feces (89.1%) within 5 days after ingestion. No radioactivity residue was found in milk.

Benefin (α,α,α-trifluoro-2,6-dinitro-*N*-butyl-*N*-ethyl-*p*-toluidine) is a chemical analog of trifluralin (α,α,α-trifluoro-2,6-dinitro-*N,N*-dipropyl-*p*-toluidine) possessing similar herbicidal properties, and is marketed under the trade name Balan. It is a selective, soil-incorporated, preemergence herbicide for many agronomic and horticultural crops, some of which may be consumed as forage. The herbicidal spectrum, which complements trifluralin, includes annual grasses and broadleaf weeds. The fate of trifluralin in soils (Probst *et al.*, 1967), plants (Golab *et al.*, 1967), and artificial rumen fluid and ruminant animals (Golab *et al.*, 1969) has been extensively investigated. A similar investigation, based on the experimental methodology employed with trifluralin, was undertaken to determine benefin's persistence, pathway of degradation, and the nature of degradation products.

MATERIALS AND METHODS

General Methods. LABELED BENEFIN. Benefin, used in this investigation, was a mixture of ^{14}C uniformly ring-labeled and trifluoromethyl-labeled species. Degradation studies conducted in our laboratories, subsequent to this investigation, revealed that the purchased ^{14}C-labeled *p*-chlorobenzoic acid, used in the synthesis of uniformly ring-labeled trifluralin (Marshall *et al.*, 1966) and benefin, is a mixture of 15% uniformly ring-labeled and 85% carboxyl-labeled *p*-chlorobenzoic acids rather than a single molecular species. Its specific activity was 14.04 μCi per mg (3.12×10^7 dpm per mg), and radiochemical purity, as determined by thin-layer chromatography using silica gel GF developed with carbon tetrachloride, was greater than 99%.

Labeled benefin used in these experiments, except in the ruminant animal (goat study), was diluted with nonlabeled material. The specific activity of ^{14}C-benefin after dilution was 0.20 μCi per mg in the soil, 1.40 μCi per mg in soil with plants, and 0.92 μCi per mg in artificial rumen fluid.

CHROMATOGRAPHIC PROCEDURES. Thin-Layer Chromatography (tlc). Standard tlc plates (20 $\times$ 20 cm), coated with a 250-μ layer of silica gel GF and activated at 110° C for 1 hr were used. The different solvent systems for one- and two-dimensional chromatography are listed:

(1) Carbon tetrachloride
(2) Benzene-ethylene dichloride (1:1, v/v)
(3) *n*-hexane-methanol (97:3, v/v)
(4) Ethyl acetate
(5) Benzene-ethyl acetate-acetic acid (60:40:1, v/v)
(6) Benzene-ethyl acetate-acetic acid (55:45:2, v/v)
(7) Benzene-methyl cellosolve (96:4, v/v)
(8) Cyclohexane-ethyl acetate (95:5, v/v).

Chromatographic chambers were presaturated only with solvent systems 2, 3, and 5. All solvent systems were used in one-dimensional chromatography, and the following combinations were utilized for two-dimensional chromatography.

I. System 2 followed by system 3 in the second dimension.
II. System 2 followed by system 8 in the second dimension.
III. System 5 followed by systems 2 and 3 in the second dimension.
IV. System 5 followed by systems 7 and 8 in the second dimension.

In one-dimensional chromatography, the model compounds were applied on a separate lane as an aid to zone identification; however, when the two-dimensional technique was employed (Golab, 1967), the model compounds were cochromatographed with the aliquots of the extracts. The model compounds were located by their natural color or, if colorless, by scanning the plate with an ultraviolet source. The 12 substances, chemically related to benefin (listed in Table I), served as model compounds.

Gas-Liquid Chromatography (glc). A Jarrell-Ash Chromatograph, Model 700, equipped with electron affinity and flame ionization detectors was fitted with borosilicate glass columns packed with 5% XE-60 on gas chrom Q, 80/100 mesh. Quantitation was established by peak height measurements. Instrument conditions used were: Column temperature: 178° C; Injector temperature: 280° C; Carrier gas—prepurified nitrogen at 90 ml per min; Electrometer output E.A.D. 1.0×10^{-9} amp full scale; Electrometer output F.I.D. 1.0×10^{-9} amp full scale; Detector temperature E.A.D.: 190° C; Detector temperature F.I.D.: 260° C.

