Metabolism of Furametpyr. 2. ^{14}C Excretion, ^{14}C Concentrations in Tissues, and Amounts of Metabolites in Rats

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^{14}C-Labeled furametpyr [*N*-(1,3-dihydro-1,1,3-trimethylisobenzofuran-4-yl)-5-chloro-1,3-dimethylpyrazole-4-carboxamide, Limber] was dosed to male and female rats at 1 (low dose) and 200 or 300 mg/kg (high dose). Elimination of furametpyr was rapid, and the dosed ^{14}C was substantially excreted within 7 days (45.5−53.3% in feces, 44.1−53.8% in urine, and 0.01% in expired air). However, ^{14}C excretion rate showed sex- and dose-related differences, more rapid in males at low dose. ^{14}C concentrations in tissues decreased rapidly to generally low levels at 7 days (<0.004 ppm with the low dose and <1.1 ppm with the high dose). Forty metabolites were detected, and 13 metabolites and 4 glucuronides were identified. A small amount of unchanged furametpyr was detected in feces (0.1−0.5% of the dose). The major metabolites in tissues were *N*-demethylated metabolites. In a bile study, 52.5−54.2% of the dosed ^{14}C was rapidly excreted into bile within 2 days. The absorption ratio was estimated to be >93.7% for the low dose (1 mg/kg). Major metabolites in bile were glucuronic acid conjugates of furametpyr hydroxides. On the basis of the results, furametpyr is substantially absorbed from the gastrointestinal tract after oral administration, rapidly distributed to tissues, extensively metabolized, and excreted into urine and bile or feces.

Keywords: *Metabolism; absorption; excretion; distribution; furametpyr; rats*

INTRODUCTION

Furametpyr [*N*-(1,3-dihydro-1,1,3-trimethylisobenzofuran-4-yl)-5-chloro-1,3-dimethylpyrazole-4-carboxamide, Limber] is a fungicide used to control rice sheath blight (Mori et al., 1997; Oguri, 1997). The metabolism of furametpyr in rats has been investigated in conjunction with toxicological studies for safety evaluation.

Metabolites of furametpyr in rats are isolated and identified by spectroanalyses (Nagahori et al., 2000). The major biotransformation reactions are proposed as (1) *N*-demethylation, (2) oxidation of the methyl group at C3 of the pyrazole ring, (3) oxidation of the methyl group at C1 of the 1,3-dihydroisobenzofuran ring, (4) hydroxylation at C3 of the 1,3-dihydroisobenzofuran ring, and (5) hydroxylation at C7 of the 1,3-dihydroisobenzofuran ring. However, it has not yet been determined how furametpyr is absorbed, metabolized, and excreted in rats.

The present paper deals with the metabolic fate (^{14}C excretion into feces, urine, expired air, and bile, ^{14}C concentrations in tissues and amounts of metabolites in excreta and tissues) of furametpyr in rats.

MATERIALS AND METHODS

Chemicals. Three lots of [*phenyl*-^{14}C]furametpyr were synthesized in our laboratory (2.85, 2.06, and 2.01 GBq/mmol). The labeled compound was purified by preparative thin-layer chromatography (TLC) with chloroform/methanol, 10:1 (v/v), and hexane/ethyl acetate, 1:3 (v/v) prior to use. Unlabeled furametpyr (purity = 99.8%) was also synthesized in our laboratory.

