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ARTICLE

Metabolism of Flufenpyr-ethyl in Rats and Mice

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The metabolism of flufenpyr-ethyl [ethyl 2-chloro-5-[1,6-dihydro-5-methyl-6-oxo-4-(trifluoromethyl) pyridazin-1-yl]-4-fluorophenoxyacetate] was examined in rats and mice. [Phenyl-^{14}C]flufenpyr-ethyl was administered to rats and mice as a single oral dose at a level of 500 mg/kg, and ^{14}C-excretion was examined. Total ^{14}C-recoveries within 7 days after administration were 93.2 to 97.5% (feces, 42.0 to 46.0%; and urine, 47.2 to 55.5%) in rats and 92.6 to 96.4% (feces, 26.7 to 32.7%; and urine, 59.9 to 69.7%) in mice. ^{14}C-Excretion into expired air was not detected in rats (expired air of mice was not analyzed). No marked species- or sex-related differences were observed in the rate of ^{14}C-elimination, but a relatively higher excretion into the urine of mice was observed compared to that in rats. ^{14}C-residues in tissue 7 days after administration were relatively high for liver, hair, skin, and kidney, but total ^{14}C-residues were low, below 0.2% of the dose. An ester cleaved metabolite (S-3153acid) was the major metabolite in feces and urine. Hydroxylation of the methyl group on the C5 of the pyridazine ring and ether cleavage were also observed. No sex-related differences were observed in ^{14}C-elimination, ^{14}C-distribution, and metabolite profiles, and metabolism of flufenpyr-ethyl in rats and mice was similar. In vitro metabolism of flufenpyr-ethyl was examined using stomach and intestinal contents and blood and liver S9 fractions (postmitochondrial supernatant fractions) in rats. S-3153acid was detected as a major metabolite in the presence of intestinal contents and blood and liver S9 fractions, and a small amount was also formed in the presence of stomach contents, indicating that the parent compound is rapidly metabolized by intestinal contents and blood and liver S9 fractions through ester cleavage.

KEYWORDS: Flufenpyr-ethyl; metabolism; absorption; excretion; distribution; rats; mice

INTRODUCTION

Flufenpyr-ethyl [ethyl 2-chloro-5-[1,6-dihydro-5-methyl-6-oxo-4-(trifluoromethyl)pyridazin-1-yl]-4-fluorophenoxyacetate, S-3153] (**Figure 1**) is a herbicide used for controling velvetleaf and morning glories in corn, soybean, and sugar cane (*1*, *2*). The mode of action of flufenpyr-ethyl is protoporphyrinogen oxidase (PPO) inhibition.

Most PPO inhibitors have relatively low acute toxicity, but hepatic PPO inhibition effects are observed with several compounds. As for flufenpyr-ethyl, acute, chronic, oncogenicity, developmental, mutagenicity, and reproductive studies of flufenpyr-ethyl have shown low acute toxicity, no evidence of oncogenicity and mutagenicity, and no reproductive or developmental toxicity (*1*, *2*).

There are many chemical families of PPO inhibitors, such as *p*-nitro diphenylethers, *N*-phenylphthalimides, and triazolinones, and the metabolism of varieties of PPO inhibitors, such as oxyfluorfen (*p*-nitro diphenylethers), carfentrazone ethyl (triazolinones), and flumioxazine (tetrahydrophthalimides), was reported previously (*3*−*6*). However, flufenpyr-ethyl does not share structural similarity with other PPO inhibitors. The structure of flufenpyr-ethyl is rather related to pyridazinone herbicides (photosynthesis inhibitor) since flufenpyr-ethyl has a pyridazinone ring and an ethyl phenoxyacetate moiety. The metabolism of pyridazinone herbicides, such as chloridazon (*7*) and norflurazon (*8*), was reported previously.

In the present investigation, the ^{14}C-excretion, the ^{14}C-tissue distribution and the metabolism of [phenyl-^{14}C]flufenpyr-ethyl in rodents were investigated in rats and mice to assess species-related differences. An in vitro metabolism study of flufenpyr-ethyl was also conducted. Ethyl groups appear to be readily removed by carboxyesterases (*9*−*11*). Therefore, the investigation was conducted using gastrointestinal contents and blood and liver S9 fractions (postmitochondrial supernatant fractions) of rats to determine whether flufenpyr-ethyl was metabolized by ester cleavage. High esterase activity in the liver, gastrointestinal contents, and blood has been previously reported in the literature (*10*, *12*, *13*). Thus, the present study investigates the comparative metabolism (^{14}C-excretion into feces, urine, and expired air, ^{14}C-concentrations in tissues, and amounts of metabolites in excreta) of flufenpyr-ethyl in rats and mice, and its in vitro metabolism in the presence of selected tissues.

