Metabolic Fate of Perfluidone Herbicide in a Lactating Cow

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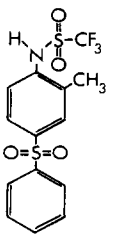
Perfluidone (1,1,1-trifluoro-*N*-[2-methyl-4-(phenylsulfonyl)phenyl-^{14}C]methanesulfonamide) was administered at 5 mg/kg as a single oral dose to a lactating cow. Radiocarbon was rapidly and essentially quantitatively excreted during a 7-day posttreatment period. About 80% of the administered dose appeared in urine, about 20% was recovered in feces, and only 0.1% of the dose was secreted into the milk. Unmetabolized perfluidone accounted for 90–95% of the total radiocarbon present in the urine and milk, and the remaining radioactivity was mainly in the form of two metabolites hydroxylated at the 3 or 4 positions of the phenylsulfonyl ring. These products were excreted almost totally in nonconjugated form. The major urinary metabolite and the only milk metabolite was 4-hydroxyperfluidone, whereas perfluidone and its 3-OH and 4-OH derivatives occurred in approximately equal amounts in feces.

Substituted fluoroalkanesulfonanilides are a new class of efficacious herbicides (Trepka et al., 1970, 1974). Among the first compounds of this group to undergo development for commercial use is perfluidone (MBR-8251, Destun, 1,1,1-trifluoro-*N*-[2-methyl-4-(phenylsulfonyl)phenyl]-methanesulfonamide), which exhibits significant herbicidal activity against many grassy and broadleaf weeds and very effectively controls the nutsedges, *Cyperus esculentus* (Gentner, 1973) and *C. rotundus*. Nutsedges are among the most serious weed pests in the world; thus, perfluidone has potential for widespread use. Since perfluidone may be applied to crops used for cattle feed, it is important to determine the metabolic fate of the compound in cattle, and to determine if perfluidone residues are found in meat and milk. These studies were initiated to elucidate the metabolic fate of perfluidone in a lactating cow.



MATERIALS AND METHODS

Chemicals. Perfluidone-^{14}C (5.2 mCi/mmol, uniformly labeled in the trisubstituted phenyl ring) was provided by the 3M Company (St. Paul, Minn.). Before use, the material was purified by preparative thin-layer chromatography (TLC) developed in a solvent mixture of benzene (saturated with formic acid) and ether (5:1). The purified perfluidone-^{14}C (>99% radiochemical purity) was subsequently diluted with unlabeled perfluidone to a specific activity of 0.11 mCi/mmol. Samples of perfluidone analogs from synthesis which were considered potential metabolites were also supplied by 3M (Table I).

Treatment and Sample Collection. A 410-kg lactating Jersey cow, producing approximately 10 kg of milk daily, was obtained from a local dairy. The animal was housed in a metabolism stall, and a flexible tube was cemented to the vulva to permit separate collection of urine and feces. The cow was hand milked at 12-hr intervals. Alfalfa hay and water were provided ad libitum, and crushed grain was fed at each milking. The perfluidone-^{14}C was mixed with a small amount of crushed grain and administered orally in a gelatin capsule with a balling gun. The treatment consisted of 2.05 g of perfluidone and 0.60 mCi of ^{14}C; the treatment level was equivalent to 5.0 mg/kg body weight. After treatment, total milk, urine, and feces samples were collected at 12-hr intervals. Aliquots (0.2 ml) of fresh milk and urine were subjected to direct liquid scintillation counting (lsc) for radiocarbon quantitation, and parts of each feces sample were analyzed by oxygen combustion. The rest of each sample was frozen for later analysis. Seven days after treatment, the cow was sacrificed, and selected tissue samples were collected and frozen for subsequent analysis.

Extraction and Analysis. Urine samples (10–100 ml, depending on radiocarbon content) were acidified to pH 2.0 with HCl and then extracted 3 times with equal volumes of ethyl acetate. Aliquots (0.2 ml) of the aqueous and organic phases were assayed by lsc for radiocarbon content, and the organic phase was dried over anhydrous sodium sulfate, concentrated, and then subjected to TLC for metabolite resolution.