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One of the authentic standards, *N*-(1,3-dihydro-3-hydroxy-1,1,3-trimethylisobenzofuran-4-yl)-5-chloro-1,3-dimethylpyrazole-4-carboxamide (Fur-HK), was synthesized in our laboratory (purity = 99.0%) and used for TLC cochromatography. The ^1H nuclear magnetic resonance (NMR) spectrum of Fur-HK [δ 8.6 (1H, s), 8.2 (1H, d), 7.4 (1H, t), 6.9 (1H, d), 3.9 (3H, s), 3.0 (1H, s), 2.5 (3H, s), 1.8 (3H, s), 1.6 (3H, s), 1.5 (3H, s)] was obtained with an NMR spectrometer (Unity 300, Varian, CA) using $CDCl_3$ as a solvent. The mass spectrometry (MS) spectrum of Fur-HK [m/z 348 $(M - H)^-$] was obtained with an APCI-MS (M-1000, Hitachi, Tokyo, Japan). Other authentic standards were prepared by purification of metabolites in excreta of rats reported by Nagahori et al. (2000).

Chromatographic Procedures. TLC was conducted essentially as described previously (Nagahori et al., 1997). Silica gel 60 F_{254} chromatoplates (20 × 20 cm, 0.25 mm layer thickness, Merck, Darmstadt, Germany) were used for analyses and isolation of metabolites with the following solvent systems: (A) ethyl acetate/formic acid/water (35:2:2); (B) toluene/ethyl formate (5:9, developed twice); and (C) 1-butanol/acetic acid/water (6:1:1). Unlabeled standards on TLC plates were detected by viewing under UV light (254 nm). The radioactive spots on TLC plates were detected by placing X-ray films (SB-5, Kodak, Rochester, NY) on plates for ∼1 week at 4 °C, followed by processing of the exposed films with a model M6B processor (Kodak).

Radioanalysis. Radioactivity in organosoluble fractions, urine, or bile was quantified with a Tri-Carb 2500TR liquid scintillation analyzer (Packard, New Haven, CT). An aliquot of each sample was added to Emulsifier Scintillator 299 (Packard) or Hionicfluor (Packard), and then radioactivity was measured by liquid scintillation counting (LSC).

Samples (100−300 mg) of fecal homogenates, unextractable fecal residues, gastrointestinal contents, and tissues were combusted with a Tri-Carb 306 sample oxidizer (Packard) prior to LSC. Eight milliliters of Carbo−Sorb and 12 mL of Permafluor E^+ (Packard) were used as the $^{14}CO_2$ absorbent and the

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scintillator, respectively. ^{14}C recovery was >96% for the combustion analysis.

Quantification of radiocarbon on TLC plates was conducted by scraping methods (Nagahori et al., 1997). After localization of radioactive zones on TLC plates by autoradiography, the appropriate silica gel regions scraped from TLC plates were added to a scintillation vial containing 10 mL of Emulsifier Scintillator 299. The radioactivity in the vials was determined by LSC. The detection limits of radioactivity were calculated on the basis of the 2 times the background dpm (SN = 2). Samples that demonstrated counts lower than the detection limit were considered to be background and not used for the calculation.

Excretion Balance Study. Male and female Crj:CD(SD) rats at the age of 6 weeks were purchased from Charles River Japan Inc. (Kanagawa, Japan) and maintained in an air-conditioned room at a temperature of 21−25 °C and a humidity of 45−65% with an alternating 12-h light and 12-h dark cycle for 1 week before use, on pelleted diet (CRF-1, Clea, Tokyo, Japan) and water ad libitum. The animals treated with low (1 mg/kg) or high (300 mg/kg for males and 200 mg/kg for females) doses of [*phenyl*-^{14}C]furametpyr suspended in 0.5% methyl cellulose aqueous suspension at a rate of 5 mL/kg were housed in Metabolica CO_2 cages (Sugiyamagen Iriki, Tokyo, Japan) to allow the separate collection of urine, feces, and expired air at 6 h (urine only) and 1, 2, 3, 5, and 7 days (expired air was collected at 1 and 2 days only). Feces of each animal collected within 3 days after administration were homogenized with ~3-fold volumes of methanol using a Waring blender, and metabolites were extracted after centrifugation at 1000*g* for 10 min. In addition, the residues were shaken with methanol twice and water once. Combined extract and residual precipitate were radioassayed separately. Feces for 3−5 days and 5−7 days were homogenized with ~2-fold volumes of water using a Waring blender.