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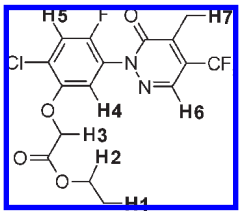

Figure 1. Proton numbers of flufenpyr-ethyl for the determination of the chemical structure by NMR spectrometry (see **Table 3**).

MATERIALS AND METHODS

Chemicals. [Phenyl-^{14}C(U)]flufenpyr-ethyl was chemically synthesized at the Environmental Health Science Laboratory (EHLS) with a specific activity of 10.8 MBq/mg. Unlabeled flufenpyr-ethyl (purity: 99.4%) was also synthesized in our laboratory. ^{1}H NMR data of flufenpyr-ethyl were obtained and compared with identified metabolites in Results and Discussion. ^{13}C NMR of flufenpyr-ethyl (CD_3OD, 68 MHz) δ = 14, 15, 63, 68, 115, 119, and 135 ppm. EI-MS analysis of flufenpyr-ethyl showed a base peak at m/z 408 (M^+). Other chemicals were of reagent grade.

Thin Layer Chromatography (TLC) Analysis. The solvent systems were as follows: A, toluene/ethyl formate/formic acid (5:7:1, v/v/v); and B, butanol/water/acetic acid (6:1:1, v/v/v). Unlabeled standards on TLC plates were detected by viewing under UV light (254 nm). Radioactive metabolites on TLC plates were detected by autoradiography using films developed with a Model M6B processor (Kodak, NY) or imaging plates processed with a BAS2000 Bioimage Analyzer (Fuji Photo Film, Kanagawa, Japan).

HPLC Analysis. HPLC analysis of samples was conducted using an L-6200 type intelligent pump (Hitachi, Tokyo, Japan), an L-4000 UV detector (Hitachi), an LB 507A HPLC radioactivity monitor (Berthold, Germany), and an 805 data station (Japan Millipore Limited, Tokyo). The wavelength of the UV detector was set at 254 nm. Preparative isolation was achieved on a 250 mm × 20 mm i.d. ODS packed column (YMC, Kyoto, Japan) with mobile phases of (a), methanol/water (70:30); (b), methanol/water (60:40); and (c), methanol/water (55:45). The flow rate was 3 mL/min. Metabolite analysis was achieved on a 150 mm × 6 mm i.d. ODS packed column (Sumipax ODS A212, Sumika Chemical Analysis Service, Ltd., Osaka, Japan) was with a mobile phase of (d), acetonitrile/water (30:70). The flow rate was 1 mL/min.

Radioanalysis. Radioactivity in organosoluble fractions or urine was quantified by liquid scintillation counting (LSC). Samples (100−300 mg) of fecal homogenates, unextractable fecal residues, and tissues were combusted with a sample oxidizer (Packard) prior to LSC after air-drying (combustion method). Quantification of radiocarbon on TLC plates was conducted by scraping methods.

Spectroscopic and Spectrometric Analysis. Chemical structures of purified metabolites were determined by NMR and MS spectrometry. ^{1}H NMR and ^{13}C NMR spectra were obtained with a JEOL GSX-270 Spectrometer (JEOL Ltd., Tokyo) with methanol-d4 as the solvent. FD-MS or EI-MS spectra were obtained with a Hitachi DF/GC/MS M-80B (Hitachi).

In Vivo Metabolism Studies. Groups of three male and female Crj:CD(SD) rats at the age of 6 weeks and five male and female mice (Crj: CD-1(ICR)) at the age of 9 weeks were purchased from Charles River Japan, Inc. (Kanagawa, Japan). All animal experimentations were conducted in accordance with the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan). All animals selected for dosing showed normal weight gain, and no abnormal clinical symptoms during 7 days of quarantine and acclimation were selected for dosing. The in-life portion of the study was conducted under the following environmental conditions: room temperature, 20−26 °C; relative humidity, 55 ± 10%; ventilation, 10 air exchanges per hour; and artificial lighting from 8:00 a.m. to 8:00 p.m. Animals had free access to pelleted diet and water through the study. Rats (231−258 g body weight for male and 194−200 g for female) and mice (36−38 g for male and 27 g for female) were orally dosed with [phenyl-^{14}C]flufenpyr-ethyl (17.7 kBq/mg) suspended in 0.5% methyl cellulose aqueous solution at 500 mg/5 mL/kg by gavage. Radiochemical purity (>99.8%) was checked by TLC with hexane/ethyl acetate (2:1, v/v), prior to use. Rats and mice dosed with the ^{14}C-labeled compound were housed individually or five mice per cage, respectively, in glass metabolism cages (Metabolica CO_2, Sugiyamagen Iriki, Tokyo, Japan). Urine and feces were separately collected for 7 days. With rats, expired air was passed through an alkaline trap containing 10% NaOH solution for two days after administration to collect expired CO_2 gas. Two male mice died on the third day after administration because of their wounds by natural fighting; therefore, the data thereafter are average values for the three surviving mice. Rats and mice were euthanized by bleeding at 7 days after administration. Their tissues and organs were dissected out and the amounts of ^{14}C distributed to tissues were measured by LSC after combustion. Detection limit was set as twice the background count. The metabolites in feces collected within 2 days after administration were extracted three times with methanol/water (9:1, v/v), and the radioactivity in supernatants and postextracted solids was analyzed by LSC. Feces collected from 3 to 7 days were homogenized with water and combusted for radioanalysis. Aliquots of the sodium hydroxide solution in which expired CO_2 was trapped within 2 days after administration were analyzed by LSC.

