Metabolism and Distribution of the Experimental Triazolone Herbicide F6285 [1-[2,4-Dichloro-5-[*N*-(methylsulfonyl)amino]phenyl]-1,4-dihydro-3-methyl-4-(difluoromethyl)-5*H*-triazol-5-one] in the Rat, Goat, and Hen

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The metabolic fate and distribution of the experimental triazolone herbicide F6285 in the rat, goat, and hen were studied. Oral doses of ^{14}C-radiolabeled F6285 administered to rats (10 mg/kg), goats (2 mg/kg, 300 ppm in diet daily, 5 days), or hens (3 mg/kg, 45 ppm in diet daily, 7 days) were quantitatively excreted in the urine, feces, or hen excreta. In all of the species studied, unchanged F6285 (<2%) and two nonconjugated metabolites were found. These metabolites were isolated and characterized by MS or NMR as the 3-hydroxymethyl derivative of F6285 (I) (88–95%) and the corresponding 3-carboxylic acid (III) (0.3–5%). The latter decomposed at high temperature or acidic pH to give the corresponding 3-demethyl compound (IV). In the rat, a minor metabolite, tentatively characterized as the 2,3-dihydro-3-hydroxymethyl derivative of F6285 (II) (0.5–5%), was also detected. Minimal residues were found in edible animal tissues, milk, and eggs.

F6285 [1-[2,4-dichloro-5-[*N*-(methylsulfonyl)amino]-phenyl]-1,4-dihydro-3-methyl-4-(difluoromethyl)-5*H*-triazol-5-one] (Figure 1) is a new and novel preemergence herbicide that provides broad-spectrum grass and broad-leafed weed control in soybeans. This compound is under consideration for development by the Agricultural Chemical Group of FMC Corp. To gain an understanding of the comparative metabolism of this herbicide in animals, a series of studies was conducted in rats, goats, and poultry. This paper summarizes the metabolic behavior of F6285 in these species.

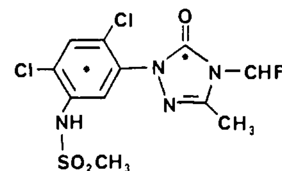

Figure 1. Structure and positions of ^{14}C radiolabels (indicated by asterisks) of F6285.

MATERIALS AND METHODS

Chemicals. F6285, uniformly labeled with ^{14}C either in the phenyl ring (radiochemical purity, 96.2%; specific activity, 20.1 mCi/mmol) or in the carbonyl carbon of the triazolinone ring (radiochemical purity, 94.0%; specific activity, 24.0 mCi/mmol), was synthesized by NEN (Boston, MA). The ^{14}C-carbonyl-labeled compound was further purified by HPLC to obtain a final radiochemical purity of 99.0% before use. Radiochemical purity was determined by TLC and autoradiography. Nonradiolabeled F6285 used for isotopic dilution was synthesized by FMC Corp. (Theodoridis, 1985). Other synthetic standards also prepared at FMC Corp. and used in this study included 1-[2,4-dichloro-5-[*N*-(methylsulfonyl)amino]phenyl]-1,4-dihydro-4-(difluoromethyl)-5-oxo-5*H*-triazol-3-carboxylic acid (F6285-3-carboxylic acid) [CIMS, m/z 373 (100.0%), 375 (68.5%); EIMS, m/z 372 (60.4%), 374 (45.9%), 293 (100.0%), 295 (74.0%); ^{1}H NMR (acetone-d_6) δ 3.15 (s, 3 H), 7.65 (t, 1 H, J = 58 Hz), 7.80 (s, 1 H) 7.88 (s, 1 H), 8.48 (s, 1 H)] and 1-[2,4-dichloro-5-[*N*-(methylsulfonyl)amino]-phenyl]-1,4-dihydro-4-(difluoromethyl)-5*H*-triazol-5-one (3-demethyl-F6285) [CIMS, m/z 373 (100.0%), 375 (67.4%); EIMS, m/z 372 (52.7%), 374 (36.6%), 293 (100.0%), 295 (64.2%); ^{1}H NMR ($CDCl_3$) δ 3.08 (s, 3 H), 7.0 (t, 1 H, J = 58 Hz), 7.62 (s, 1 H), 7.80 (s, 1 H), 7.90 (s, 1 H), 6.9 (s, 1 H)]. Solvents used for chromatography and extractions were of HPLC grade and were purchased from J. T. Baker (Phillipsburg, NJ).

Animal Dosing and Sample Collection. The in-life phase of the rat metabolism study was conducted at Xenobiotic Laboratories, Inc., Princeton, NJ. The corresponding phases of the goat and hen metabolism studies were conducted at Biological Test Center, Irvine, CA. Biological samples (urine, feces, organs) generated by these studies were shipped frozen at −78 °C to FMC Corp. for further analyses.