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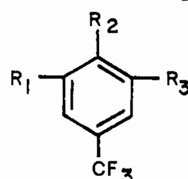
Table I. Model Compounds



Experimental Number	Name	R_1	R_2	R_3
33	Benefin	NO_2	$N(C_4H_9)(C_2H_5)$	NO_2
3	2,6-Dinitro-4-trifluoromethylaniline	NO_2	NH_2	NO_2
4	α,α,α-Trifluoro-5-nitrotoluene-3,4-diamine	NO_2	NH_2	NH_2
12	2,6-Dinitro-α,α,α-trifluoro-*p*-cresol	NO_2	OH	NO_2
17	3,5-Dinitro-4-methoxybenzotrifluoride	NO_2	$O—CH_3$	NO_2
26	α,α,α-Trifluorotoluene-3,4,5-triamine	NH_2	NH_2	NH_2
34	*N*-Butyl-2,6-dinitro-α,α,α-trifluoro-*p*-toluidine	NO_2	$N(C_4H_9)(H)$	NO_2
35	*N*-Ethyl-α,α,α-trifluoro-2,6-dinitro-*p*-toluidine	NO_2	$N(C_2H_5)(H)$	NO_2
36	N^4-Butyl-N^4-ethyl-α,α,α-trifluoro-5-nitrotoluene-3,4-diamine	NO_2	$N(C_4H_9)(C_2H_5)$	NH_2
37	N^4-Butyl-N^4-ethyl-α,α,α-trifluorotoluene-3,4,5-triamine	NH_2	$N(C_4H_9)(C_2H_5)$	NH_2
38	N^4-Butyl-α,α,α-trifluoro-5-nitrotoluene-3,4-diamine	NO_2	$N(C_4H_9)(H)$	NH_2
39	N^4-Butyl-α,α,α-trifluorotoluene-3,4,5-triamine	NH_2	$N(C_4H_9)(H)$	NH_2
40	N^4-Ethyl-α,α,α-trifluoro-5-nitrotoluene-3,4-diamine	NO_2	$N(C_2H_5)(H)$	NH_2

The extracts were purified on column or thin-layer chromatography prior to glc analysis.

Column Chromatography (cc). Extracts were chromatographed on Florisil columns (100/200 mesh) and were eluted successively with *n*-hexane (benefin fraction), benzene (derivatives more polar than benefin), and benzene-methanol (polar material) until no radioactivity remained on the column.

THIN-LAYER RADIOAUTOGRAPHY (tlr). Aliquots of all radioactive extracts subjected to tlc in both one- and two-dimensional systems were exposed to 8 × 10 cm Kodak Blue Brand Medical X-Ray Films. Exposure time ranged from several to over 100 days. The developed films revealed darkened spots, which were matched with the position of the unlabeled reference substances.

COUNTING OF RADIOACTIVE SAMPLES. All radioactive measurements were made with a Packard Tri-Carb Spectrometer, Model 3000. The aliquots from each sample, taken before and after extraction, were combusted in a quartz tube surrounded by an electrical furnace. The carbon dioxide formed was absorbed in a 2-aminoethanol-methyl cellosolve (30:70, v/v) solution. The solution was diluted with appropriate scintillation fluid and counted. The aliquots of organic sol-

vent extracts were dissolved in toluene scintillation fluid and counted. Zones or spots from tlc plates were scraped into scintillation vials, eluted with approximately 0.2 ml of methanol, diluted with scintillation fluid, and counted. The following scintillation fluids were used. For nonaqueous samples: solution of 5 g of PPO (2,5-diphenyloxazole) and 0.3 g of dimethyl POPOP {1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene} in 1 l. of toluene (Hayes, 1963). For combusted samples ($^{14}CO_2$ trapped in an ethanolamine-methyl cellosolve solution): scintillation fluid prepared according to Bruno and Christian (1961). For aqueous samples: solution described by Kinard (1957) and modified by Herberg (1960).

Counting efficiencies were determined by external standardization, internal standardization with toluene 1-C-14, or by channel-ratio standardization. Depending on the level of radioactivity in the samples, counting times ranged from 10 to 30 min.