The 0−2 day urine was subjected to TLC using solvent system A. The 0−2 day fecal extracts after administration of [phenyl-^{14}C]flufenpyr-ethyl were subjected to TLC analyses using solvent systems A and B. Metabolites were identified by TLC cochromatography with flufenypyr-ethyl metabolites previously isolated from rat urine and identified as described below. The other areas were scraped, and the radioactivity in these areas was summed up as others. Polar metabolites retained near the origin on a TLC plate using solvent system A were well separated using solvent system B.

Isolation and Purification of Metabolites. Two male Crj:CD(SD) rats purchased from Charles River Japan (Kanagawa) were used for dosing at the age of 7 weeks. The environmental conditions were as described for the in vivo metabolism study. After the ^{14}C-labeled compound was diluted to a concentration of approximately 50 kBq/mg specific activity using an unlabeled compound, it was suspended in corn oil at 100 mg/mL dosing solution. Urine was collected from the two rats following repeated daily oral administration of the ^{14}C-labeled compound 5 mL/kg-body weight for 3 days. Urine was collected during the administration period and for 2 days after the last administration of ^{14}C-labeled dosing solution. Urine was directly applied to XAD-2 (Amberlite, Organo, Tokyo) column chromatography, and the metabolites were eluted with water and methanol. Methanol eluates were concentrated and dissolved in water and added to an equal volume of ethyl acetate. Water and ethyl acetate layers were designated as D1 and D2, respectively. D1 was adjusted to pH 2 with 2 M HCl and added to an equal volume of ethyl acetate. The metabolites were distributed, and the water layer and ethyl acetate layer were designated as D1−1 and D1−2, respectively. D1−2 and D2 were applied to preparative TLC with solvent system A and separated into five and four fractions, respectively. These fractions were designated as D1−2−1 to 5 and D2−1 to 4, respectively. Fraction D1−2−1 was applied to HPLC using mobile phases a and b. The main peak was designated as M1 at T_R = 23.6 min using mobile phase b. Fraction D1−2−2 was applied to HPLC using mobile phases a, b, and c. The main peak was designated as M2 at T_R = 23.5 min using analytical system c. Fraction D2−2 was applied to HPLC using analytical system a, and the main peak designated as M3 at T_R = 12.2 min was purified. The structures of all three isolated metabolites were identified by spectroanalyses. The metabolites were then used as standards.

In Vitro Metabolism in Blood, Liver, and Stomach and Intestinal Contents of Rats. Blood was collected from two male Crj:CD(SD) rats (Charles River Japan Inc., Kanagawa, Japan) from the abdominal artery under anesthesia with diethyl ether, and the liver, stomach, and intestinal contents were collected. Each liver was combined, weighed, and homogenized with Potter-Elvehjem homogenizer with a 3-fold volume of 0.05 M Tris-HCl (pH 7.4) buffer. Each liver homogenate was adjusted to 50 mL with Tris-HCl buffer and centrifuged at 9,000g for 20 min at 4 °C. The supernatants were collected as S9 fractions (postmitochondrial supernatant fractions), and concentrations of protein were measured with a protein assay kit (Bio-Rad Laboratories, Richmond, CA) and adjusted to 16.6 mg/mL. Stomach and intestinal contents were diluted with ultrapure water to make 50% (w/v) suspensions. Reaction mixtures containing 120 nM [phenyl-^{14}C]flufenpyr-ethyl (10 μL in ethanol) with or without