For the rat study, Sprague-Dawley rats (BR/Charles River Laboratory, Inc.) (150–250 g) were used. Two males and two females were administered oral doses of [*carbonyl*-^{14}C]- and [*phenyl*-^{14}C]-F6285 (10 mg/kg, 10 μCi/animal) in corn oil (0.75 mL), respectively. The animals were housed in glass metabolism cages and supplied with certified Purina Rodent Chow and water ad libitum. Urine and feces were collected under dry ice every 24 h for 72 h. Expired CO_2 was trapped in a solution of ethanolamine/2-ethoxymethanol (1:1 v/v) and was collected at 24, 48, and 72 h after dosing. In another experiment, groups of five male and five female rats were given oral doses of [*carbonyl*-^{14}C]- and [*phenyl*-^{14}C]-F6285 (100 mg/kg, 10 μCi/animal), respectively. Animals were housed in stainless steel cages. Urine and feces were collected under dry ice at 12, 24, and 48 h. The dose regimen was repeated, and excreta samples were again collected for another 48 h. No CO_2 monitoring was performed. Collected samples were stored at −20 °C until analysis. The purpose of the latter high-dose experiment was to generate a sufficient quantity of metabolites for identification purposes.

For the goat study, two lactating diary goats (identified as the black (42 kg) and the brown (43 kg) goat, Power's Place, Anza, CA) in good milk production were each administered oral doses of [*phenyl*-^{14}C]-F6285 (90 mg, 200 μCi/animal) in gelatin capsules daily for 5 consecutive days. This dose rate was equivalent to a dietary intake of ca. 90 ppm on the basis of an average daily feed intake of 1000 g/goat. Animals were housed in individual metal metabolism stanchions and were given a 1.5-kg daily ration consisting of goat pellets (O. H. Kruse, El Monte, CA) and hay cubes which were divided and fed twice daily. Actual feed consumption of each goat was monitored. Water was provided ad libitium. The goats were catheterized at day 0 for the collection of urine. Feces and urine (freeze-trapped) were collected daily, while milk was collected twice daily. The animals were sacrificed

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approximately 24 h after the last dose. Whole organs (kidney, liver) and approximately 200 g of selected tissues (renal and omental fat, leg and flank muscle) were removed. All samples were stored at -20 °C until anaylsis.

For the poultry study, 10 white leghorn laying hens (McWilliams Egg Ranch, Mira Loma, CA) (1.3-1.6 kg) were each given oral doses of [*phenyl*-^{14}C]-F6285 (4 mg, 20 μCi) daily in gelatin capsules for 7 days. This dose rate was equivalent to a dietary intake of 40 ppm on the basis of an average daily feed consumption of 100 g/hen. The birds were individually housed in laying cages and were provided with O. H. Cruse Cage Layer Chow and water ad libitium. Actual feed consumption of each hen was measured. Eggs and excreta were collected daily for 7 days. The birds were sacrificed approximately 24 h after the final dose. Whole livers and selected tissues (pectoral and adductor muscle, fat) were removed. Samples were stored at -20 °C until analysis.

Analytical Procedures. Samples were combusted on a Harvey biological sample oxidizer (Model OX-300, Harvey Instrument, Hillsdale, NJ). Thin-layer chromatography (TLC) was performed on analytical or preparative silica gel TLC plates (silica gel 60, F254, 20 × 20 cm, 0.25 or 2 mm, Merck). Preparative samples were applied with a TLC streaker (Alltech Associates, Deerfield, IL). Solvent systems used for TLC were methylene chloride/methanol/ammonium hydroxide 75:25:1 (system A) or 85:15:1 (v/v/v) (system B). Autoradiograms were developed by using Kodak diagnostic films (X-Omat). High-performance liquid chromatography (HPLC) was performed on either a Beckman System Gold HPLC system equipped with a programmable solvent Module 116, a programmable detector Module 166 UV (set at 254 nm), and a Model 171 radioisotope detector or a Waters 600E Powerline multisolvent delivery system with a 990 photodiode array detector, a U6K injector, and a Radiomatic A-110 Flo-one radioactive flow detector. Analyses were performed by using a Du Pont Zorbax ODS column (5 μm, 4.6 mm × 25 cm) equipped with a Brownlee Labs RP-18 guard column (5 μm, 4.6 mm × 3 cm). Gradient elution programs of acetonitrile/water/acetic acid ranging from 25:75:0.4 to 60:40:0.4 in 45 min (system 1) or from 0:100:0.4 to 45:55:0.4 in 45 min (system 2) at 1 mL/min were employed. For semipreparative HPLC, a Du Pont Zorbax C8 column (10 μm, 9.4 mm × 25 cm) using system 1 at 3 mL/min was employed. Eluents from the HPLC column were collected with a Pharmacia Frac-100 fraction collector. Samples for HPLC analysis were filtered with microfilterfuge tubes with 0.45-μm nylon 66 membrane filters and a Tomy HF-120 microcentrifuge (Rainin Instrument Co. Inc., Woburn, MA). Liquid scintillation counting was performed on a Beckman LS 5801 or on a Packard Tri-carb 2200CA liquid scintillation counter. Mass spectra were obtained on a VG 7070 EQ or a Finnigan Incos 50 instrument. Analyses were performed in the electron impact (EI) or chemical ionization (CI) (isobutane) mode. Proton nuclear magnetic resonance spectra (NMR) were obtained on a General Electric NT-300 instrument.