Whole milk (2 l. from the combined 12- and 24-hr samples) was centrifuged to separate the aqueous and cream fractions. Preliminary studies indicated that acidification of milk fractions did not improve extractability of radiocarbon residues, and this step was omitted. The aqueous milk fraction was extracted 3 times with ethyl acetate, and the combined extracts were dried over sodium sulfate. The solvent was removed under reduced pressure, and the residue was taken up in a small amount of acetonitrile, which was then partitioned twice with hexane to remove residual butterfat. The hexane contained no radiocarbon and was discarded. The acetonitrile was subsequently concentrated under a gentle stream of nitrogen for TLC analysis. The cream fraction was subjected directly to an acetonitrile–hexane partition. The hexane phase contained no ^{14}C and was discarded. The acetonitrile phase was analyzed by TLC.

Feces samples (25 g) were homogenized in 50 ml of water with a Polytron homogenizer (Brinkman Instruments, Westbury, N.Y.). The samples were then acidified to pH 2 with HCl and extracted 3 times with 100-ml volumes of ethyl acetate by homogenization and subsequent centrifugation to break the emulsion. Radiocarbon in each of the three fractions (combined organic, aqueous, and residue) was quantitated by lsc or by oxygen combustion followed by lsc of the trapped $^{14}CO_2$. The organic phase was concentrated and the residue taken up in acetonitrile which was subsequently partitioned with hexane. The hexane was discarded because it contained no radiocarbon, and the acetonitrile was concentrated for TLC analysis. The nature of radioactivity in the aqueous and residue phases of the feces samples was not determined.

Fluoroalkanesulfonanilides are unusually acidic due to the electron-withdrawing effect of the fluoroalkanesulfonyl

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Table I. TLC Behavior of Perfluidone Analogs

Chemical name	Abbreviated name	R_f in indicated solvent system[a,b]						
		A	B	C	D	E	F	G
1,1,1-Trifluoro-*N*-[2-methyl-4-(phenylsulfonyl)phenyl]-methanesulfonamide	Perfluidone	0.43	0.15	0.50	0.06	0.07	0.39	
1,1,1-Trifluoro-*N*-[4-[(2-hydroxyphenyl)sulfonyl]-2-methylphenyl]methanesulfonamide	2-Hydroxyperfluidone	0.35	0.14	0.42	0.04	0.05	0.37	
1,1,1-Trifluoro-*N*-[4-[(3-hydroxyphenyl)sulfonyl]-2-methylphenyl]methanesulfonamide	3-Hydroxyperfluidone	0.25	0.12	0.36	0.04	0.04	0.37	
1,1,1-Trifluoro-*N*-[4-[(4-hydroxyphenyl)sulfonyl]-2-methylphenyl]methanesulfonamide	4-Hydroxyperfluidone	0.21	0.11	0.32	0.04	0.05	0.38	
1,1,1-Trifluoro-*N*-[3-hydroxy-2-methyl-4-(phenylsulfonyl)-phenyl]methanesulfonamide	3′-Hydroxyperfluidone	0.49	0.15	0.53	0.07	0.08	0.37	
1,1,1-Trifluoro-*N*-[5-hydroxy-2-methyl-4-(phenylsulfonyl)-phenyl]methanesulfonamide	5′-Hydroxyperfluidone	0.35	0.11	0.44	0.03	0.05	0.35	
2-Methyl-1-nitro-4-(phenylsulfonyl)benzene	Nitroperfluidone	0.57	0.62	0.59	0.56	0.58	0.61	
2-Methyl-4-(phenylsulfonyl)-benzenamine	Aminoperfluidone	0.27	0.45	0.38	0.32	0.42	0.53	
2-Nitro-5-(phenylsulfonyl)-benzoic acid	2′-Carboxyperfluidone	0.21	0.02	0.25	0.01	0.01	0.17	
3-Methyl-4-[[(trifluoromethyl)-sulfonyl]amino]benzenesulfonic acid	Perfluidone sulfonic acid	0.0	0.0	0.0	0.0	0.0	0.0	0.62

[a] Brinkman Silplate F-22 (0.25 mm gel thickness). [b] Solvent systems as follows: (A) benzene (HCOOH)–ether (5:1); (B) hexane–ethyl acetate–methanol (2:2:1); (C) benzene (HCOOH)–ethyl acetate–chloroform (1:1:1); (D) ether; (E) benzene–acetone (2:1); (F) ethyl acetate–acetic acid (49:1); (G) butanol–acetic acid–water (3:1:1).

moiety (Trepka et al., 1970, 1974); thus, sample acidification resulted in suppression of ionization and more ready partitioning of radiocarbon into the organic phase. Extraction and analysis of nonacidified urine and feces samples indicated that acid treatment did not result in chemical degradation of perfluidone or its metabolites.