DERIVATIVE FORMATION. Evidence from tlc suggested that model compound 12 (2,6-dinitro-α,α,α-trifluoro-*p*-cresol) may be present in soil and plant extracts. The close proximity of compound 12 to the origin on thin-layer chromatoplates makes identification difficult. To alter the chromatographic behavior of compound 12, some of the extracts were subjected to methylation with diazomethane according to the procedure of Vogel (1966). The resulting methyl derivative, which was displaced from the origin, served as a criterion to verify identity of compound 12.

Soil Experiment in Anaerobic State. INCORPORATION OF ^{14}C-BENEFIN IN SOIL. A 10-mg quantity of ^{14}C-benefin with a specific activity of 0.20 μCi per mg was dissolved in 20 ml of acetone and poured into a glass jar containing 600 g of air-dried Brookston silty clay loam soil. After mixing the contents of the jar for 2 hr and permitting the residue to evaporate overnight, the soil then was mixed thoroughly with an additional 1900 g of air-dried Brookston soil. The mixed soil containing 4 ppm of benefin was placed in a plastic container and adjusted to the appropriate field moisture capacity (F.C.). In this study the soil was flooded with tap water (1 in. above the soil surface) and was incubated at 80° F.

SAMPLING AND EXTRACTION PROCEDURES. A sample equivalent to approximately 100 g of air-dried soil was removed at 0, 1, 2, 3, 4, 5, 6, 8, 10, 13, and 16 days, respectively, and extracted in an Omni-mixer with 120 ml water-methanol (1:1, v/v) for 5 min. After filtering and washing the filter cake with 50 ml of methanol, 250 ml of 10% NaCl was added to a filtrate, and the mixture was partitioned in a separatory funnel with methylene chloride (three times with 400-ml portions). The methylene chloride extracts were concentrated in vacuum to 2 ml and examined by tlc (visible, ultraviolet, and radioautographic analysis), glc, and direct-liquid scintillation counting (lsc). A portion of each extract was chromatographed on the Florisil column, and the fractions were counted for radioactivity and analyzed by glc.

Soil and Plant Experiments. PLANTING AND HARVESTING PROCEDURE. Two different concentrations of ^{14}C-benefin were incorporated in greenhouse soil (1 part of sand and 1 part of silty clay loam) in the manner previously described. With peanut plants grown to maturation, nondiluted ^{14}C-benefin (spec. act. 14.03 μCi per mg) was incorporated at 2.33 ppm.

Soil containing 2.33 ppm of labeled benefin was placed in a plastic-lined wooden box and planted with peanut seeds. The plants were maintained in a growth room with 12-hr light (80° F) and dark (70° F) periods. At time intervals of 28, 34, 61, 95, 118, 129, 192, 262, and 352 days, soil samples were removed at depths of 0 to 2, 2 to 4, and 4 to 6, or 4 to 8 in. and analyzed. After 129 days, mature peanut plants were harvested and separated into roots, stems, leaves, and nuts. The roots and nuts were washed with running water and dried. Nuts were separated into hulls and meat. All parts were kept frozen until analyzed.

In a second portion of soil, diluted ^{14}C-benefin (spec. act. 1.40 μCi per mg) was incorporated to a concentration of 2.08 ppm, and the soil was divided into several boxes. Half of the boxes were seeded with peanuts and the other half with alfalfa. Peanut plants were harvested at successive time intervals of 28, 34, 49, 61, 76, 95, and 118 days and then were analyzed. Alfalfa was harvested and analyzed at 61, 76, 95, 118, 161, and 227 days. Peanut and alfalfa plants grown in nontreated soil were used as controls at each harvesting time interval.

SAMPLING AND EXTRACTION PROCEDURES. Soil. Soil samples of 10 to 15 g were extracted with methanol in a Soxhlet apparatus for 3 to 5 hr or were extracted with methanol in a Mason jar. The methanol extracts were concentrated on a rotary vacuum evaporator and analyzed for benefin and its degradation products.

Plant Parts. Between 15- and 25-g portions of wet peanut tissues (roots, stems, leaves, hulls, and meat) or alfalfa cuttings were weighed and blended with 200-ml portions of methanol four consecutive times for 5 min each. Where practicable, concentrated extracts were analyzed for radiochemical activity using tlc in conjunction with radioautography and liquid scintillation counting. In some instances, it was necessary to purify some samples by the column chromatography technique previously described. Colored extracts from leaves, stems, and roots were decolorized with an activated charcoal (Darco G-60) prior to analysis.