Radioanalysis. Feces, hen excreta, and tissues were homogenized in liquid nitrogen with a Tekmar (Cincinnati, OH) Model A-20 grinder. Sample aliquots (50-200 mg) were combusted. Evolved $^{14}CO_2$ was trapped in carbon-14 cocktail (Harvey Instrument) and counted by liquid scintillation counting. Eggs were separated into whites and yolks, blended, and combusted similarly (100-mg aliquots). Detection limits were 0.002 ppm for goat tissue, 0.004 ppm for fat, 0.0005 ppm for poultry tissue, 0.001 ppm for egg. Aliquots of urine, milk, and CO_2 trapping solutions (0.1-1 mL) were counted directly in Insta-gel cocktail (Packard Instrument, Dowers Grove, IL) or in Hydrocount cocktail (J. T. Baker). Counting was performed for 10 min or until a statistical accuracy of ±2% was obtained.

Analysis of Urine. Rat (0-24 h) and brown goat urine samples (days 2 and 3) were partitioned three times with equal volumes of ethyl acetate. The aqueous layers were separated, acidified with 36% HCl to pH 1, and partitioned again three times with equal volumes of ethyl acetate to remove radiolabeled residues with acidic functionalities. Significant radiolabeled residues that remained in the acidic aqueous layers were heated under reflux for 2 h followed by partitioning with ethyl acetate to release heat-labile conjugates. The respective ethyl acetate fractions were combined, dried (anhydrous Na_2SO_4), and concentrated in vacuo. Aliquots of the samples were evaporated under nitrogen to near dryness, reconstituted with acetonitrile (200-400 μL), and subjected to TLC and HPLC analyses.

Analysis of Milk. Milk samples (280 mL, days 4 and 5) from the brown goat were used for extractions, which were performed similarly to the procedures described above. Brief centrifugation at ca. 2000 rpm was required to obtain complete separation of the layers. The ethyl acetate fractions before acidification (ca. 1000 mL) were combined, dried, and evaporated in vacuo to give an oily residue (ca. 25 mL) to which hexane (100 mL) was added. The hexane layer was partitioned four times with equal volumes of acetonitrile. The combined acetonitrile fraction (400 mL) was washed with hexane (400 mL) and concentrated in vacuo (15 mL). A white precipitate, which developed during cold storage of the sample, was filtered (0.45-μm Nylon Acrodisc, Gelman Sciences). Precipitation continued during concentration of the sample under nitrogen, necessitating refiltration. The final sample, reconstituted in 400 μL of acetonitrile, was analyzed by TLC and HPLC. The acidic ethyl acetate fraction (ca. 750 mL) was dried and concentrated in vacuo and under nitrogen to give an oily residue (3.5 mL). Precipitates formed during the concentration process were filtered. Hexane (2 mL) was added to the oily residue and partitioned twice with acetonitrile (6 mL). The acetonitrile fraction was concentrated under nitrogen to a small volume (200 μL) and assayed by HPLC and TLC.

Analysis of Feces and Tissues. Rat feces (low dose, 10-15 g, 0-24 h), black goat kidney (106 g), and hen excreta (160 g, day 7) were homogenized and stirred in 3 volumes of acetonitrile/water (8:2 v/v). The solution was filtered under vacuum with repetitive rinsing of the residue. The filtrate was evaporated in vacuo to remove the acetonitrile portion to give the aqueous fraction, which was partitioned three times with equal volumes of ethyl acetate. The remaining aqueous layers were acidified (pH 1) with 36% HCl and repartitioned three times with equal volumes of ethyl acetate. If a significant amount of radioactivity remained in the acidic aqueous layer, it was heated under reflux for 2 h and repartitioned with ethyl acetate. The respective ethyl acetate fractions were combined, dried, and concentrated to a small volume (200-500 μL). A similar procedure was employed for the extraction of the goat kidney. Hexane (3 mL) was added to the final oily residue followed by partitioning with equal volumes of acetonitrile. The combined acetonitrile fraction was washed with hexane (10 mL) and concentrated to a small volume (500 μL). The final samples were assayed by HPLC and TLC.