Isolated perfluidone metabolites suspected to be conjugates were analyzed by the following procedure. The products were isolated by TLC, eluted from the gel with methanol, and subsequently incubated with a mixture of glucuronidase–arylsulfatase (Calbiochem, Los Angeles, Calif.). Incubations were for 24 hr at 37° in 5.0 ml of sodium acetate buffer (0.1 *M*, pH 4.5) with enzyme activity of 50,000 Fishman units and 5000 Whitehead units. After incubation, the mixture was extracted with ether and the ether phase was quantitated by lsc and analyzed by TLC.

Thin-Layer Chromatography. TLC was used to resolve perfluidone metabolites in milk, urine, and feces. The plates were Brinkman precoated chromatoplates (Silplate-F22, 0.25 mm gel thickness). Routine metabolite separations were made by developing the plates in solvent mixture A (Table I). Radioactive gel regions were detected by radioautography; quantitation was by lsc. Metabolite cochromatography studies were conducted by mixing the appropriate metabolite standard (Table I) with the radioactive metabolite and developing two-dimensionally in six solvent systems (A–F, Table I). The position of metabolite standards on the plate was determined by viewing under short-wavelength ultraviolet light. Identical chromatographic behavior of the ^{14}C-labeled metabolite and authentic standard in each of the six solvent systems constituted tentative metabolite characterization.

Gas–Liquid Chromatography/Mass Spectrometry. Metabolite identifications on TLC were confirmed by gas–liquid chromatography (GLC)/mass spectral studies. The instrument was a Varian-Mat-CH-7 spectrometer coupled with a Varian 2700 gas chromatograph and 620 L Varian computer. Compounds were resolved on a 1.8 m × 2 mm (i.d.) glass column packed with 3% AN-600 on 80–100 mesh Gas-Chrom Q. Operating parameters were as follows: injector, 290°; column, 240–260°; detector oven, separator and inlet, 300°; ion source, 275°; helium flow, 50 ml/min; ionizing voltage, 70 eV.

Radioactive products isolated by TLC were taken up in ether solution and allowed to react with diazomethane to generate *N*-methyl or aryl-*O*-methyl derivatives. An acetone solution of the derivatized products was then injected on column. Methylated perfluidone was analyzed at a column temperature of 240°; its derivatized metabolites were run at 260°. Identical GLC retention times and mass spectra of the derivatized radioactive metabolites and standard compounds from syntheses were considered proof of structure.

RESULTS AND DISCUSSION

Excretion. Radiocarbon was rapidly and almost quantitatively excreted after oral treatment of the cow with perfluidone-^{14}C (Figure 1). The urine and feces were the major pathways for radiocarbon elimination, and only about 0.1% of the total dose was recovered in milk. Elimination was al-

Table II. Radioactive Products in Urine, Milk, and Feces after Oral Treatment of a Lactating Cow with Perfluidone-^{14}C at 5.0 mg/kg

	Days after treatment	Radiocarbon in indicated sample as each product, %						
		Origin[a]	Unknown[b]	4-Hydroxy-perfluidone	3-Hydroxy-perfluidone	Perfluidone	Water solubles	Unextractable
Urine	1	0.6	0	4.1	0.4	94.9	0	
	2	0.6	0	5.0	0.4	94.0	0	
	3	1.4	0	7.3	0.6	90.7	0	
	4	0	0	11.4	1.2	87.4	0	
	5	0	0	10.1	0.6	89.3	0	
Milk[c]	1	0	0	5.2	0	94.8	0	
Feces	1	0	0	18.2	26.6	20.0	1.1	34.1
	2	1.9	3.1	22.6	23.3	22.2	3.2	23.7
	3	0	0.5	18.8	19.2	16.2	1.6	43.7
	4	0	0	24.2	20.5	19.2	0	36.1
	5	0	0	33.4	27.9	24.2	0	14.5

[a] Product(s) remaining at the origin following chromatoplate development in solvent system A (Table I). [b] R_f 0.15 in solvent system A (Table I). [c] Including cream and aqueous phases (see text).

