Fate of the Plant Growth Regulator Mefluidide [*N*-[2,4-Dimethyl-5-[[(trifluoromethyl)sulfonyl]amino]phenyl]acetamide] in a Cow and Sheep

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The plant growth regulator mefluidide [*N*-[2,4-dimethyl-5-[[(trifluoromethyl)sulfonyl]amino]phenyl]-acetamide], in radiolabeled form, was administered as 5 consecutive daily oral doses to a cow and a sheep. The compound was rapidly absorbed and rapidly excreted by the animals; elimination was primarily through the urine and almost totally in the form of unmetabolized mefluidide. No detectable radiocarbon residues were secreted into the milk of the mefluidide-treated cow, and retention of radiocarbon residues by the tissues of both the cow and the sheep was minimal. Results in these studies suggest that potential interactions of mefluidide with ruminants from its use in agriculture will result neither in toxicological hazards to the exposed animals nor in contamination of meat or milk used for human consumption.

The plant growth regulator mefluidide, *N*-[2,4-dimethyl-5-[[(trifluoromethyl)sulfonyl]amino]phenyl]acetamide, has potential for several agricultural and nonagricultural uses. These include use on certain pasture grasses to improve forage quality by suppressing seed head formation and increasing digestibility, sugar, and protein content (Glenn et al., 1980), the treatment of sugarcane to facilitate ripening and increase yields of recoverable sugar (Gates, 1975; Hagman et al., 1977; Zamora and Rosario, 1977; Rostron, 1977), use as a herbicide to control Johnson grass and certain other problem weeds in soybeans and peanuts (Gates, 1975, 1976; Gruenhagen et al., 1975; Harrison et al., 1976), use on small grains, including wheat, to increase seed yields (Miles et al., 1977), and use to suppress vegetative growth of trees and woody ornamentals (Gates, 1975). Mefluidide has several other potential applications that have been summarized by Truelove et al. (1977).

Certain of the proposed mefluidide uses may result in exposure of livestock to its residues through the consumption of feeds derived from treated crops or forage or from the consumption of contaminated field or runoff waters. It is therefore prudent to evaluate the interactions of mefluidide with food-producing ruminants. The current studies were undertaken to define the fate of mefluidide in a cow and a sheep, particularly its metabolic behavior and potential for secretion of residues into milk and their retention by edible tissues.

MATERIALS AND METHODS

Chemicals. Uniformly ^{14}C ring labeled mefluidide (>98% radiochemical purity) was provided for these studies by the 3M Co., St. Paul, MN. Before administration to the test animals, the specific activity of the ^{14}C-labeled preparation was adjusted by the addition of nonradioactive technical-grade mefluidide (>99% purity) to appropriate levels (vide infra). In addition to mefluidide, three nonradioactive mefluidide analogues were provided by the 3M Co. for comparative studies as possible mefluidide metabolites. These included *N*-(2,4-dimethyl-5-aminophenyl)acetamide, 2,4-dimethyl-5-[[(trifluoromethyl)sulfonyl]amino]aminobenzene, and 2,4-dimethyl-1,5-diaminobenzene.

Animals. A 352-kg lactating Jersey cow, producing ~10 kg of milk daily, was obtained directly from the milking herd of a local dairy. The animal was catheterized and held in a metabolism stanchion throughout the study period. It was provided coastal Bermuda grass hay and water ad libitum and was fed ~2 kg of dairy concentrate ration just before each milking. The cow was milked at 12-h intervals with a pneumatic milking machine.

A 64-kg castrated male (wether) sheep of mixed breed was obtained from a flock maintained at this laboratory. The animal was held in a metabolism stanchion that permitted separate collection of urine and feces without catheterization. The sheep was provided hay and water ad libitum and was fed ~0.5 kg of a commercial sheep concentrate ration twice daily.