Isolation of Metabolites in Rat Urine. The ethyl acetate fractions of the high-dose male rat urine samples (0-12 and 12-24 h, 20 mL each) were combined and concentrated in vacuo and under nitrogen to a small volume (ca. 500 μL). A precipitate that formed during the concentration process was removed by filtration. The final sample was purified by preparative TLC using solvent system A. Individual bands on the TLC plate corresponding to images on the autoradiogram were scraped. The silica gel was stirred in ethyl acetate (40 mL) and filtered under vacuum. The final filtrate containing metabolite I was concentrated under nitrogen for HPLC, MS, and NMR analysis. Similarly, the ethyl acetate fractions after acidification of the urine samples were concentrated in vacuo and under nitrogen to a small volume (400 μL) and purified by TLC on an analytical plate using 100% ethyl acetate. The major radioactive band was scraped and stirred in a mixture of ethyl acetate/methanol 2:1 (v/v) (15 mL), and the silica gel was filtered under vacuum. The final filtrate containing metabolite III was concentrated under nitrogen for analyses by HPLC and MS. The ethyl acetate fractions of the high-dose female rat urine (0-24 h, 60 mL) were worked up similarly to procedures described above. The partially purified sample after preparative TLC was subjected to additional purification by HPLC. Aliquots of the sample (50 μL in acetonitrile) were injected repetitively, and peaks of interest were collected on the basis of their elution profile. Fractions for each peak of interest were combined, evaporated under nitrogen to remove the acetonitrile portion of the mobile phase, and partitioned with three times equal volumes of ethyl acetate. The ethyl acetate layers were dried and concentrated for HPLC and MS analysis.

Table I. Elimination of Radiocarbon in Urine and Feces of Rats and Goats and in the Excreta of Hens after Oral Administration of [^{14}C]-F6285

	cumulative % of dose eliminated									
	rat[a]									
	male[b]			female[c]			goat[d]			hen[e]
day	urine	feces	total	urine	feces	total	urine	feces	total	excreta
1	88.4	5.0	93.3	78.4	13.4	91.8	20.8	0.0	20.8	14.5
2	91.7	5.6	97.2	82.1	14.0	96.1	42.5	0.1	42.6	26.8
3	92.5	5.6	98.1	83.2	14.2	97.4	62.5	0.1	62.6	39.6
4							81.5	0.3	81.8	43.5
5							102.3	0.5	102.8	55.5
6										68.5
7										86.2

[a] Results were average values of two rats each given a single oral dose of [^{14}C]-F6285 (10 mg/kg). Percentage of dose eliminated as $^{14}CO_2$ was negligible (<0.05%). [b] Treated with [*carbonyl*-^{14}C]-F6285. [c] Treated with [*phenyl*-^{14}C]-F6285. [d] Brown goat treated with daily oral doses of [*phenyl*-^{14}C]-F6285 (2 mg/kg) for 5 days. The animal was sacrificed approximately 24 h after the last dose. [e] Combined samples of 10 hens; each was given daily oral doses of [*phenyl*-^{14}C]-F6285 (3 mg/kg) for 7 days.

RESULTS

Excreta Balance. Table I gives the excretion pattern of [*carbonyl*-^{14}C]- and [*phenyl*-^{14}C]-F6285 in male and femal rats after a single oral dose of 10 mg/kg. A majority of the dose (>90%) was excreted in urine and feces within 24 h after dosing. The recovery at the end of 72 h was nearly quantitative (>97%). The percentage of the dose eliminated as radioactive $^{14}CO_2$ was negligible (<0.05%). Excretion in urine was found to be the major route of elimination (83.2–92.5%). There appeared to be a slight difference in the excretion pattern of radioactive residues between the male and the female. The elimination of radioactive residues in the goat and the hen with dietary intake of [*phenyl*-^{14}C]-F6285 is also summarized in Table I. It should be noted that when actual feed consumption was measured, the average dietary dosages were calculated to be 300 and 45 ppm for the goat and the hen, respectively. At the end of the 5-day feeding period, orally administered F6285 and its metabolites in the goat were quantitatively recovered in the urine (102.8%). A majority of the orally administered radioactivity in the hen was also recovered in the excreta at the end of the 7-day feeding period (>86%).

Metabolic Fate. *Rat Urine and Feces.* Most of the radioactivity was readily extractable into organic solvents (85.9–97.6%), suggesting that xenobiotic conjugation was not a major step in the metabolism of F6285 in the rat. A small percent of the radioactive residues (1.1–5.7%) was extractable into organic solvents upon acidification of the residual aqueous fraction, suggesting the presence of metabolites with acidic functionalities. The aqueous soluble residues (1.2–4.0%) and, in the case of the feces, the nonextractable (bound) residues (2.7–5.1%) were not analyzed further.