Bile Study. Seven-week-old male and female Crj:CD(SD) rats were bile-duct cannulated and treated at a rate of 1 mg/kg with [*phenyl*-^{14}C]furametpyr suspended in a 0.5% methyl cellulose aqueous solution (5 mL/kg). Dosed rats were housed in Bollman cages (Sugiyamagen Iriki) and given electrolytic fluid (Solita-T3, Shimizu Pharmacy, Shizuoka, Japan) ad libitum. Urine, feces, and bile were collected on the first and second day after administration, and radioactivity was measured according to the procedure described under Excretion Balance Study. The rats were sacrificed on the second day after administration, and gastrointestinal contents were collected.

Distribution of Metabolites to Tissues. Seven-week-old male and female Crj:CD(SD) rats were orally dosed with a low (1 mg/kg) or high (300 mg/kg for males and 200 mg/kg for females) dose of [*phenyl*-^{14}C]furametpyr suspended in 0.5% methyl cellulose aqueous solution at 5 mL/kg. Three rats each were sacrificed by bleeding at 0.5, 4, 8, 24, and 168 h after administration of the low dose and at 8, 24, 48, 72, and 168 h after administration of the high dose. Their tissues and organs were dissected out, and the amounts of ^{14}C distributed to tissues were measured.

Identification and Quantification of Metabolites. Urine, bile, and fecal extracts were pooled for each group and each sex. Blood, kidney, and liver samples were also pooled and homogenized with a Waring blender after the addition of an equivalent amount of distilled water. Thereafter, the homogenate was extracted with ~3-fold volume of methanol and centrifuged at 1000*g* for 10 min. The precipitates of kidney and liver were shaken additionally twice with methanol, and the precipitates of blood were shaken three times.

Metabolites in the urine, bile, fecal, and tissue extracts were separated and identified by two-dimensional TLC cochromatography with authentic standards using solvent systems B (first) and A (second) and quantified by LSC. The amounts of metabolites in urine, fecal extracts, bile, and tissues were quantified by scraping methods.

The identification of conjugates was carried out as follows. Metabolites were isolated by TLC using solvent system C and added to 1 mL of 0.2 M acetate buffer solution (pH 5.0) and β-glucuronidase (bovine liver, type B-1, Sigma, St. Louis, MO) or sulfatase (Limpets, type VII, Sigma). Hydrolysis was carried out by incubation at 37 °C overnight. D-Saccharic acid 1,4-lactone (Sigma) was added as a β-glucuronidase inhibitor to inhibit the glucuronidase activity in sulfatase. After incubation, the metabolites were extracted with methanol from the residues after the removal of water by nitrogen gas and analyzed by two-dimensional TLC cochromatography with authentic standards using solvent systems B (first) and A (second).

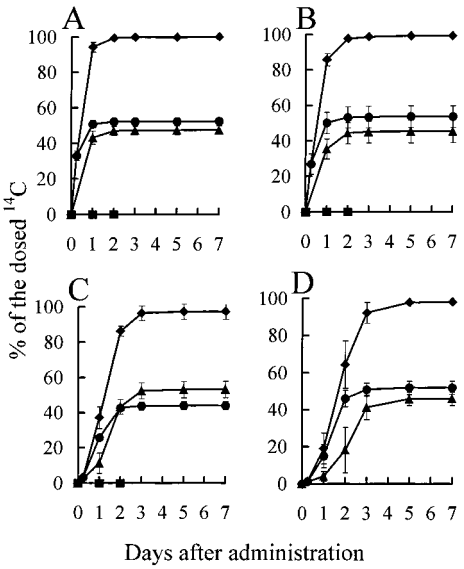

Figure 1. Cumulative ^{14}C excretion after a single oral administration of [*phenyl*-^{14}C]furametpyr to rats: (A) male, 1 mg/kg; (B) male, 300 mg/kg; (C) female, 1 mg/kg; (D) female, 200 mg/kg: (◆) total ^{14}C; (●) ^{14}C in urine; (▲) ^{14}C in feces; (■) ^{14}C in expired air. Points and bars are the mean values and standard deviations for data from five animals.