The combined methanol extracts were concentrated to 200 ml. To each extract, 500 ml of 5% NaCl aqueous solution was added, and the mixture was partitioned five times in a separatory funnel with 700-ml portions of methylene chloride. The combined methylene chloride phases were chromatographed over a Florisil column, and the radioactivity was successively eluted with *n*-hexane, ethyl acetate, and methanol. The aqueous phase was extracted again successively with ethyl acetate and chloroform at pH 1 and 10, respectively. The balance of radioactivity before and after extraction or purification was determined by liquid scintillation counting.

^{14}C-BENEFIN INCORPORATION INTO ARTIFICIAL RUMEN FLUID. The effect of artificial rumen fluid on ^{14}C-labeled benefin was determined by the method of Golab *et al.* (1969), previously used with trifluralin. Labeled benefin, adsorbed on cellulose, was incubated with the two-component mixture of fresh rumen fluid and artificial saliva solution prepared according to the formula of Hubbert *et al.* (1958). Immediately after mixing and purging the aspirator bottle with CO_2, an initial 50-ml aliquot was withdrawn (0 time). Subsequent aliquots were removed at 1/4, 1/2, 3/4, 1, 1 1/2, 2, 2 1/2, 3, 4, 5, 6, 7, 8, 9, 10, and 11 hr. These aliquots were extracted according to the procedure of Golab *et al.* (1969), and were subjected to radiochemical, thin-layer, and gas chromatographic analyses.

^{14}C-BENEFIN EXCRETION RATE IN LACTATING GOATS. Using the same facilities, techniques, and procedures described by Golab *et al.* (1969) for trifluralin, one lactating goat was fed unlabeled benefin for 8 days at 1 ppm incorporated in the ration, labeled benefin for 1 day, and unlabeled benefin again for 14 days. The second goat served as a con-

trol and received no benefin throughout the experimental period.

Total radioactivity was determined in aliquots of milk, urine, feces, and blood from the daily collections. One-ml aliquots of urine were diluted with 20 ml of appropriate scintillation solution, and the radioactivity was determined. Radioactivity in feces and milk samples (1-g and 1-ml aliquots, respectively) was determined by combustion. Recovery of radioactivity from tissues was determined by combusting appropriate aliquots of tissue with known amounts of ^{14}C-stearyl alcohol followed by determination of radioactivity by lsc. Recoveries of 96% were obtained using this technique.

RESULTS AND DISCUSSION

Persistence of ^{14}C-Benefin in Soil under Anaerobic Conditions. Under a type of anaerobic condition, that is, soil flooded with water to reduce oxygen tension, benefin decomposed rapidly as compared with normal moist soil. After 16 days, only 4.6% of the originally incorporated benefin was detectable under these conditions. Figure 1 shows the time-rate degradation of benefin and the formation of its major degradation products, namely, compound 36 (benefin with one nitro group reduced), compound 37 (benefin with both nitro groups reduced), and compound 26 (benefin with both nitro groups reduced and both alkyl groups removed). The radioactivity associated with compound 36 accumulated to a maximum of 44% of the total after 5 to 6 days, then declined to 1.7% within 16 days. The other two quantitatively significant degradation products, compounds 37 and 26, revealed a radioactivity maximum after 13 days and represented 8 and 9% of the total radioactivity, respectively. Compounds 34, 35, 38, 39, and 4 (Table I), detected by glc and tlc in conjunction with radiochemical methods, are considered as minor degradation products constituting less than 5% of the total radioactivity. As observed with trifluralin under similar conditions (Probst *et al.*, 1967), the largest portion of extractable radioactivity is represented as polar products which increase with time and constitute 22.6% of the original radioactivity at the conclusion of the experiment. The nonextractable radioactivity in the residual soil, determined by combustion, increased with time to 62% of the total radioactivity.

Based on the reductive cleavage of trifluralin polar products, previously described by Golab *et al.* (1969), benefin extractable polar products are also assumed to be a mixture of aromatic amine condensation products. The nature of the tightly soil-bound nonextractable radioactivity is unknown. This radioactivity is not amenable to manipulation by conventional methods for direct identification, and is the subject of a separate investigation. The pattern of benefin degradation under this type of anaerobic condition is remarkably similar to trifluralin—namely, initial reduction of the nitro groups followed by dealkylation with aromatic amine condensation continuously occurring.