The nature of the radioactivity in different fractions was investigated by TLC and HPLC. Parts a–c of Figure 2 give HPLC analyses of the organic fractions of male and female urine. It is apparent that in the male neutral organic fraction only one single metabolite (I) (rt = 14.5 min) was present. This metabolite was also found to be the major metabolite in the female, where a second metabolite (II) (rt = 12.5 min), not present in the male, was also found. A third metabolite (III) (rt = 3.3 min), probably acidic in nature, was present in both male and female organic fractions after acidification of the aqueous samples. The metabolic profile was slightly different in the feces. Both I and II were found in the male, whereas only I and a trace of parent F6285 (rt = 28 min), were found in the female. Again, III was a common metabolite in both genders. The distribution of these metabolites in urine and feces is given in Table II. When the radioactive residues were expressed as percent of the applied dose, the following distribution was obtained: I (male, 94.6%; female, 87.5%), II (male, 0.5%, female, 5.4%), III (male, 1.7%; female, 1.3%), and unchanged F6285 (female, 0.5%).

The metabolic profiles of F6285 in the high-dose rat study (100 mg/kg) (data not shown) were similar to those described above. These samples were therefore used for the isolation of metabolites for identification purposes. The major metabolite in male urine (0–24 h), I, was purified by preparative TLC (R_f 0.58) to afford 16 mg of pure material for structural elucidation by MS and NMR. The CI mass spectrometric data (Table III) showed molecular ions (M + 1) of 403 (96.5%) and 405 (46.6%), which corresponded to a hydroxylated F6285 and its ^{37}Cl isotope, respectively. The EI mass spectrum showed ions of 402 (M^+, 62.0%); 367 [$(M - Cl)^+$, 41.3%], and 323 [$(M - SO_2 - CH_3)^+$, 100.0%]. The presence of the ion at m/z 323 suggested that the methylsulfonyl moiety was not modified. NMR data obtained were ($CDCl_3$) δ 3.0 (3 H, s, SO_2CH_3), 4.7 (2 H, s, CH_2OH), 6.8 (1 H, s, N*H*), 7.0 (1 H, t, J = 58.3 Hz, CHF_2); 7.5 (1 H, s, Ar*H*), and 7.7 (1 H, s, Ar*H*). These data were consistent with 3-(hydroxymethyl)-F6285 as the proposed structure of this metabolite.

Metabolite III (present in organic fractions after acidification of the aqueous layer) in male urine (0–24 h) was partially purified by TLC, where the major radioactive band (R_f 0.08) was isolated. A M + 1 ion of 373 in the CI mass spectrum (Table III) suggested that the metabolite was a demethylated derivative of F6285. However, due to the polar characteristics of this metabolite (as evidenced by TLC and HPLC), it was concluded that the apparent molecular ion (M + 1) of 373 was most likely the result of the decarboxylation of the corresponding 3-carboxylic acid in the mass spectrometer. This conclusion was further supported with the availability of the authentic F6285-3-carboxylic acid standard, which gave chromatographic characteristics and CI mass spectrum identical with those of III. The metabolite was found to undergo acid-catalyzed decarboxylation (1.0 N HCl, 90 °C, 2 h) to generate IV, identified as 3-demethyl-F6285 on the basis of its identical chromatrographic characteristics as the synthetic standard.

Metabolite II, found in femal rat urine and male feces, was isolated from female urine (0–24 h) by preparative TLC. This compound was found to have an R_f value identical with that of I (R_f 0.58). Therefore, the radioactive band consisting of II and I was further subjected to semipreparative HPLC purification. The CI mass spectrum of the final sample (Table III) gave a molecular (M + 1) ion of 405, which corresponded to a two-electron (two-proton) reduced product of a hydroxylated metabolite of