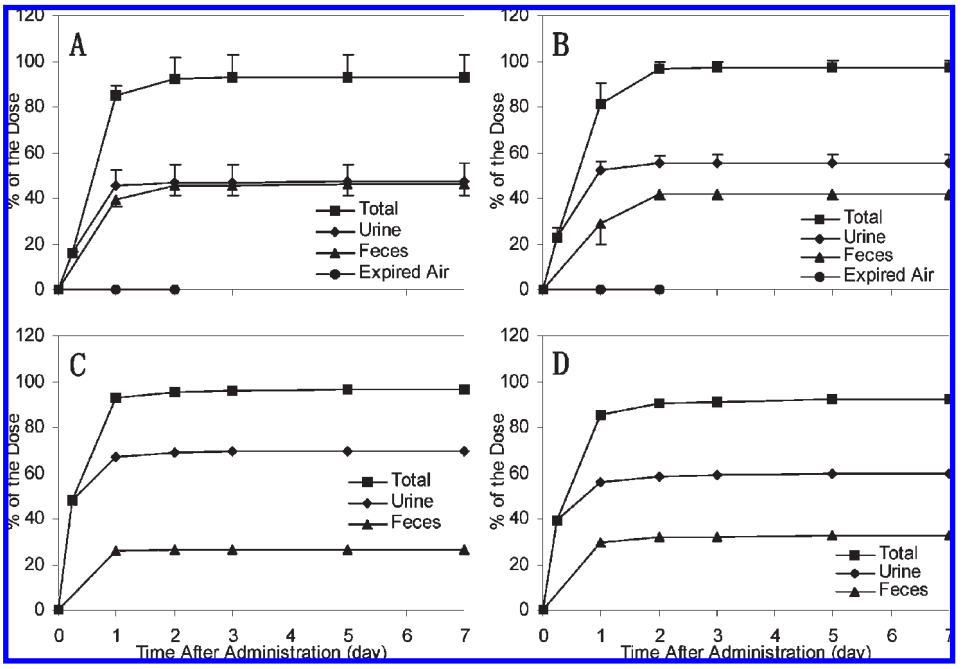

Figure 2. Cumulative ^{14}C-excretion after single oral administration of [phenyl-^{14}C]flufenpyr-ethyl to male rats (**A**), female rats (**B**), male mice (**C**), and female mice (**D**) at 500 mg/kg body weight. Points and bars are the mean values and standard deviations for data from three rats or the mean values for three to five mice.

3 mM of NADPH in 1 mL of liver S9 fractions, blood, or 50% suspension of stomach and intestinal contents were incubated at 37 °C for 12 h. The metabolites were extracted three times with 3 mL of ethanol, more than 88% of the radioactivity thereby being removed, and analyzed by TLC using solvent system A.

RESULTS AND DISCUSSION

^{14}C-excretion profiles into feces, urine, and expired air during 7 days after single oral administration of [phenyl-^{14}C]flufenpyr-ethyl are shown in **Figure 2**. ^{14}C was rapidly and almost completely excreted into urine and feces in rats and mice. ^{14}C-Excretion into expired air was not detected in rats (expired air of mice was not analyzed). ^{14}C-exretion within 7 days after administration was 93.2% (feces, 46.0%; and urine, 47.2%) in male rats, 97.5% (feces, 42.0%; and urine, 55.5%) in female rats, 96.4% (feces, 26.7%; and urine, 69.7%) in male mice, and 92.6% (feces, 32.7%; and urine, 59.9%) in female mice. No marked species- or sex-related differences were observed in the rate of ^{14}C-elimination, but a relatively higher excretion into urine of mice was observed compared to that in rats.

^{14}C-tissue residues in rats and mice on the seventh day after administration of the ^{14}C-labeled compound are shown in **Table 1**. Values were generally low and less than 4.4 μg equivalents of flufenpyr-ethyl/g wet tissue (ppm). The liver showed the highest concentration of 0.7−1.9 ppm in rats, and hair and skin the highest, 1.4−4.4 ppm, in mice. ^{14}C-residues in liver, hair, skin, and kidneys were higher than in other tissues, which had values < 0.3 ppm or below the detection limit. ^{14}C-elimination was almost complete, and total ^{14}C-residues in tissues 7 days after administration accounted for below 0.2% of the total. Tissues containing high radioactivity were generally the same in both species and sexes.

Table 2 shows the amounts (% of the dosed ^{14}C) of fecal and urinary metabolites in rats and mice. Representative TLC autoradiograms are shown in **Figure 3**. At least 2 and 6 metabolites were detected in the urine and feces, respectively. Two metabolites (M1 and M2) were identified as S-3153acid and S-3153acid-5′-CH_2OH (**Figure 4**). S-3153acid was the major metabolite in both

Table 1. ^{14}C-Concentrations in Tissues of Male and Female Rats and Mice at 7 Days after a Single Oral Administration of [Phenyl-^{14}C]flufenpyr-ethyl at 500 mg/kg[a]

