the decomposition of recalcitrant molecules. The inability of pure cultures of microorganisms to degrade a chemical can not be taken as proof that the substance is resistant to microbial attack; it may, in fact, be readily destroyed by the combined action of two or more organisms. A synergistic relationship between microorganisms has also been noted in the degradation of cycloparaffinic hydrocarbons (Beam and Perry, 1974) and other pesticides (Bordeleau and Bartha, 1971; Gunner and Zuckerman, 1968; Pfaender and Alexander, 1972).

LITERATURE CITED

Alexander, M., Aleem, M. I. H., *J. Agric. Food Chem.* **9**, 44 (1961).
Arnow, L. E., *J. Biol. Chem.* **118**, 531 (1937).
Bartha, R., Pramer, D., *Soil Sci.* **100**, 68 (1965).
Beam, H. W., Perry, J. J., *J. Gen. Microbiol.* **82**, 163 (1974).
Blackburn, R. D., *Weeds* **11**, 21 (1963).
Bollag, J.-M., Briggs, G. G., Dawson, J. E., Alexander, M., *J. Agric. Food Chem.* **16**, 829 (1968).
Bordeleau, L. M., Bartha, R., *Soil Biol. Biochem.* **3**, 281 (1971).
Bounds, H. C., Colmer, A. R., *Weeds* **13**, 249 (1965).
Burger, K., MacRae, I. C., Alexander, M., *Soil Sci. Soc. Amer. Proc.* **26**, 243 (1962).
Evans, W. C., Smith, B. S. W., Fernley, H. N., Davies, J. I., *Biochem. J.* **122**, 543 (1971).
Frank, P. A., Hodgson, R. H., Comes, R. D., *Weeds* **11**, 124 (1963).
Gaunt, J. K., Evans, W.-C., *Biochem. J.* **122**, 519 (1971).
Gunner, H. B., Zuckerman, B. M., *Nature (London)* **217**, 1183 (1968).
Horvath, R. S., *Bull. Environ. Contam. Toxicol.* **5**, 537 (1971).
Iwasaki, I., Utsumi, S., Ozawa, T., *Bull Chem. Soc. Jpn.* **25** 226 (1952).
Loos, M. A., Roberts, R. N., Alexander, M., *Can. J. Microbiol.* **13**, 679 (1967).
Praender, F. K., Alexander, M., *J. Agric. Food Chem.* **20**, 842 (1972).
Tiedje, J. M., Duxbury, J. M., Alexander, M., Dawson, J. E., *J. Agric. Food Chem.* **17**, 1021 (1969).

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Fate of 2-Chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene (Oxyfluorofen) in Rats

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Two albino rats were dosed orally with [^{14}C]trifluoromethyl-labeled 2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene for 7 consecutive days. Only trace amounts of radioactivity (2–4%) were recovered in the urine and tissues. The major route of dose elimination was through the feces (~95%). About 75% of the fecal radioactivity was the unchanged dosed compound. Other compounds present in feces were: 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrophenol, 4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-ethoxybenzenamine, *N*-[4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-ethoxyphenyl]acetamide, and *N*-[4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-hydroxyphenyl]acetamide.

The use of diphenyl ethers, such as fluorodifen and nitrofen, as herbicides has been reported (Rogers, 1971; Nakagawa and Crosby, 1974; Yih and Swithenbank, 1975). The diphenyl ether, 2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene, is an experimental herbicide of broad spectrum activity known as RH-2915 (hereafter compound A). The Weed Society of America has adopted the common name of oxyfluorfen and a trademark of GOAL has been established for RH-2915. Compound A has been shown to provide a high degree of weed control and a great versatility of application (Yih and Swithenbank, 1975). In order to determine the fate of ^{14}C-labeled compound A in an animal system, a material balance and metabolism study was conducted with rats using ^{14}C-labeled material.

MATERIALS AND METHODS

Synthesis of Compounds. *Compound A. 2-Chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl)benzene.* Compound A was synthesized by a procedure similar to that of Yih and Swithenbank (1975) with a ^{14}C label in the CF_3 group. Radiopurity was established as 99% using thin-layer chromatography and radioautography. The compound was used at a sp act. of 0.99 mCi/g or 2220 dpm/μg.

Rohm and Haas Company, Research Laboratories, Spring House, Pennsylvania 19477.