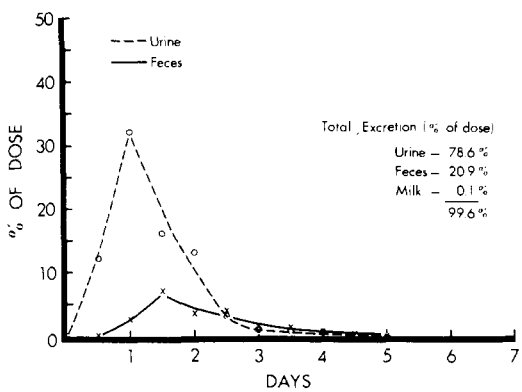

Figure 1. Radiocarbon excretion after oral treatment of a lactating cow with perfluidone-^{14}C at 5.0 mg/kg.

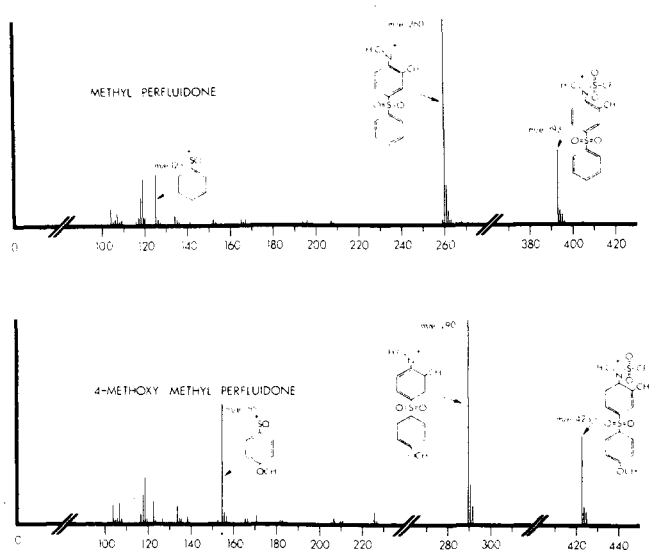

Figure 2. Electron impact mass spectra of perfluidone and its major metabolite after methylation and GLC–mass spectral analysis.

most total within 4 days after treatment, and radioactive residues could not be detected in urine and feces after 6 days. Detectable residues were found in milk in only the 12- and 24-hr samples.

Combustion analysis of approximately 20 tissues collected when the animal was sacrificed 7 days after treatment confirmed that radiocarbon excretion was total or essentially so after perfluidone-^{14}C administration. None of the tissue samples analyzed contained detectable radiocarbon residues. The sensitivity limit of the combustion procedure used was 0.03 ppm.

Metabolite Characterization. Ethyl acetate extraction of urine samples after acidification resulted in quantitative recovery of radiocarbon in the organic phase. TLC analysis of the urinary radiocarbon revealed four radioactive products, of which the major one corresponded on TLC to perfluidone (Table II). The unmetabolized parent compound comprised about 90–95% of the total ^{14}C-labeled residues in all urine samples analyzed. The major metabolite cochromatographed in six solvent systems with 1,1,1-trifluoro-*N*-[4-[(4-hydroxyphenyl)sulfonyl]-2-methylphenyl]methanesulfonamide (4-hydroxyperfluidone). The two additional urinary metabolites observed were 1,1,1-trifluoro-*N*-[4-[(3-hydroxyphenyl)sulfonyl]-2-methylphenyl]-methanesulfonamide (3-hydroxyperfluidone), which appeared in all samples, and a product remaining at the origin on TLC, which was not seen in samples taken later than 3 days posttreatment.

The urinary radiocarbon remaining at the origin after TLC development consisted primarily of conjugates of 4-hydroxy- and 3-hydroxyperfluidone. Incubation of this metabolite from the 1-day urine sample with glucuronidase–arylsulfatase yielded 48% 4-hydroxyperfluidone and 38% 3-hydroxyperfluidone, and 14% remained unextractable from the water phase. Incubation of the urinary radioactivity remaining at the origin on TLC with enzyme that had been deactivated by boiling yielded no significant conversion of radioactivity into ether-soluble compounds.