Persistence of ^{14}C-Benefin in Soil under Aerobic Conditions. Soil maintained at moisture contents that support the growth of peanut plants in this context being designated as aerobic conditions was systematically sampled to determine benefin degradation. Benefin degradation under aerobic conditions was slower than its degradation under the anaerobic-type state. However, during the first 28 days, the total radioactivity and benefin content decreased to 68.5 and 47.4%, respectively—a rate distinctly more rapid than that for the remaining period of the study (Figure 2). Qualitative evidence indicated that a portion of the loss was attributed to volatility from soil. After 352 days, the total radioactivity in soil decreased to approximately 32%. The composition of this remaining soil radioactivity was 10% benefin (0.233 ppm), 6% degradation products which match the model compounds (0.140 ppm), 2% extractable polar products (0.047 ppm), and

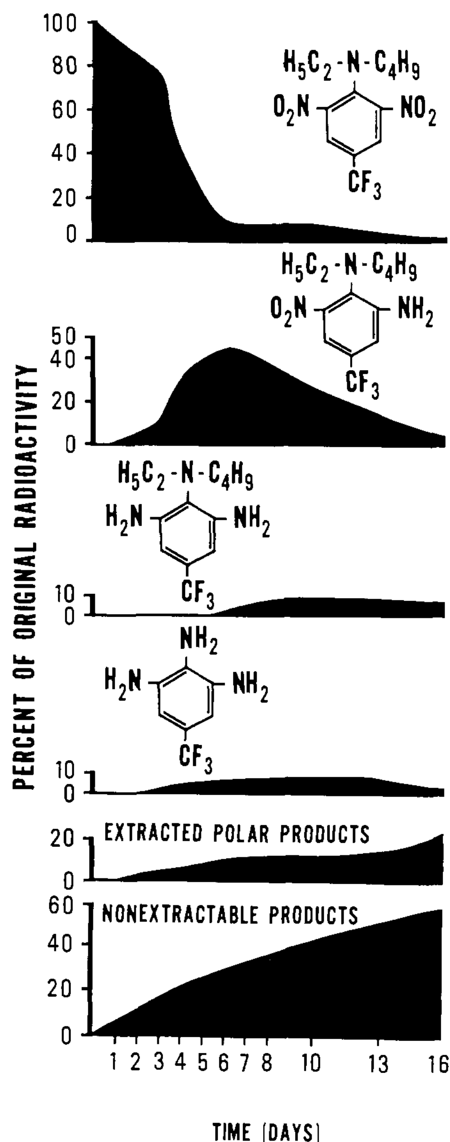


Figure 1. Degradation of ^{14}C-labeled benefin in soil flooded with water

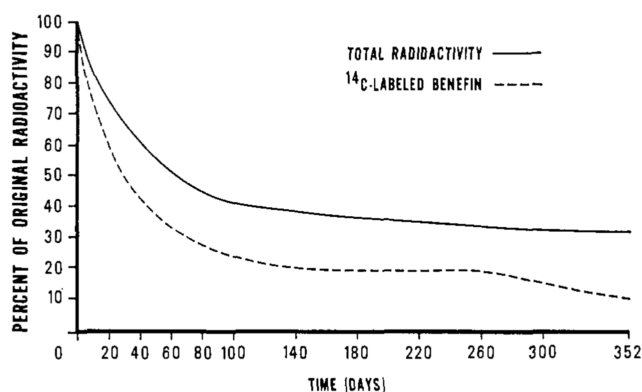


Figure 2. Persistence of ^{14}C-labeled benefin in soil under growth room conditions

Table II. Total Radioactivity Content in Peanut Tissue Calculated as Ppm Benefin

Time (Days)	Stems	Leaves	Roots
28[a]	0.444	0.444	5.361
34	0.295	0.582	5.286
49	0.368	0.567	8.114
61	0.547	0.702	5.103
76	2.186	1.774	4.103
95	0.281	0.825	2.374
118	0.198	0.481	1.304
129[b]	0.580	0.800	3.270

[a] Treatment with ^{14}C-benefin at spec. act. of 1.40 μCi/mg and at 2.08 ppm level. [b] Treatment with ^{14}C-benefin at spec. act. of 14.04 μCi/mg and 2.33 ppm level. After 129 days, the amount of radioactivity calculated as ppm benefin in hulls and meat was 1.756 and 0.158, respectively.