Treatment. The [^{14}C]mefluidide treatments of the cow and the sheep consisted of administering 5 equal doses of the radiochemical to the animals daily at 24-h intervals. The appropriate amounts of [^{14}C]mefluidide plus nonradioactive mefluidide were mixed with a small amount of the appropriate concentrate ration and then were orally administered in gelatin capsules with a balling gun. The cow was treated with daily doses of 35.2 mg of total mefluidide [0.1 mg of mefluidide (kg of body weight)$^{-1}$ day^{-1}], and the specific activity of the preparation was ~10 500 dpm/μg. The sheep was treated similarly, except that the daily dosage was 64 mg of total mefluidide/day [1.0 mg of mefluidide (kg of body weight)$^{-1}$ day^{-1}], with a specific activity of ~6500 dpm/μg.

Sample Collection and Analysis. After initiation of the [^{14}C]mefluidide treatments, total urine and feces samples were collected at 24-h intervals. Aliquots (0.2 mL) of freshly collected urine were subjected to liquid scintillation counting (LSC) for quantitation of the radiocarbon present, and larger aliquots of each sample were frozen in plastic bags for later analysis. Fecal samples were mixed thoroughly (water was added to the fecal samples from the sheep to facilitate softening and subsequent mixing), and then several aliquots (1.0 g wet weight) were removed from each sample for subsequent analysis by oxygen combustion. The [^{14}C]carbon dioxide resulting from combustion was bubbled through a trapping solution of equal parts of

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Table I. Radiocarbon Elimination by a Cow and a Sheep Treated Orally with [^{14}C]Mefluidide for 5 Consecutive Days[a]

	radiocarbon eliminated, cumulative % of total administered ^{14}C, at indicated day after first treatment								
sample	1	2	3	4[b]	5	6	7	8	9
cow[c]									
urine	14.4	32.3	51.9	70.1	88.6	92.3	92.9	93.1	93.2
feces	0.4	1.6	4.1	5.8	7.7	9.1	9.4	9.6	9.7
milk[d]	0	0	0	0	0	0	0	0	0
sheep[e]									
urine	11.8	25.7	43.0	69.7	83.2[f]				
feces	0.2	0.7	1.3	1.8	2.0[f]				

[a] Dosage levels were 0.1 and 1.0 mg of mefluidide (kg of body weight)$^{-1}$ day^{-1} for the cow and sheep, respectively. [b] Fifth and final dose administered. [c] Cow sacrificed 5 days after the final [^{14}C]mefluidide dose. [d] No milk samples contained detectable ^{14}C residues. [e] Sheep sacrificed 0.5 day after the final [^{14}C]mefluidide dose. [f] Final samples collected 0.5 0.5 day after the final [^{14}C]mefluidide dose.

Carbsorb II (Packard) and scintillation cocktail and then was quantitated by LSC (Oehler and Ivie, 1980). Appropriate corrections were made for combustion and counting efficiency and for quench. Larger fecal samples were held frozen. The cow was milked every 12 h throughout the study, and aliquots (0.2–1.0 mL) of the fresh milk samples were analyzed by LSC. Several 100-mL portions of milk from each sample were frozen for possible later analysis.

The cow was killed 9 days after the first [^{14}C]mefluidide dose (5 days after the fifth and final dose) was administered. Numerous tissue samples were collected at that time; small portions (up to 1.0 g) were excised and dried for combustion analysis, and larger portions were held frozen. The sheep was killed 12 h after the fifth and final [^{14}C]mefuidide dose, and tissue samples were collected and processed similarly to those from the cow.

Urine samples from the cow and the sheep either were spotted directly on thin-layer chromatographic (TLC) plates for resolution of the ^{14}C-labeled constituents present or were adjusted to pH 1–2 with HCl and then extracted 4 times with equal volumes of ethyl acetate. Radiocarbon in organic and aqueous phases was quantitated by LSC; then the organic phases were dried over anhydrous Na_2SO_4, concentrated, and subjected to TLC analysis. Fecal samples (5 g) were extracted with acetonitrile or methanol by blending with a Polytron homogenizer, followed by centrifugation and decanting off the extract. The residue was extracted 4 additional times, then the radiocarbon in each phase was quantitated by an appropriate technique (LSC or combustion analysis), and the extracts were analyzed by TLC. Tissue samples (5 g) that contained sufficient ^{14}C-labeled residues for more detailed analyses were extracted by blending with acetonitrile and then combining the extracts (total of 5), followed by removal of lipids by partitioning with hexane. Radiocarbon in the liquid fractions was quantitated by LSC, and that in the extracted tissue residues by oxygen combustion. The acetonitrile phases were then subjected to TLC analysis.