RESULTS

^{14}C Elimination. The cumulative ^{14}C excretion rates are shown in Figure 1. After a single oral administration of [*phenyl*-^{14}C]furametpyr to male and female rats at 1 mg/kg for the low dose and at 300 mg/kg (males) and 200 mg/kg (females) for the high dose, ^{14}C was rapidly excreted in all groups within 3 days after administration. ^{14}C excretion into feces, urine, and expired air within 7 days after administration was 97.4−100.1% of the dose (45.5−53.3% in feces, 44.1−53.8% in urine, and 0.01% in expired air). Excretion into expired air was negligible and not examined in high-dose females.

The period of ^{14}C excretion was extended with the high dose, recovery within 2 days after administration being 97.7−99.3% for the low dose and 64.4−86.3% for the high dose.

Sex dependence was also observed. ^{14}C recoveries within 1 day after administration of the low dose were 94.3 ± 2.82% in male rats and 85.7 ± 3.49% in female rats. The respective values after administration of the high dose were 37.2 ± 6.32% in male rats and 19.1 ± 8.35% in female rats.

^{14}C Concentrations in Tissues. The ^{14}C concentration−time profiles in tissues after a single oral administration of [*phenyl*-^{14}C]furametpyr at low and high doses are shown in Tables 1 and 2, respectively.

At the low dose, the ^{14}C concentrations of tissues other than gastrointestinal tracts were highest at the first sampling time, 0.5 h after administration, and thereafter decreased rapidly. Kidney and liver showed relatively high ^{14}C concentrations, accounting for 1.2 and

Table 1. ^{14}C Concentrations in Tissues of Rats Orally Dosed with [*phenyl*-^{14}C]Furametpyr at 1 mg/kg