The exceedingly small quantities of radiocarbon which appeared in milk after perfluidone treatment required the analysis of a relatively large milk sample. When 2 l. of milk from the combined 1-day sample was separated into aqueous and cream phases, about 35% of the total milk radioactivity remained with the cream. These products, however, transferred to acetonitrile when the cream was partitioned between hexane and acetonitrile, and TLC analysis showed only two radioactive compounds, unmetabolized perfluidone (98.4%) and 4-hydroxyperfluidone (1.6%). Radiocarbon in the aqueous milk phase also consisted totally of perfluidone (92.8%) and its 4-hydroxy metabolite (7.2%).

Radioactivity was not readily extractable from feces samples, and about 14–44% of the total feces radiocarbon remained with the residue after the extraction procedure

(Table II). In the organic phase, unmetabolized perfluidone and its 3-OH and 4-OH metabolites were present in all samples analyzed in roughly equal amounts. Two additional metabolites, the material at the origin and a minor product at R_f 0.15 (solvent system A), were found only in small quantity. They did not co-chromatograph with any of the available metabolite standards and were not studied further.

GLC–mass spectral studies with the radioactive products identified by TLC (perfluidone and 3-hydroxy- and 4-hydroxyperfluidone) confirmed their chemical identity. Authentic perfluidone, when methylated and analyzed by GLC–mass spectroscopy, gave an identical GLC retention time (4.9 min) and mass spectrum (Figure 2) as the methylated perfluidone-^{14}C recovered from samples of urine, milk, and feces. Similar studies with 3-hydroxy- and 4-hydroxyperfluidone-^{14}C isolated from urine, milk, and feces confirmed their chemical identity with the respective standards from synthesis. GLC retention times were 4.2 min for methylated 3-hydroxyperfluidone and 5.7 min for 4-hydroxyperfluidone. As expected, the methylated derivatives of the two isomeric perfluidone metabolites exhibited essentially identical mass spectra, but distinction between the two products could be made with certainty when their TLC and GLC behavior was considered.

Although perfluidone has a high degree of resistance to metabolic attack by ruminants, the absorbed compound is nonetheless rapidly excreted, and the current study suggests that perfluidone will likely show little or no tendency toward retention or accumulation in tissues. Of perhaps equal importance was the observation that perfluidone and its metabolic products are not secreted into the milk to any appreciable extent.

The appearance of perfluidone and its hydroxylated metabolites in urine in a predominantly nonconjugated form indicates that conjugation mechanisms are not involved to any appreciable extent in the ruminant metabolism of this compound. Enzymatic cleavage of the trifluoromethylsulfono moiety of perfluidone did not occur to any detectable degree; hydroxylation of the phenylsulfonyl ring was the only metabolic pathway of significance. Paulson et al. (1973) found that in rats unmetabolized perfluidone was by far the major product excreted in urine after oral treatment with perfluidone-^{14}C. The 3-OH metabolite was observed in feces, but 4-hydroxyperfluidone was not.

The current studies and those by Paulson et al. (1973) strongly suggest that most perfluidone residues in tissues of animals exposed to perfluidone in the environment will be in the form of unaltered parent compound or its 3-OH or 4-OH metabolites. If such is the case, GLC–mass spectroscopy should prove useful in confirming perfluidone and its metabolites at the residue level. The mass spectra of methylperfluidone and the dimethyl derivatives of 3-hydroxy- and 4-hydroxyperfluidone (Figure 2) give characteristic and intense molecular and base peak ions at relatively high m/e values. In the studies reported here, GLC–mass spectroscopy confirmed the identity of the single perfluidone metabolite in milk (4-hydroxyperfluidone) even though its concentration in the whole milk sample analyzed was only 0.005 ppm. Residues of these compounds can likely be studied at levels considerably lower than those encountered here, given the high sensitivity attainable in GLC–mass spectroscopy and the intense, diagnostic ions generated on electron impact of derivatized perfluidone and its major metabolites.

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