14% nonextractable radioactivity (0.327 ppm). Benefin degradation products detected by two-dimensional tlc radioautographs using the model compounds as references were compounds 3, 4, 12, 26, 34, 35, 36, 38, and 39. Of the various degradation products, compounds 12 and 39 occurred in highest concentrations of approximately 35 ppb, compounds 4 and 26 were intermediate at 15 ppb, and all other compounds were at 10 ppb or less. Trace radioactivity on the radioautographs represented less than 20 ppb including compound 37 and 40, which were estimated at less than 1 ppb.

The presence of compound 12, α,α,α-trifluoro-2,6-dinitro-*p*-cresol in soil as a degradation product of benefin has not been observed previously in similar trifluralin studies. Theoretically, the cresol could be derived from benefin and trifluralin. The existence of compound 12 was substantiated by methylating soil extracts, according to the procedure of Vogel (1966), converting the cresol into the corresponding methyl ether. The derivative was matched with a synthetic reference compound. This difference was attributed to the soil type and conditions, as compound 12 was detectable to a lesser degree in a subsequent experiment with trifluralin incorporated in the same soil.

Peanuts and Alfalfa Grown in ^{14}C-Benefin Treated Soil. The total radioactivity content calculated as ppm of benefin in peanut plant tissues harvested at different times was variable, but generally decreased over the experimental period. Table II summarizes the results of two separate benefin application rates. With 2.33 ppm, incorporation into plant tissue harvested after 129 days was higher than with 2.08 ppm incorporation and harvest after 118 days. Peanut meat contained the lowest level of radioactivity, 0.158 ppm calculated as benefin. As expected, the radioactivity in the roots and hulls was higher than that of other plant parts that were examined. The variable radioactivity present in the stems and leaves, particularly after 61 days, may be due to surface contamination.

The distribution of the radioactivity remaining after 129 days in the nonextractable and extractable portion of plant parts was determined. The nonextractable radioactivity, representing endogenous material recovered only by combustion, expressed as percent of total radioactivity was: 75% in stems, 43% in leaves, 64% in roots, 14% in hulls, and 37% in meat. In the extractable fraction, which contains benefin and its recognizable degradation products, most of the radioactivity was polar material. The concentration of benefin was estimated as 0.005 ppm in stems, 0.001 ppm in leaves, 0.154 ppm in roots, 0.037 ppm in hulls, and a trace (less than 1 ppb) in meat. The degradation products found in plant tissues essentially reflect those products found in soil, but in very low

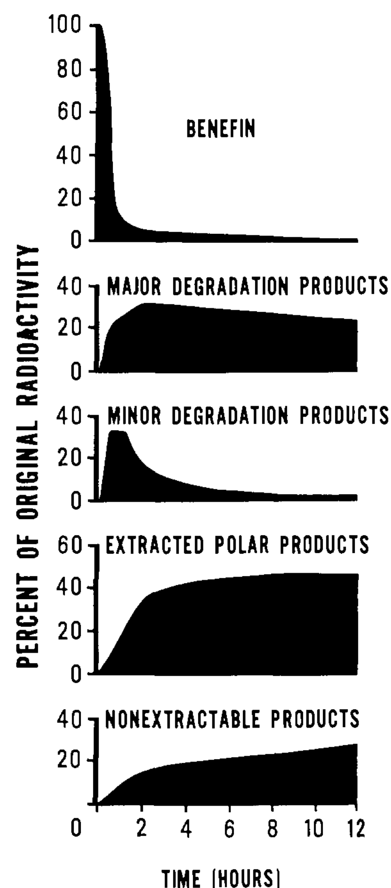


Figure 3. The rate of ^{14}C-benefin degradation and the formation of degradation products in artificial rumen fluid

to trace concentrations. Only stems, roots, and hulls contained significant amounts of decomposition products. These ranged from 9 to 39 ppb for compound 4, 2 to 57 ppb for compound 12, and 8 to 33 ppb for compound 26. Roots consistently exhibited the highest concentration. The other degradation products represented in the model compound series occurred in amounts of less than 2 ppb.