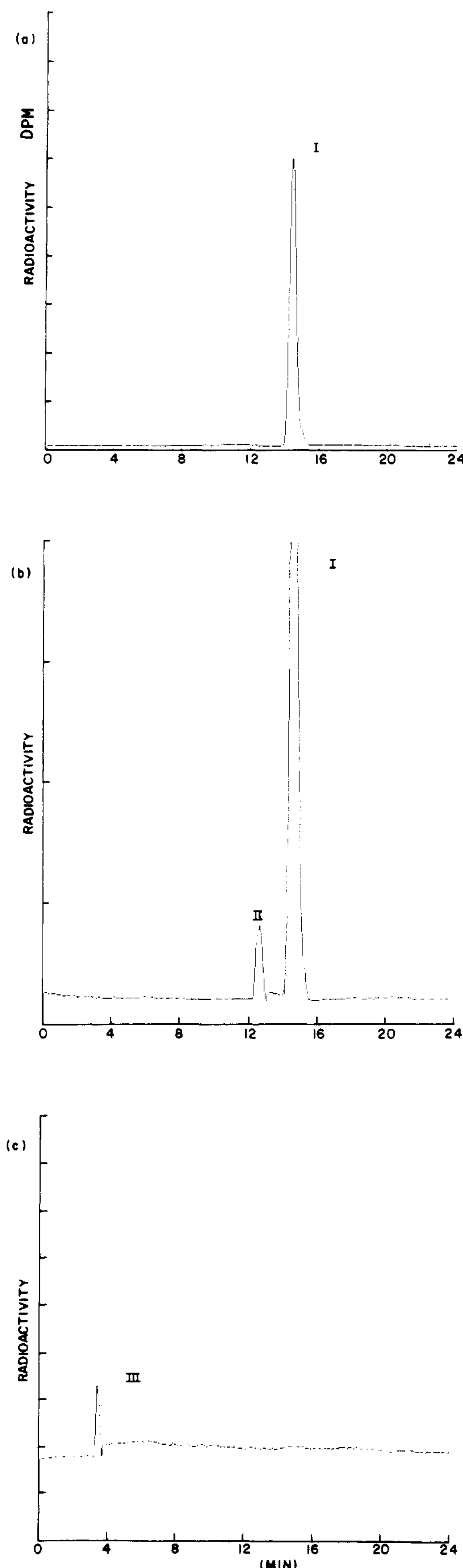


Figure 2. HPLC (system 1) (see Analytical Procedures for details) chromatograms of organic fractions of rat urine (0–24 h): (a) male [treated with a single oral dose of [*phenyl*-^{14}C]-F6285 (10 mg/kg)] ethyl acetate fraction; (b) female [treated with a single oral dose of [carbonyl-^{14}C]-F6285 (10 mg/kg)] ethyl acetate fraction; (c) male [treated with a single oral dose of [*phenyl*-^{14}C]-F6285 (10 mg/kg)] acidic ethyl acetate fraction.

Table II. Nature and Distribution of Radiocarbon in Urine and Feces of Rats, Urine, Milk, and Kidney of Goats, and Excreta of Hens after Oral Administration of [^{14}C]-F6285

sample		% radioactivity				
		I[g]	II[h]	III[i]	IV[j]	F6285
rat urine	male[a]	97.3	ND[k]	1.5	ND	ND
	female[b]	91.1	6.5	1.1	ND	ND
rat feces	male	82.3	8.1	5.2	ND	ND
	female	82.4	ND	2.8	ND	3.4
goat[c] urine		91.0	ND	5.0	0.2	1.9
hen[d] excreta		95.3	ND	0.3	0.1	2.1
goat[e] milk		71.2	ND	5.1	ND	0.5
goat[f] kidney		15.6	ND	3.9	ND	70.5

[a] Treated with a single oral dose of [*carbonyl*-^{14}C]-F6285 (10 mg/kg). Results were obtained from the 0–24-h urine sample. [b] Treated with [*phenyl*-^{14}C]-F6285 (10 mg/kg). Results were obtained from the 0–24-h urine sample. [c] Treated with daily oral doses of [*phenyl*-^{14}C]-F6285 (2 mg/kg). Results were obtained from brown goat day 2 and 3 urine samples. [d] Treated with daily oral doses of [*phenyl*-^{14}C]-F6285 (3 mg/kg). Results were obtained from combined day 7 excreta. [e] Brown goat milk samples (p.m.) days 4 and 5. [f] Black goat kidney. [g] I, 3-(hydroxymethyl)-F6285. [h] II, 2,3-dihydro-3-(hydroxymethyl)-F6285. [i] III, F6285-3-carboxylic acid. [j] IV, 3-demethyl-F6285. [k] ND, nondetectable.

Table III. Mass Spectrometric Analyses of Metabolites of F6285 Isolated in Rat Urine and Feces[a]

metabolites		ions, m/z (rel intensity)
I[b]	CI	403 $(M + 1)^+$ (100.0%); 405 $(M + 1)^+$ ^{37}Cl (58.4%)
	EI	402 (M^+) (62.0%); 404 (M^+) ^{37}Cl (45.9%); 367 $(M - Cl)^+$ (41.3%); 323 $(M - SO_2 - CH_3)^+$ (100.0%); 325 $(M - SO_2 - CH3)^+$ ^{37}Cl (62.6%)
II[c]	CI	405 $(M + 1)^+$ (96.5%); 407 $(M + 1)^+$ ^{37}Cl (46.6%)
	EI	404 (M^+) (10.3%); 369 $(M - Cl)^+$ (9.0%); 325 $(M - SO_2 - CH_3)^+$ (44.0%); 290 $(M - SO_2 - CH_3 - Cl)^+$ (26.2%)
III[d]	CI	373 $(M + 1)^+$ (17.7%); 375 $(M + 1)^+$ ^{37}Cl (12.1%); 338 $(M + 1 - Cl)^+$ (20.9%)

[a] Groups of five rats each given a single oral dose of [*carbonyl*-^{14}C]- (male) or [*phenyl*-^{14}C]-F6285 (female) (100 mg/kg). Urine and feces were collected for 72 h. [b] I, 3-(hydroxymethyl)-F6285. Isolated from male urine (0–24 h). [c] II, 2,3-dihydro-3-(hydroxymethyl)-F6285. Isolated from female rat urine. [d] III, F62853-carboxylic acid. Isolated from male urine (0–24 h).