Compound B. 5-[2-Chloro-4-(trifluoromethyl)phenoxy]-2-nitrophenol. 1,3-Bis[2-chloro-4-(trifluoromethyl)phenoxy]-4-nitrobenzene (51.2 g, 0.1 M) was treated with potassium hydroxide (26.4 g, 0.4 M) in water (200 mL) at 50–60 °C for 4 h in dimethyl sulfoxide (800 mL). The mixture was diluted with water, acidified with dilute acid, and extracted with ether. The extract was extracted with 10% potassium hydroxide solution and this extract acidified and extracted with ether. The ethereal solution was dried with magnesium sulfate and the solvent removed to give a residue which was distilled at 0.4 mm up to 100 °C to give 2-chloro-4-trifluoromethylphenol. The distillation residue was recrystallized from benzene–hexane to give 3-[2-chloro-4-(trifluoromethyl)phenoxy]-4-nitrophenol (21.5 g). The solvent was thoroughly removed from the above mother liquors and this residue extracted with warm hexane (40 °C, 500 mL) and the extract cooled to 10 °C to give B (9.1 g), mp 68–70 °C. Anal. Calcd for $C_{13}H_7ClF_3NO_4$: C, 46.78; H, 2.10; Cl, 10.63; F, 17.09; N, 4.20. Found: C, 46.28; H, 2.07; Cl, 10.61; F, 17.69; N, 4.05.

Compound C. 4-[2-Chloro-4-(trifluoromethyl)phenoxy]-2-ethoxybenzenamine. Compound A (50.0 g, 0.138 M), platinum(II) oxide (200 mg), and 1000 mL of absolute ethyl alcohol were charged to a 2-L Parr shaking autoclave. Hydrogen was added at room temperature at an initial pressure of 40 psi and was recharged until a constant pressure was maintained. The reaction mix was filtered and the filtrate concentrated at 100 °C and 20 mm to yield

44.7 g of purple oil which was dissolved in 200 mL of toluene. Treatment of the toluene solution with gaseous hydrogen chloride precipitated a solid that was separated by filtration. This solid was treated with 400 mL of 10% sodium hydroxide, extracted with 200 mL of toluene, and after washing the toluene solution with 3 × 300 mL of water, concentrated at 100 °C and 20 mm to yield 22.8 g of oil that solidified on cooling (mp 70–72 °C). Recrystallization from isooctane gave 16.3 g of light purple solid, compound C, mp 73–75 °C. Anal. Calcd for $C_{15}H_{13}ClF_3NO_2$: C, 54.24; H, 3.96; Cl, 10.70; F, 17.40; N, 4.22. Found: C, 54.61; H, 4.01; Cl, 10.74; F, 17.12; N, 4.23.

Compound D. N-[4-[2-Chloro-4-(trifluoromethyl)-phenoxy]-2-ethoxyphenyl]acetamide. Compound C (34.1 g, 0.103 M), triethylamine (12.9 g, 0.128 M, Aldrich), and toluene (100 mL) were charged to a 500-mL flask equipped with a stirrer, thermometer, addition funnel, and condenser. Acetyl chloride (8.0 g, 0.128 M, Aldrich) was added with cooling and agitation over a period of 10 min (25–30 °C). The resulting slurry was filtered and the filtrate washed with 2 × 300 mL of water, followed by vacuum distillation to remove solvent to give 32.0 g of brown oil which crystallized on cooling, mp 110–112 °C, and was recrystallized from isooctane to yield 17.5 g of a tan solid, compound D, mp 111–113 °C. Anal. Calcd for $C_{17}H_{15}ClF_3NO_3$: C, 54.62; H, 4.02; Cl, 9.49; F, 15.26; N, 3.72. Found: C, 54.61; H, 3.97; Cl, 9.59; F, 15.24; N, 3.73.

Rat Dosing and Sample Collection. For 7 days, doses of 15.8 mg of $^{14}CF_3$-labeled compound A were administered orally by syringe and stomach tube to one female and one male albino rat each weighing 178 g (~100 mg/kg based on average body weight). Both specimens were housed in separate cages that allowed for the separate collection of urine, feces, and exhaled CO_2 (Roth, 1956). A 3-day withdrawal interval followed the dosing period. Food and water were available ad libitum.

Analysis of Samples. *Radioassay.* Aliquots (0.1 mL) from the daily urine output were measured into polyethylene counting vials containing 15 mL of Aquasol (New England Nuclear Corp.) and analyzed by liquid scintillation counting on a Packard Model 3320 Liquid Scintillation Spectrometer.

Feces were weighed, powdered with dry ice in a Vir-Tis Homogenizer, and held frozen in vials pending analysis. Subsamples (0.25 g) were weighed into zircon combustion boats and combusted in a furnace (Peets et al., 1960) on a 90-min cycle. CO_2 produced during the cycle was continually purged from the furnace by a sweep of air and trapped in 10 mL of 5 M ethanolamine in 2-methoxyethanol. Aliquots of the CO_2 trapping solution were assayed by liquid scintillation spectrometry as described above.