Five successive cuttings of alfalfa, grown in soil containing 2.08 ppm ^{14}C-labeled benefin, had a total radioactivity content which ranged from 0.5 to 1 ppm, calculated as ^{14}C-benefin. At the final harvest time, 227 days post-planting, the total radioactivity decreased to 0.337 ppm. Sixty-five percent of the radioactivity was extractable from the alfalfa, but almost all of this radioactivity was polar material. The alfalfa extract had a benefin content of 0.023 ppm plus traces of compounds 4, 12, and 26 which were detected by radioautographs of thin-layer chromatograms.

Benefin Degradation in Artificial Rumen Fluid. Benefin degradation and the formation of degradation products in artificial rumen fluid is summarized graphically in Figure 3. The curves represent the average values obtained from radiochemical analysis and gas-liquid chromatographic measurements. After 12 hr incubation, 99.9% of the benefin was degraded. The major identifiable degradation products were compounds 26, 36, and 37. Compounds 26 and 36 accumulated to maximum concentrations of 20 and 6%, respectively, after 1 hr and then declined to 14 and 1.5% after 12 hr. Compound 37 accumulated maximally to 15% after 4 hr, then slowly declined to 10% after 12 hr. The extracted polar products, defined as the extracted radioactivity remaining at the origin of thin-layer chromatographs, and the nonextractable

radioactive products increased continuously, suggesting that the radioactivity of labeled benefin ultimately accumulates in the latter heterogeneous mixture.

The rate of accumulation and decline of these major identifiable degradation products suggests that compound 36 is the primary degradation product of benefin and, in turn, is possibly converted to compounds 37 and 26, as well as polar products. Conversion of compound 36 to these latter degradation products exceeds its formation from benefin; therefore, the reduction of one nitro group is the rate limiting reaction in benefin degradation. A similar degradation sequence was observed previously with trifluralin, an analog of benefin (Golab *et al.*, 1969) in which the major identifiable degradation products were analogs of compounds 36 and 37. The quantitative differences observed with the two analogs, benefin and trifluralin, are attributed to differences in the compounds as well as the microbial population of the rumen fluids used in the individual experiments.

Other identified compounds 4, 34, 35, and 39 and some unidentified products are designated as "minor degradation products" in Figure 3. The total of these compounds, detected only by radiochemical methods, constituted 31% of the radioactivity after 1 hr incubation, and then declined to approximately 2% after 12 hr. The amounts of the minor identifiable compounds ranged in different samples as follows: compound 4 (0.4 to 5.5%); compound 34 (0.1 to 0.6%); compound 35 (0.5 to 2.5%); and compound 39 (0.4 to 2.6%). Model compounds 3, 38, and 40 were not detected. The extractable polar products increased continuously, accounting for 47% of the total radioactivity after 12 hr incubation. The radioactivity in the spent aqueous rumen fluid, designated as "nonextractable products" in Figure 3, gradually increased from 2 to 27% during the time-course of the experiment. The ethyl acetate extracts and the spent aqueous rumen fluid contained 99% of the total radioactivity. Thus benefin was not degraded to radioactive gases such as carbon dioxide, methane, etc. The nature of the radioactivity designated as "extractable polar products" and "nonextractable products" is unknown and is the subject of special investigation. The polar product mixture from benefin degradation is probably formed as a result of aromatic amine condensation as suggested by Golab *et al.* (1969) for the formation of polar products from trifluralin. A similar condensation has been previously reported by Bartha and Pramer (1967).

Radioactivity Excretion in the Lactating Goat Fed ^{14}C-Labeled Benefin. The rate and total amount of excretion of radioactivity following oral administration of ^{14}C-labeled benefin to a goat is shown in Figure 4. Although the amount fed was 40 times greater than the highest benefin residue level found in forage material grown in soil treated with benefin at the recommended rate, almost complete recovery of the administered radioactivity was obtained in urine (10.8%) and feces (89.1%) within 5 days.

Statistical analysis according to the method of Redman *et al.* (1965), which supports a 90% certainty for the presence of radioactive residues, revealed that the radioactivity was above the normal background level in the urine for 3 days and in the feces for 4 days. The same statistical analysis applied to data obtained from milk samples taken before, during, and after the administration of the labeled material provided confidence that no radioactivity above the normal level was present in the milk at any time. Although the nature of the radioactivity in the urine and feces was not examined, because of the similarity of their degradation in other systems we may assume that the excretory products following benefin ingestion would be analogous to those found with trifluralin in a similar experiment.