tissue	μg equiv of furametpyr/g of wet tissue (ppm) at				
	0.5 h postdose	4 h postdose	8 h postdose	24 h postdose	168 h postdose
			Male		
blood	0.4 ± 0.06	0.1 ± 0.01	0.1 ± 0.02	0.01 ± 0.000	0.002 ± 0.0002
bone	0.1 ± 0.02	0.0 ± 0.01	0.0 ± 0.01	0.00 ± 0.000	0.000 ± 0.0000
bone marrow	0.3 ± 0.02	0.3 ± 0.01	0.1 ± 0.04	0.12 ± 0.019	<0.001
brain	0.2 ± 0.05	0.0 ± 0.02	0.0 ± 0.01	0.00 ± 0.001	<0.000
cecum	0.4 ± 0.02	1.8 ± 0.58	4.7 ± 0.98	0.32 ± 0.054	<0.000
cecum content	0.2 ± 0.02	10.6 ± 1.33	19.7 ± 2.67	1.45 ± 0.440	NE
fat	0.4 ± 0.10	0.1 ± 0.04	0.0 ± 0.01	0.00 ± 0.000	0.001 ± 0.0014
heart	0.5 ± 0.09	0.1 ± 0.03	0.0 ± 0.02	0.01 ± 0.001	0.001 ± 0.0000
kidney	1.2 ± 0.16	0.5 ± 0.14	0.2 ± 0.09	0.02 ± 0.002	0.002 ± 0.0002
large intestine	0.5 ± 0.14	0.6 ± 0.39	1.8 ± 1.28	0.16 ± 0.124	<0.000
large intestine content	0.3 ± 0.07	1.8 ± 0.78	9.5 ± 2.00	1.43 ± 0.269	NE
liver	3.8 ± 0.34	1.2 ± 0.06	0.6 ± 0.26	0.09 ± 0.004	0.004 ± 0.0007
lung	0.5 ± 0.03	0.1 ± 0.03	0.0 ± 0.02	0.01 ± 0.001	0.001 ± 0.0001
muscle	0.3 ± 0.05	0.1 ± 0.02	0.0 ± 0.01	0.00 ± 0.001	0.002 ± 0.0005
skin	0.3 ± 0.02	0.1 ± 0.02	0.1 ± 0.03	0.02 ± 0.013	0.002 ± 0.0021
small intestine	1.9 ± 1.33	1.5 ± 0.04	1.6 ± 0.68	0.12 ± 0.031	0.001 ± 0.0001
small intestine content	4.8 ± 0.70	18.9 ± 6.23	6.0 ± 2.04	0.56 ± 0.173	NE
spleen	0.4 ± 0.06	0.1 ± 0.02	0.0 ± 0.02	0.01 ± 0.001	0.001 ± 0.0002
stomach	7.3 ± 4.81	1.5 ± 0.49	1.3 ± 0.88	0.02 ± 0.012	0.000 ± 0.0001
stomach content	46.0 ± 33.60	9.6 ± 2.21	2.2 ± 2.26	0.17 ± 0.276	NE
testis	0.3 ± 0.08	0.1 ± 0.03	0.0 ± 0.01	0.00 ± 0.001	0.000 ± 0.0001
thyroid	0.5 ± 0.23	0.4 ± 0.27	0.1 ± 0.03	0.01 ± 0.001	<0.005
			Female		
blood	0.5 ± 0.03	0.2 ± 0.05	0.1 ± 0.01	0.02 ± 0.003	0.003 ± 0.0006
bone	0.2 ± 0.01	0.1 ± 0.01	0.0 ± 0.00	0.01 ± 0.002	0.001 ± 0.0001
bone marrow	0.1 ± 0.01	0.1 ± 0.02	0.0 ± 0.00	0.01 ± 0.003	<0.001
brain	0.5 ± 0.08	0.1 ± 0.04	0.0 ± 0.00	0.00 ± 0.000	<0.000
cecum	0.8 ± 0.23	1.9 ± 0.75	3.0 ± 0.45	0.61 ± 0.033	0.000 ± 0.0001
cecum content	0.3 ± 0.07	11.2 ± 6.77	13.4 ± 2.42	2.81 ± 0.515	NE
fat	0.7 ± 0.04	0.2 ± 0.08	0.0 ± 0.01	0.01 ± 0.002	<0.000
heart	0.7 ± 0.03	0.2 ± 0.05	0.1 ± 0.03	0.01 ± 0.004	0.001 ± 0.0001
kidney	1.2 ± 0.09	0.7 ± 0.10	0.2 ± 0.03	0.09 ± 0.068	0.002 ± 0.0003
large intestine	0.9 ± 0.23	0.6 ± 0.28	2.0 ± 0.90	0.46 ± 0.334	<0.000
large intestine content	0.3 ± 0.09	2.7 ± 1.10	13.6 ± 4.56	2.52 ± 1.903	NE
liver	4.2 ± 0.30	2.2 ± 0.53	0.9 ± 0.01	0.17 ± 0.114	0.004 ± 0.0005
lung	0.7 ± 0.04	0.2 ± 0.06	0.1 ± 0.01	0.01 ± 0.003	0.001 ± 0.0002
muscle	0.4 ± 0.02	0.2 ± 0.04	0.1 ± 0.01	0.01 ± 0.001	0.001 ± 0.0002
ovary	0.6 ± 0.02	0.2 ± 0.06	0.1 ± 0.01	0.01 ± 0.003	0.001 ± 0.0002
skin	0.4 ± 0.04	0.2 ± 0.05	0.1 ± 0.01	0.04 ± 0.005	0.002 ± 0.0009
small intestine	2.0 ± 0.44	3.6 ± 1.53	1.0 ± 0.42	0.19 ± 0.082	0.001 ± 0.0001
small intestine content	2.8 ± 0.42	16.1 ± 7.88	4.8 ± 1.25	0.96 ± 0.068	NE
spleen	0.5 ± 0.06	0.2 ± 0.02	0.1 ± 0.01	0.01 ± 0.003	0.001 ± 0.0001
stomach	12.2 ± 6.02	0.6 ± 0.20	0.4 ± 0.23	0.07 ± 0.026	0.000 ± 0.0001
stomach content	63.4 ± 11.95	5.4 ± 3.87	0.6 ± 0.66	0.34 ± 0.554	NE
thyroid	4.7 ± 3.56	0.2 ± 0.08	0.1 ± 0.01	0.02 ± 0.012	<0.006
uterus	0.4 ± 0.14	0.2 ± 0.04	0.0 ± 0.01	0.01 ± 0.003	0.000 ± 0.0001