	μg equivalents of flufenpyr-ethyl/g wet tissue (ppm)			
	rat		mouse	
	male	female	male	female
blood	0.3 ± 0.20	<0.2	0.1 ± 0.09	<0.2
blood cell	0.2 ± 0.04	<0.2	0.1 ± 0.04	<0.4
plasma	0.2 ± 0.01	<0.2	0.2 ± 0.10	<0.2
hair and skin	0.7 ± 0.33	0.2 ± 0.10	4.4 ± 2.48	1.4 ± 1.16
kidney	0.2 ± 0.04	<0.2	0.4 ± 0.04	0.8 ± 0.29
liver	1.9 ± 0.36	0.7 ± 0.12	1.4 ± 0.10	1.2 ± 0.28
mandibular gland	<0.1	<0.2	0.1 ± 0.09	<0.6
residual carcass	0.2 ± 0.10	0.2 ± 0.03	0.1 ± 0.01	0.1 ± 0.00

[a] ^{14}C-Concentrations in adrenal glands, bone, bone marrow, brain, cecum, eye, fat, heart, large intestine, lung, muscle, ovary, pancreas, pituitary, sciatic nerve, small intestine, spinal cord, spleen, stomach, testis, thymus, thyroid, and uterus are below the detection limit. Data are the means ± standard deviation for values from three rats, three male mice, and five female mice.

feces and urine, and small amounts of S-3153acid-5′-CH_2OH were also detected. The parent compound was detected only in feces and accounted for < 13% of the dose. The amount of other metabolites were summed up and shown as others in **Table 2**. Marked species- and sex-related differences were not observed in the metabolism of flufenpyr-ethyl in rats and mice.

^{1}H NMR data for the flufenpyr-ethyl and identified metabolites are shown in **Table 3**. Proton numbers of flufenpyr-ethyl are shown in **Figure 1**. The signals of H4 and H5 were assigned by comparing the ^{1}H−^{19}F spin coupling constant in ^{1}H NMR data.

M1. The H atom in the ethyl group of flufenpyr-ethyl showed triplet and quartet signals at 1.3 and 4.3 ppm in the ^{1}H NMR spectrum. However, no signal was observed with M1. On the basis of the spectral data, this metabolite was concluded to have lost the ethyl group by cleavage of the ester linkage of flufenpyr-ethyl. ^{13}C NMR (CD_3OD, 68 MHz) δ = 13, 67, 115, 119, and

Table 2. Amounts of Metabolites in the Urine and Feces of Rats and Mice within 2 Days after a Single Oral Administration of [Phenyl-^{14}C]flufenpyr-ethyl at 500 mg/kg[a]

	amount (% of the dosed ^{14}C)			
	rats		mice	
	male	female	male	female
Urine				
S-3153acid (M1)	45.5 ± 7.63	54.2 ± 3.49	67.9	56.6
S-3153acid-5′-CH_2OH (M2)	1.2 ± 0.26	0.7 ± 0.06	0.7	1.2
others	0.3 ± 0.02	0.4 ± 0.06	0.5	0.5
subtotal	47.0 ± 7.87	55.2 ± 3.53	69.0	58.3
Feces				
flufenpyr-ethyl	12.8 ± 5.84	12.5 ± 8.64	11.1	6.1
S-3153acid (M1)	27.3 ± 0.22	24.5 ± 5.71	12.2	23.2
S-3153acid-5′-CH_2OH (M2)	0.2 ± 0.05	0.1 ± 0.04	0.1	0.1
others	1.2 ± 0.16	0.8 ± 0.07	1.1	1.0
not extracted	4.0 ± 1.56	3.7 ± 2.85	2.1	1.8
subtotal	45.5 ± 4.45	41.6 ± 2.30	26.6	32.2
total	92.5 ± 9.28	96.8 ± 2.92	95.6	90.5

[a] Data are the means ± standard deviation for values from three rats and the mean values for five mice.