None of the milk samples from the [^{14}C]mefluidide-treated cow contained detectable ^{14}C-labeled residues (vide infra); thus, these samples were not further analyzed.

Chromatography. Radiocarbon-labeled components in urine, feces, and tissues were resolved by TLC with Brinkman precoated silica gel plates (Silplate F-22, 0.25 mm thick, with fluorescent indicator). The ^{14}C-labeled constituents in these samples were resolved by developing the plates in one or more of the following solvent systems: 1 (ethyl acetate–acetic acid, 49:1); 2 (chloroform–methanol–acetic acid, 45:5:1); 3 (benzene saturated with formic acid–ethyl acetate–chloroform, 1:2:1); 4 (benzene saturated with formic acid–ether, 1:1); 5 (benzene–ethyl acetate methanol, 15:5:1); 6 (benzene–acetone–chloroform, 2:1:1). After development of the plates in an appropriate solvent system(s), ^{14}C-labeled components were detected by radioautography (Kodak No-Screen X-ray film).

Radiolabeled components from extracts of urine, feces, or tissues from the [^{14}]mefluidide-treated animals were identified by direct TLC cochromatography with the authentic nonradioactive standards available. The positions of the nonradioactive compounds on the plate were determined by viewing under short-wavelength ultraviolet light, and positions of the ^{14}C-labeled compounds were determined by radioautography. Cochromatography in each of the six solvent systems studied was considered to be sufficient evidence of ^{14}C-labeled compound identity.

RESULTS

Excretion and Retention of Radiocarbon Residues. Treatment of the cow and the sheep with successive daily doses of [^{14}]mefluidide was followed by rapid excretion of radiocarbon in each animal, primarily through the urine (Table I). Within 1 day after the fifth and final [^{14}C]-mefluidide treatment of the cow, ~95% of the total administered ^{14}C had been eliminated in the urine and feces. At the time the cow was sacrificed, 5 days after the final dose, essentially all of the administered radiocarbon had been eliminated in the urine and feces (Table I). LSC analysis of milk collected throughout the study showed that detectable levels of radiocarbon were not present in any milk sample (Table I).

Radiocarbon excretion patterns after treatment of the sheep with [^{14}C]mefluidide were similar to those of the cow, except that the sheep eliminated less of the dose through the feces (Table I). At the time the sheep was sacrificed, 12 h after the final [^{14}C]mefluidide dose, ~85% of the administered radiocarbon had been eliminated in the urine and feces.

Although numerous tissue samples were collected from the cow 5 days after the final [^{14}C]mefluidide dose, only two (kidney and liver) contained detectable levels of radiocarbon. Residues in these two tissues corresponded to 0.005 ppm of mefluidide equivalents, whereas other samples analyzed (brain, fat, heart, muscle, ovary, skin, spleen, tongue, and udder) did not contain ^{14}C-labeled residues of sufficient magnitude to be detected. The sensitivity limit of the combustion procedure used was 0.005 ppm of mefluidide equivalents. Each of the analyzed tissues from the sheep, which received a 10-fold higher level of [^{14}C]-mefluidide than the cow and was sacrificed only 12 h after the final dose, contained quantifiable levels of radiocarbon. Residues were highest in kidney (0.76 ppm of mefluidide equivalent) and liver (0.39 ppm), and levels in other tissues were as follows: brain (0.03); fat (0.02); heart (0.06); lung (0.30); muscle (0.08); spleen (0.16); tongue (0.21).