Table IV. Concentration of Radiocarbon in Milk of Goats and Eggs of Hens Treated with [*Phenyl*-^{14}C]-F6285

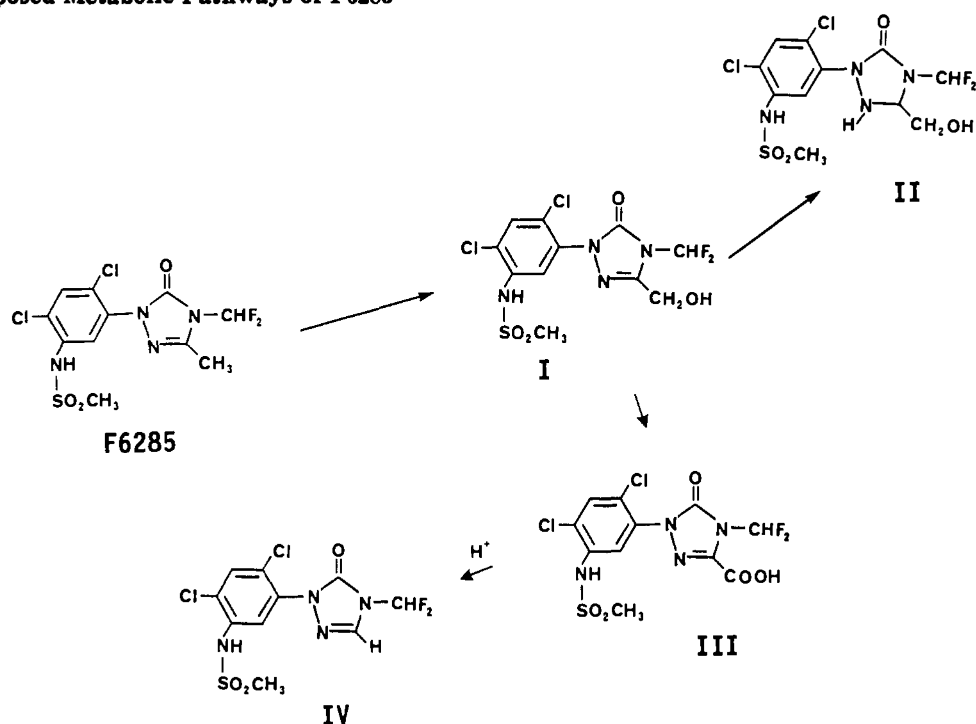
	concn of radioactive residues, ppm equiv of F6285			
	goat[a] milk		hen[b] eggs	
day	black goat	brown goat	whites	yolks
1	0.030	0.018	0.039	0.003
2	0.031	0.027	0.055	0.024
3	0.031	0.029	0.089	0.041
4	0.022	0.041	0.053	0.038
5	0.032	0.045	0.098	0.051
6			0.065	0.060
7			0.130	0.089

[a] Treated with daily oral doses of F6285 (2 mg/kg) for 5 days. Results were obtained from p.m. milk samples, which showed the highest concentration of residues. [b] Treated with daily oral doses of F6285 (3 mg/kg) for 7 days. Results were obtained from combined egg samples of 10 hens.

F6285. This metabolite was thus tentatively characterized as the 2,3-dihydro-3-(hydroxymethyl)triazolidinone compound.

Goat Urine and Hen Excreta. Most of the radioactivity was extracted into organic fractions before (93.5–98.0%)

Scheme I. Proposed Metabolic Pathways of F6285



and after (1.3–6.0%) acidification of the aqueous fractions. These results again suggested that a majority of the radioactive residues were in the form of free (nonconjugated) metabolites.

Characterizations of the radioactive residues by HPLC showed that the 3-hydroxymethyl metabolite (I) was again the dominant metabolite in both species. Unchanged F6285, F6285-3-carboxylic acid (III), and trace quantities of the decarboxylated product (IV) were also found. The distribution of these metabolites in goat urine and hen excreta is summarized in Table II. When the radioactive residues were expressed as percent of the total applied doses (data not shown), they were found to be very similar to those calculated previously for the rats.

Milk and Eggs. The concentration of radioactivity in goat milk (p.m.) (phenyl-^{14}C) is summarized in Table IV. The p.m. milk was found to contain the highest level of radioactivity. Residue levels ranging from 0.018 ppm at day 1 to 0.045 ppm at day 5 were observed. The total recovered radioactivity during the test period represented only 0.01% of the administered dose. Again, a majority of the radioactivity was readily soluble in organic solvents before (72.9%) and after (14.6%) acidification of the aqueous fractions. Characterizations of these radioactive residues by HPLC and TLC revealed the presence of metabolite I, unchanged F6285, and III (Table II). The residual aqueous fractions were not analyzed further.