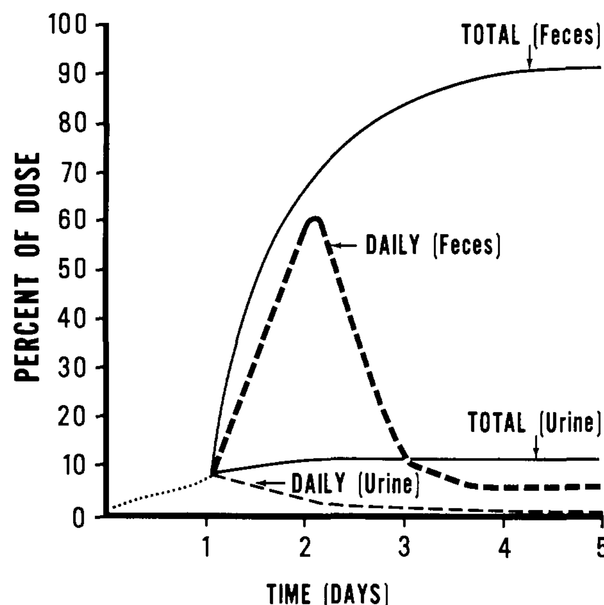


Figure 4. Excretion of radioactivity in goat urine and feces after ingestion of ^{14}C-labeled benefin

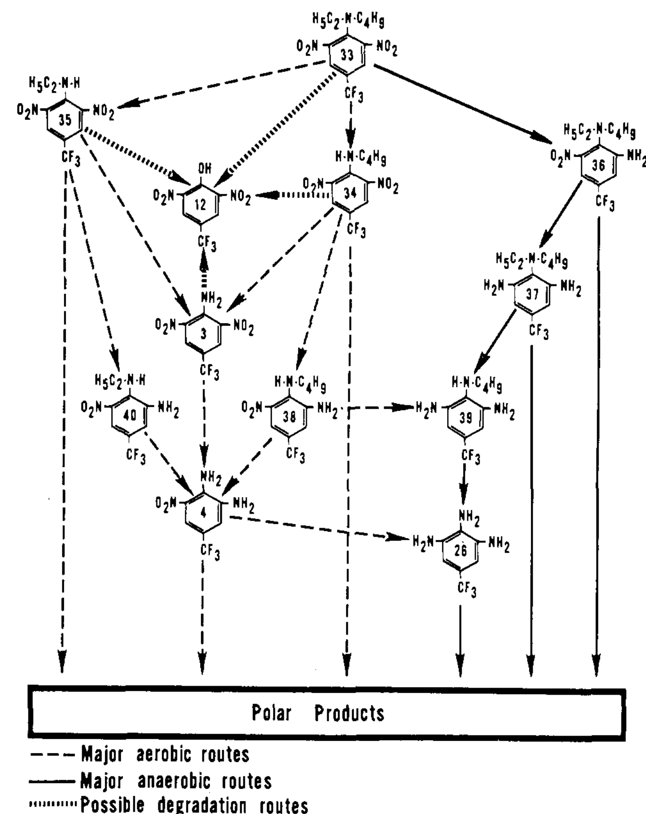


Figure 5. Postulated pathway of fate of benefin under aerobic and anaerobic conditions

CONCLUSION

The experimental results confirm that the fate of benefin in soil, plants, artificial rumen fluid, and the ruminant animal follows a pattern similar to that previously observed with trifluralin. Some differences in the rate of degradation and/or

formation of degradation products were observed which may be associated with the nature of the alkyl substituents of the two analogs.

The use of model compounds provided a means of postulating the sequential steps involved in the degradation process. Benefin degradation, like trifluralin, can be divided into two major routes: aerobic—occurring primarily in normal moist soil; and anaerobic—occurring predominantly in soil under water, artificial rumen fluid, or in ruminant animals. The conditions defined as "aerobic or anaerobic" are not absolute. For example, degradation products which occur in major amounts in the aerobic route are present in minor quantities in the anaerobic route and *vice versa*. Figure 5 illustrates the postulated pathway of benefin degradation by these two routes. The aerobic route is characterized by oxidative dealkylation reactions, and the anaerobic route by a sequence of reductive reactions. Both routes result in formation of the polar products. Aromatic amines formed from either dealkylation or reduction probably serve as precursors of polar products. The formation of these polar products appears to be the main pathway for the ultimate degradation of benefin into carbon dioxide and water.

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