Characterization of Residues. Analysis of ^{14}C-labeled residues in urine and feces of the mefluidide-treated animals showed that mefluidide is eliminated essentially unchanged by these animals. Ethyl acetate extraction of

acidified cow urine collected 1, 3, or 5 days after initiation of the [^{14}C]mefluidide treatments gave >98% partitioning of the radiocarbon into the organic phase, and TLC analysis of these extracts showed that the radiocarbon present was in each sample almost totally (>98%) mefluidide. As many as six other radioactive components were present in the urine extracts in trace amounts, and one of these cochromatographed with 2,4-dimethyl-5-[[(trifluoromethyl)sulfonyl]amino]aminobenzene. However, this product comprised only ~0.1–0.2% of the total radiocarbon in any sample, and the product may have resulted from an impurity in the [^{14}C]mefluidide administered to the cow. In the sheep, the administration of a considerably larger amount of [^{14}C]mefluidide resulted in urine residues of sufficient magnitude to permit direct analysis of whole urine by TLC. These analyses showed that in all samples, mefluidide was the only ^{14}C-labeled component present in sufficient amounts to be detected.

Acetonitrile or methanol extraction of fecal samples from the [^{14}C]mefluidide-treated cow and sheep recovered 63–85% of the radiocarbon present. On the basis of TLC analysis, extracts of sheep feces contained only mefluidide. In the cow, >75% of the extracted fecal ^{14}C was mefluidide, but as much as 15% of the ^{14}C remained at the origin after TLC in solvent systems 1 and 2. Two additional ^{14}C-labeled products were present in the fecal extracts of the cow in minor (2–6%) amounts. These products did not chromatograph with any of the compounds of known structure available, and they were not studied further because of both their availability in only small amounts and the large amounts of interfering material present in the fecal extracts.

Although none of the tissue samples from the [^{14}C]mefluidide-treated cow contained sufficient levels of radiocarbon to permit further study, sheep kidney, liver, and muscle samples were extracted and subjected to TLC analysis. Acetonitrile extraction recovered approximately 97% (kidney), 72% (liver), and 80% (muscle) of the total radiocarbon in these samples, and subsequent cleanup of the extracts with hexane removed only 10% or less of the radiocarbon in the samples. TLC analysis of the acetonitrile fractions showed that mefluidide was the only ^{14}C-labeled component detectable in samples of each of the tissues studied.

DISCUSSION

Results in the current studies show that the plant growth regulator mefluidide readily crosses the gastrointestinal barrier after oral administration to ruminants and is then rapidly excreted, unmetabolized, in the urine. Mefluidide probably is excreted by the kidneys without difficulty because it is an acidic compound ($pK \sim 4.5$) due to the (trifluoromethyl)sulfonyl moiety, and it thus exists largely in the ionized state at physiological pH.

Studies with the cow suggest that mefluidide may be subjected to at least a minor degree of metabolism, although the very low levels of possible metabolites seen in the urine of the treated animal might be attributed to impurities (or their metabolites) present in the [^{14}C]mefluidide sample given to the cow. More likely, the higher levels of mefluidide transformation products seen in fecal samples of the cow arose through legitimate metabolic transformations, presumably microbial in origin.

There was little tendency for mefluidide to be secreted into the milk, as evidenced by the fact that none of the milk samples analyzed contained detectable radiocarbon residues. On the basis of sensitivity levels for radiocarbon detection in milk and the amount of milk produced during the study, the total secretion of mefluidide into milk of the treated cow did not exceed 0.0003% of the total administered radiocarbon.

Although residues of mefluidide occur in tissues of a sheep sacrificed shortly after cessation of mefluidide exposure, residues are in all tissues low and are primarily if not totally in the form of the unmetabolized parent compound. Further, studies with the cow indicate that such residues are rapidly eliminated and are not retained in appreciable amounts by edible tissue.

On the basis of the current studies, and upon previous determinations that mefluidide is low in toxicity in mammals (Bandal, 1980), it appears most likely that low-level oral exposure of ruminants to this plant growth regulator will not result in a toxicological hazard to the exposed animals or in appreciable contamination of meat or milk used for human consumption.

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