The concentration of radioactivity in egg whites and yolks is given in Table IV. The levels ranged from 0.039 to 0.13 ppm in whites and from 0.003 to 0.089 ppm in yolks. The total recovered radioactivity represented only 0.039% and 0.010% of the administered dose in whites and yolks, respectively. These samples were not analyzed further.

Tissue Residues. The concentration of radioactive residues in selected organs and tissues of the goat was determined. No residue was detected in renal, omental fat, and flank muscle. Detectable levels were found in leg muscle (0.048 ppm), liver (0.054–0.067 ppm), and kidney (0.059–0.16 ppm). In the hen, the highest concentration was found in the liver (0.11 ppm) and fat (0.11 ppm). Levels in pectoral (0.021 ppm) and adductor muscle (0.008 ppm) were minimal.

Further analyses were performed with black goat kidney (0.16 ppm). Essentially all of the radioactivity (>98%) was soluble in the organic fraction. Characterization of the radioactive residues by HPLC and TLC again revealed the presence of unchanged F6285 and metabolites I and III (Table II).

DISCUSSION

Orally administered F6285 was shown to be eliminated rapidly and essentially quantitatively, primarily in the urine of the rat and goat and in the excreta of the hen. A majority of the dose in all three species was excreted as the nonconjugated 3-hydroxymethyl metabolite (I) (88–95%), which presumably was further oxidized to the 3-carboxylic acid (III) (0.3–5%) (Scheme I) via a transient aldehyde intermediate. The 3-demethyl compound (IV), found in small quantities in some instances, was most likely an acid-catalyzed decomposition product of the 3-carboxylic acid metabolite (III). Similar acid-catalyzed decarboxylation reactions, presumably via an arenium ion intermediate, have been observed in certain aromatic carboxylic acids (Longridge and Long, 1968; Schubert et al., 1954).

The tentatively identified 2,3-dihydro-3-hydroxymethyl metabolite (II) (0.5–5%) found in male rat feces and female rat urine was possibly the result of further metabolism of the 3-hydroxymethyl metabolite (I) by the gut microflora, which are known to carry out reduction reactions of xenobiotics such as sulfinpyrazone and digoxin (Lindenbaum et al., 1981; Renwick et al., 1982). It should be noted that metabolite II was not detected in the goat or the hen. Although this metabolite was found in the rat, the excretion patterns of II in the sexes were different; namely, it was found in the feces of the male (0.5%) and in the urine of the female rats (5.4%). The implications of these observations are twofold. First, there appear to be species differences in the metabolism of F6285, noteably between the goat/hen and the rat. This raises the question of selecting the appropriate animal model which is most

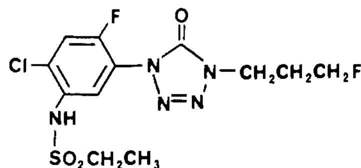


Figure 3. Structure of F5231.

applicable to humans for metabolism studies. Second, there appears to be sex differences in the rat in the metabolism of F6285. Although the mechanism underlying this sex selectivity is not clear at present, the results again further support the necessity of including both males and females in metabolism studies, in particular when toxicity assessments of metabolites are to be considered.

The metabolic profiles of F6285 were very similar in urine, feces, milk, and egg. The exception was in goat kidney, where unchanged F6285 was the major residue. A previous study on the microbial transformation of the structurally similar tetrazolinone herbicide F5231 [1-[4-chloro-2-fluoro-5-[(ethylsulfonyl)amino]phenyl]-1,4-dihydro-4-(3-fluoropropyl)-5*H*-tetrazol-5-one] (Figure 3) (Schocken et al., 1989) showed that N-4-dealkylation and aliphatic hydroxylation of the ethylsulfonylamino moiety were dominant pathways in the metabolism of the compound. Similar metabolic pathways have not been observed with F6285 in microbial cultures (unpublished data) or in animals in the present study. This discrepancy suggests that the C-3-methyl substituent is the preferential site of oxidation (presumably by the cytochrome P-450 system) in the F6285 molecule.

Even when these exaggerated dietary doses (>100×) were administered, the residue levels found in milk, eggs, and various tissues of the goat and the hen were relatively low. It was observed that there was a steady rise in the concentration of radioactive residues with time in goat milk and in eggs. Therefore, it seems desirable that collection of these milk and egg samples be performed for an extended period of time for the radioactive residues to reach a steady-state concentration. Nevertheless, these residue data, together with the excretion data obtained in the three animal species, suggest that residues of F6285 and its metabolites in animal feed would pose a minimal bioaccumulation problem in animals.

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