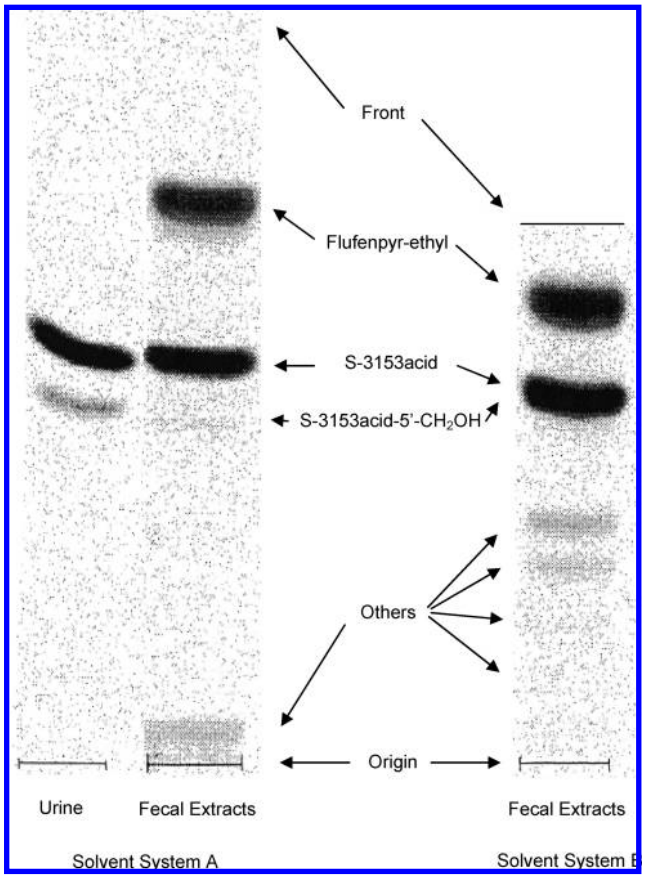

Figure 3. Representative TLC autoradiograms of urine and fecal extracts of male rats after single oral administration of [phenyl-^{14}C]flufenpyr-ethyl at 500 mg/kg body weight. The metabolites are developed with solvent systems A, toluene/ethyl formate/formic acid (5:7:1, v/v/v), and B, butanol/water/acetic acid (6:1:1, v/v/v).

134 ppm. Further, EI-MS analysis showed a molecular ion peak at m/z 380 (M^+, 60%) and a base peak at m/z 335. On the basis of these results, M1 was identified as [2-chloro-4-fluoro-5-(5-methyl-6-oxo-4-trifluoromethyl-1,6-dihydropyridazin-1-yl) phenoxy]acetic acid (S-3153acid).

M2. From consideration of M1 (S-3153acid), M2 was hypothesized to be an ester-cleaved metabolite of flufenpyr-ethyl. The H atom of the methyl group at position-5′ of the pyridazine ring showed a singlet signal at 2.4 ppm in the ^{1}H NMR spectrum of flufenpyr-ethyl, but the signal was shifted to a lower magnetic field of 4.7 ppm, and the signal intensity decreased from 3 protons to 2 protons in the ^{1}H NMR spectrum of M2. On the basis of these results, the metabolite was concluded to be a hydroxylated metabolite of M1 at the methyl group on the 5′-position of the pyridazine ring of M1. ^{13}C NMR (CD_3OD, 68 MHz) δ = 54, 115, 119, and 135 ppm. EI-MS analysis showed a base peak at m/z 396 (M^+). On the basis of these results, M2 was identified as [2-chloro-4-fluoro-5-(5-hydroxymethyl-6-oxo-4-trifluoromethyl-1,6-dihydropyridazin-1-yl)phenoxy]acetic acid (S-3153acid-5′-CH_2OH).

M3. From consideration of M1 (S-3153acid), this metabolite was hypothesized to be an ester-cleaved metabolite of flufenpyr-ethyl. Further, though the H atom in the methylene group of acetate showed a singlet signal at 4.9 ppm in the ^{1}H NMR spectrum of the flufenpyr-ethyl, no signal was observed in this area in the ^{1}H NMR spectrum of M3. EI-MS analysis showed a base peak at m/z 322 (M^+). On the basis of the results, the metabolite was concluded to be formed by cleavage of the ether linkage of flufenpyr-ethyl. M3 was therefore identified as 2-(4-chloro-2-fluoro-5-hydroxy)phenyl-4-methyl-5-trifluoromethyl-2,3-dihydropyridazin-3-one (S-3153−1-OH).

Flufenpyr-ethyl was incubated with stomach or intestinal contents and blood or liver S9 fractions of rats, for 12 h, and the amounts of flufenpyr-ethyl and its metabolites were quantified by TLC. The results are shown in **Table 4**. Flufenpyr-ethyl was almost entirely metabolized to S-3153acid by the intestinal contents and blood and liver S9 fractions, up to 85.0−92.5% of the applied dose. Amounts of other metabolites were low (2.6−4.5%). However, smaller amounts of flufenpyr-ethyl were metabolized to S-3153acid by the buffer (20.7%) and the stomach contents (4.8−4.9%). On the basis of these results, an appreciable metabolism of flufenpyr-ethyl was apparent within intestinal contents and blood and liver, but not stomach contents and buffer.

The present study revealed that following a single oral administration of [phenyl-^{14}C]flufenpyr-ethyl to rats and mice the radiocarbon was rapidly excreted into feces and urine, at 26.7−46.0% and 47.2−69.7%, respectively, with total ^{14}C recoveries of 93−98%. Total ^{14}C-residues in tissues were below 0.2%, though relatively high levels were observed in liver, hair, skin, and kidneys. S-3153acid was the major metabolite in feces and urine. Small amounts of S-3153acid-5′-CH_2OH were also detected. No sex-related differences were observed in ^{14}C-elimination, the ^{14}C-distribution, and metabolite profile, and the metabolism of flufenpyr-ethyl was similar in rats and mice. Elimination was very rapid, and tissue residues were low after the administration of flufenpyr-ethyl, likely due to its extensive metabolism. Flufenpyr-ethyl was metabolized to S-3153acid and then rapidly excreted into urine and feces.