[a] Data are the mean ± SD of values for three rats. SD, standard deviation; NE, not examined.

3.8 ppm in males, respectively, and 1.2 and 4.2 ppm in females. The time profile for ^{14}C concentrations in tissues was biphasic as in the blood. The biological half-lives of ^{14}C in blood within 24 h after administration were 5 h in males and females, whereas those between 24 and 168 h were 69 h in males and females. ^{14}C concentrations in tissues on the seventh day after administration were generally low, accounting for <0.004 ppm in males and females.

At the high dose, the ^{14}C concentrations of tissues other than gastrointestinal tracts reached peaks at 8 (first sampling time) or 24 h after administration. Liver and thyroid (only in female) showed relatively high ^{14}C concentrations, accounting for 559 and 55 ppm in males, respectively, and 207 and 333 ppm in females. The time profile for ^{14}C concentrations in all tissues was like that in blood; the biological half-lives between 24 and 48 h after administration were 6 h in males and females, and those between 48 and 168 h were 35 h in males and 69 h in females. ^{14}C concentrations in tissues on the seventh day after administration were generally low, accounting for <1.1 ppm in males and females.

Amounts of Metabolites in Excreta. Amounts of metabolites (percent of the dose) in urine and feces within 3 days after a single oral administration of [*phenyl*-^{14}C]furametpyr are presented in Table 3.

About 40 metabolites were detected in feces and urine, and 12 metabolites and 4 glucuronides were identified. A small amount of unchanged furametpyr was detected only in feces accounting for 0.1−0.5% of the dose. Major metabolites in feces were DM-Fur-COOH, DM-Fur-CH_2-OH, and 3-CH_2OH-DM-Fur-CH_2OH with the low dose, accounting for 1.5−4.3%, and DM-Fur-OH, DM-Fur-COOH, and Fur-COOH with the high dose, accounting for 1.5−3.9%. Other identified metabolites in feces were <2.8%. Major metabolites in urine were DM-Fur-COOH, DM-Fur-HK-CH_2OH, Fur-COOH, and 3-CH_2-OH-DM-Fur-CH_2OH with the low dose, accounting for 2.1−7.8%, and DM-Fur-HK-OH, DM-Fur-HK-CH_2OH, and Fur-COOH with the high dose, accounting for 2.8−

Table 2. ^{14}C Concentrations in Tissues of Rats Orally Dosed with [*phenyl*-^{14}C]Furametpyr at 300 mg/kg (Males) or 200 mg/kg (Females)[a]