Exhaled CO_2, which was trapped daily in 100 mL of 5 M ethanolamine in 2-methoxyethanol, was likewise counted in polyethylene vials with 15 mL of a scintillator solution (1285 mL of methanol, 1715 mL of toluene, 15.0 g of 2,5-diphenyloxazole, 0.90 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene).

After the withdrawal period, the rats were sacrificed and skinned. Selected organs and tissues were assayed by combustion and liquid scintillation spectrometry similar to feces.

Feces Extraction. The primary route of elimination of compound A and metabolites was through the feces; only trace amounts of radioactivity were absorbed in the tissues or eliminated in the urine (Table I).

The radioactivity in rat feces was readily extractable with methanol (94–99%). From each powdered feces sample, 0.25 g was removed to a 1-pint Waring Blendor jar containing 100 mL of methanol. The mixture was blended at low speed for 3 min and suction filtered in a Buchner funnel containing Whatman No. 1 filter paper. An aliquot of the filtrate was radioassayed as described above.

The feces residues were oxidized in a Packard Tri-Carb Sample Oxidizer Model 306.

The methanol extract was concentrated by flash evaporation to 5 mL and quantitatively transferred to an Alltech mini-vial. The volume was further reduced to 0.5 mL with a gentle stream of nitrogen.

Thin-Layer Chromatography. Each concentrated methanol extract was applied with a Drummond Microcap spot applicator to a 20 cm × 20 cm 250 μ silica gel LQF TLC plate with preadsorbent layer (Quantum Industries). Authentic reference compounds were concurrently spotted on the same plate and cochromatographed. TLC plates were developed to 10 cm by the ascending technique in an 11.5 in. × 10 in. × 3.5 in. glass tank. Two solvent systems, (A) 2% methanol–benzene and (B) 40% heptane–benzene (v/v), were utilized. Elution of the solvent through the preadsorbent layer concentrated each extract into a single band at the origin before selectively separating the various components along the silica gel surface.

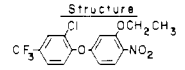
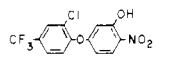
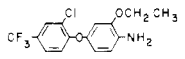
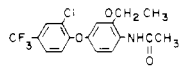
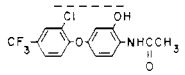
Radioautography. To locate the radiolabeled metabolites, TLC plates were exposed to Eastman Kodak SB54 blue-sensitive x-ray film and secured for a sufficient time period for visualization.

Table I. ^{14}C Compound A Material Balance in Rats

Sample	% recovery of total administered dose[a] Male	Female
CO_2	0.1	0.2
Feces	102.9	86.1
Urine	1.9	4.2
Cage washings	0.5	3.3
Tissues	0.3	0.3

[a] Average % recovery, 99.9.

TABLE II. PERCENT DISTRIBUTION OF RADIOACTIVITY

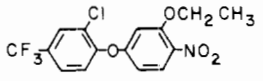
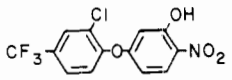
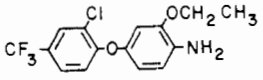
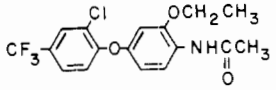
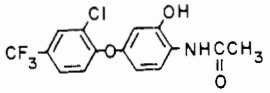
Rf (cm)	Compound	5 Days[a]	6 Days	7 Days	8 Days	9 Days	Structure
9.6	A	71.5	66.0	71.5	71.0	75.2	
7.6[b]	B	~1	~1	~1	~1	~1	
8.7	C	10.5	10.9	5.7	5.4	6.7	
5.9	D	0.9	0.7	1.4	TRACE	1.0	
3.8	E[c]	2.3	1.2	1.5	1.6	0.9	------
2.8	F[c]	2.2	1.7	1.4	1.6	1.6	------
1.0	G	6.0	6.6	8.6	7.5	5.8	
0	H[c]	6.6	12.9	9.9	12.5	8.7	------

[a] TIME (DAYS) AFTER ONSET OF DOSING
[b] SOLVENT SYSTEM B; ALL OTHERS FROM A
[c] E, F, AND H ARE UNKNOWN AT THIS TIME

RESULTS

Radioautographs of female and male fecal metabolite distributions were closely aligned. Quantitation of corresponding TLC plates by scraping the silica gel radioactive areas of the plates into counting vials, followed by radioassay provided data for metabolite percentages and distribution (Table II). The daily fluctuations of me-

TABLE III. MASS SPECTRAL CHARACTERISTICS OF COMPOUND A AND METABOLITES

Compound and Structure	m/e	% of Peak Base
 A	69	86
	131	25
	219	24
	252	100
	300	25
	361	18
 B	63	100
	69	80
	252	90
	333	45
 C	29	83
	52	92
	79	56
	123	100
	274	100
	302	98
	331	100
 D	124	23
	274	22
	302	35
	331	30
	373	20
 G	234	55
	235	6.0
	250	100
	303	25
	345	5

tabolite percentages varied only superficially. Control samples produced blank radioautographs.