Ester cleavage is one of the major metabolic reactions in PPO inhibitors, and herbicidal activity is associated with carboxylic acid formation (*3*). Flufenpyr-ethyl was metabolized to S-3153acid by intestinal contents, blood, and liver, consistent with previous reports of high esterase activity in intestinal contents, blood, and liver (*10*, *12*, *13*). However, significant amounts of S-3153acid were interestingly also formed in buffer suggesting that flufenpyr-ethyl was degraded to S-3153acid via nonenzymatic pathways. Flufenpyr-ethyl was stable with

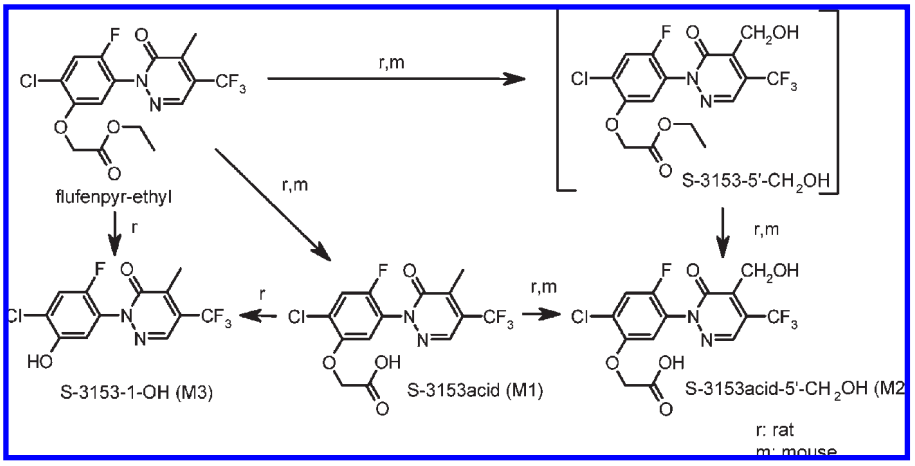

Figure 4. Proposed metabolic pathways for flufenpyr-ethyl in rats and mice.

Table 3. ^{1}H-NMR Data for Flufenpyr-ethyl and Isolated Metabolites[a]

	flufenpyr-ethyl		S-3153acid (M1)		S-3153acid-5′-CH_2OH (M2)		S-3153-1-OH (M3)	
proton no.	δ (CD_3OD)	J (Hz)	δ (CD_3OD)	J (Hz)	δ (CD_3OD)	J (Hz)	δ (CD_3OD)	J (Hz)
H1	1.28 (3H, t)	6.9	———	———	———	———	———	———
H2	4.28 (2H, q)	6.9	———	———	———	———	———	———
H3	4.85 (2H, s)	———	4.79 (2H, s)	———	4.76 (2H, s)	———	———	———
H4	7.28 (1H, d)	6.2	7.27 (1H, d)	6.2	7.21 (1H, d)	6.2	7.01 (1H, d)	6.1
H5	7.52 (1H, d)	9.1	7.51 (1H, d)	9.1	7.50 (1H, d)	9.1	7.36 (1H, d)	9.5
H6	8.18 (1H, s)	———	8.20 (1H, s)	———	8.28 (1H, s)	———	8.20 (1H, s)	———
H7	2.39 (3H, s)	———	2.42 (3H, s)	———	4.65 (2H, s)	———	2.33 (3H, s)	———

[a] s, singlet; d, doublet; q, quartet; ———, none.

Table 4. Amounts of Flufenpyr-ethyl and Its Metabolites in Reaction Mixtures After Incubation for 12 h

	Amount of metabolites (% of the applied ^{14}C)								
	Buffer	Stomach cont.		Intestinal cont.		Blood		Liver	
Metabolite	-	+	-	+	-	+	-	+	-
Flufenpyr-ethyl	66.5	91.0	89.1	0.6	0.7	0.5	0.4	1.9	1.6
S-3153acid (M1)	20.7	4.9	4.8	94.1	92.5	87.3	85.0	92.4	92.8
Others	11.7	2.1	3.2	3.2	4.5	3.2	2.6	3.3	3.0
Unextractable	1.1	2.0	2.9	2.1	2.3	9.0	12.0	2.3	2.6

The reaction mixtures were incubated with (+) or without (-) 3 mM NADPH and 120nM flufenpyr-ethyl.

stomach contents, likely due to stability under acidic conditions. Flufenpyr-ethyl was stable in acidic conditions (unpublished observation).

The oxidative metabolite, S-3153acid-5′-CH_2OH, was not detected in the present in vitro study. The results were comparable to the in vivo metabolism study where S-3153acid-5′-CH_2OH was detected at only 0.8−1.4% of the dose in rodents. Because the oxidation is much slower than ester cleavage, S-3153acid-5′-CH_2OH would not be expected to be produced under in vitro conditions. This would be consistent with the lack of remarkable differences in the metabolite profile with or without NADPH since ester cleavage does not require this cofactor in contrast with P450 oxidation. S-3153-1-OH was not detected in rats and mice and in the in vitro study, even though it was isolated from rat urine and identified by MS and NMR spectrometry suggesting that it may be formed in very small amounts. Ether cleavage and phenol formation were reported previously with other PPO inhibitors, such as azafenidin (*14*).

The pyridazinone ring was not opened in rats and mice as well as pyridazinone herbicides (*7*, *8*). It seems that the pyridazinone ring is metabolically stable in mammals.

On the basis of the metabolites identified in the present study, the major biotransformation reactions of flufenpyr-ethyl are as follows: (1) cleavage of the ester linkage; (2) hydroxylation of the methyl group on C5 of the pyridazine ring; and (3) cleavage of the ether linkage. On the basis of these observations, proposed metabolic pathways of flufenpyr-ethyl in rats and mice are shown in **Figure 4**.

LITERATURE CITED

(1) Federal Register 68 (September 19, 2003): 54834.
(2) Federal Register 68 (June 25, 2003): 37813.
(3) Aizawa, H.; Brown, H. M. Metabolism and Degradation of Porphirin Biosynthesis Inhibitor Herbicides. In *Peroxidizing Herbicides*; Boger, P., Wakabayashi, K., Eds.; Springer-Verlag: Berlin, Germany, **1999**; pp 347−377.
(4) Adler, I. L.; Jones, B. M.; Wargo, J. P.Jr. Fate of 2-chloro-1-(3-ethoxy-4-nitrophenoxy)-4-(trifluoromethyl) benzene (oxyfluorofen) in rats. *J. Agric. Food Chem*. **1977**, *25*, 1339–1341.
(5) Matsunaga, H.; Isobe, N.; Kaneko, H.; Nakatsuka, I.; Yamane, S. Metabolism of pentyl 2-chloro-4-fluoro-5-(3,4,5,6-tetrahydrophthalimido)phenoxyacetate (flumiclorac pentyl, S-23031) in rats. 2. Absorption, distribution, biotransformation, and excretion. *J. Agric. Food Chem*. **1997**, *45*, 501–506.
(6) Matsunaga, H.; Tomigahara, Y.; Kaneko, H.; Nakatsuka, I.; Yamane, S. Identification of a reduced form metabolite of flumiclorac pentyl (S-23031) in rats. *J. Pestic. Sci*. **1997**, *22*, 133–135.
(7) Roberts, T. R. *Metabolic Pathways of Agrochemicals Part 1: Herbicides and Plant Growth Regulators*; The Royal Society of Chemistry: London, U.K., **1998**; pp 407−409.
(8) Quistad, G. B.; Saunders, A. L.; Skinner, W. S.; Reuter, C. C.; Collier, K. D. Metabolism of norflurazon by rats. *J. Agric. Food Chem*. **1989**, *37*, 1412–1416.

(9) Hosokawa, M.; Furihata, T.; Yaginuma, Y.; Yamamoto, N. Koyano, N.; Fujii, A.; Nagahara, Y.; Satoh, T.; Chiba, K. Genomic structure and transcriptional regulation of the rat, mouse, and human carboxylesterase genes. *Drug Metab. Rev*. **2007**, *39* 1–15.
(10) Imai, T. Hydrolysis by carboxylesterase and disposition of prodrug with ester moiety. *Yakugaku Zasshi* **2007**, *127*, 611–619.
(11) Satoh, T.; Hosokawa, M. The mammalian carboxylesterases: from molecules to functions. *Annu. Rev. Pharmacol. Toxicol*. **1998**, *38*, 257–288.
(12) McCracken, N. W.; Blain, P. G.; Williams, F. M. Human xenobiotic metabolizing esterases in liver and blood. *Biochem. Pharmacol*. **1993**, *46*, 1125–1129.
(13) Goldin, B. R. Intestinal microflora: metabolism of drugs and carcinogens. *Ann Med*. **1990**, *22*, 43–48.
(14) Aizawa, H. *Metabolic Maps*; Academic Press: Oxford, U.K., **2001**; p 159.

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