Identification of Rat Feces Metabolites. Metabolites which cochromatographed with authentic reference compounds in both solvent systems were tentatively identified by comparison of R_f values. Confirmation was obtained by GLC and mass spectral analyses on a Finnigan Model 1015 S/L Quadrupole Mass Spectrometer. Table III lists the identified compounds and their mass spectral data. The tentative identification of metabolite G, *N*-[4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-hydroxyphenyl]acetamide, is based on mass spectroscopic–GLC analyses alone. Metabolite B, 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrophenol, was present in feces to the extent of only 1%. Metabolite C was identified as the amino derivative of compound A, or 4-[2-chloro-4-(trifluoromethyl)phenoxy]-2-ethoxybenzenamine. Metabolite D was determined as *N*-[4-[2-chloro-4-(trifluoromethyl)-phenoxy]-2-ethoxyphenyl]acetamide. Trace metabolites E and F are unidentified at this time.

DISCUSSION

As seen in Table I, no appreciable amounts of ^{14}C radioactivity were recovered in the CO_2, urine, cage washings, or tissues of either the male rat (2.8%) or the female rat (8.1%). The major route of dose elimination was through the feces.

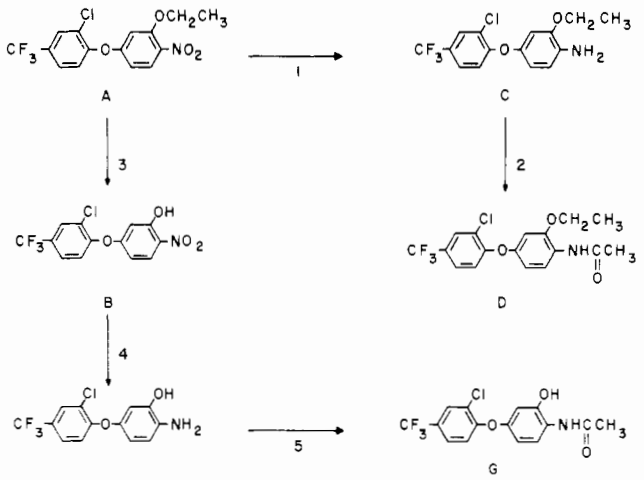


Figure 1. Proposed metabolic pathway of compound A in rats.

A scheme for the transformations of compound A in rat is presented in Figure 1. Reduction of the nitro substituent in herbicides has been observed with diphenyl ethers (Gutenmann and Lisk, 1967) and other herbicides (Probst et al., 1967; Fisher et al., 1965; St. John et al., 1965) as in the case of the degradation of compound A to metabolite C (1). Subsequent acetylation yields metabolite D (2). Deethylation of an ethoxy group has been observed with *p*-phenetidine in the dog (Williams, 1959, p 328) and in other examples. This simple chemical transformation (3) most likely occurs in forming B. Pathways 4 and 5, the consecutive conversion of a nitro group to an amino group to an acetamido group (Williams, 1959, p 423), is suspected in the formation of metabolite G.

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LITERATURE CITED

Fisher, D. E., St. John, L. E., Jr., Gutenmann, W. H., Wagner, D. G., Lisk, D. J., *J. Dairy Sci.* **48**, 1711–1715 (1965).
Gutenmann, W. H., Lisk, D. J., *J. Dairy Sci.* **50**, 1616–1518 (1967).
Nakagawa, M., Crosby, D. G., *J. Agric. Food Chem.* **22**, 843–853 (1974).
Peets, E. A., Florini, J. R., Buyske, D. A., *Anal. Chem.* **32**, 1465–1467 (1960).
Probst, G. W., Golab, T., Herberg, R. J., Holzer, F. J., Parka, S. J., Schans, C. Vander, Tepe, J. B., *J. Agric. Food Chem.* **15**, 592–599 (1967).
Rogers, R. L., *J. Agric. Food Chem.* **19**, 32–35 (1971).
Roth, L. J., *Nucleonics* **14**, 104 (1956).
St. John, L. E., Jr., Ammering, J. W., Wagner, D. G., Warner, R. G., Lisk, D. J., *J. Dairy Sci.* **48**, 502–503 (1965).
Williams R. T., "Detoxication Mechanisms", Wiley, New York, N.Y., 1959.
Yih, R. Y., Swithenbank, C., *J. Agric. Food Chem.* **23**, 592–593 (1975); Rohm and Haas U.S. Patent 3798276, March 19, 1974.

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