	μg equiv of furametpyr/g of wet tissue (ppm) at				
tissue	8 h postdose	24 h postdose	48 h postdose	72 h postdose	168 h postdose
			Male		
blood	34 ± 5.7	38 ± 23.6	3 ± 0.4	3 ± 0.8	0.4 ± 0.08
bone	10 ± 3.5	14 ± 11.5	1 ± 0.2	1 ± 0.1	0.2 ± 0.04
bone marrow	36 ± 6.9	33 ± 23.8	1 ± 0.2	1 ± 0.1	0.2 ± 0.02
brain	42 ± 10.6	27 ± 22.3	0 ± 0.1	0 ± 0.1	0.2 ± 0.06
cecum	205 ± 26.8	547 ± 166.6	79 ± 47.3	100 ± 112.0	0.3 ± 0.09
cecum content	624 ± 72.3	1620 ± 571.1	478 ± 195.2	1210 ± 88.2	NE
fat	110 ± 31.9	88 ± 67.0	1 ± 0.6	1 ± 0.3	0.3 ± 0.14
heart	58 ± 10.8	53 ± 27.6	1 ± 0.4	2 ± 0.6	0.3 ± 0.08
kidney	78 ± 11.9	91 ± 47.7	5 ± 2.2	8 ± 2.1	0.5 ± 0.12
large intestine	115 ± 17.4	373 ± 27.1	57 ± 31.2	52 ± 30.5	0.2 ± 0.05
large intestine content	522 ± 92.6	1410 ± 1194	619 ± 154.2	1230 ± 543.2	NE
liver	142 ± 25.4	559 ± 640.7	18 ± 5.3	21 ± 6.7	1.1 ± 0.24
lung	58 ± 11.2	55 ± 29.9	2 ± 0.4	2 ± 0.6	0.3 ± 0.10
muscle	34 ± 7.9	33 ± 21.4	1 ± 0.1	1 ± 0.3	0.4 ± 0.13
skin	35 ± 7.7	34 ± 17.6	2 ± 0.7	5 ± 2.0	4.5 ± 2.99
small intestine	170 ± 29.0	360 ± 39.5	19 ± 5.4	38 ± 12.4	0.5 ± 0.24
small intestine content	436 ± 91.6	1130 ± 283.6	196 ± 65.5	255 ± 47.4	NE
spleen	42 ± 7.6	40 ± 23.3	1 ± 0.2	1 ± 0.4	0.4 ± 0.17
stomach	276 ± 13.2	260 ± 41.4	4 ± 3.5	10 ± 0.9	0.2 ± 0.07
stomach content	5120 ± 3035	4320 ± 2804	19 ± 61.6	91 ± 63.7	NE
testis	37 ± 7.9	40 ± 12.6	1 ± 0.2	1 ± 0.3	0.2 ± 0.07
thyroid	55 ± 11.5	55 ± 12.4	2 ± 0.8	2 ± 0.1	3.8 ± 3.87
			Female		
blood	26 ± 5.4	44 ± 12.0	3 ± 0.5	2 ± 0.2	0.5 ± 0.09
bone	9 ± 1.6	16 ± 3.7	1 ± 0.0	1 ± 0.1	0.1 ± 0.07
bone marrow	29 ± 7.1	46 ± 13.6	2 ± 0.4	1 ± 0.1	0.7 ± 0.26
brain	38 ± 6.3	48 ± 21.6	1 ± 0.7	0 ± 0.1	<0.1
cecum	113 ± 25.5	299 ± 99.1	97 ± 5.6	159 ± 61.4	0.2 ± 0.11
cecum content	335 ± 150	924 ± 270.4	558 ± 139.1	897 ± 150.5	NE
fat	94 ± 21.3	120 ± 51.9	1 ± 0.2	6 ± 7.1	0.3 ± 0.13
heart	46 ± 7.0	74 ± 21.4	2 ± 1.0	1 ± 0.2	0.3 ± 0.12
kidney	56 ± 8.8	106 ± 13.1	7 ± 1.7	5 ± 0.7	0.5 ± 0.12
large intestine	96 ± 28.4	193 ± 100.8	52 ± 19.6	94 ± 21.0	0.2 ± 0.07
large intestine content	294 ± 101.2	636 ± 520.3	601 ± 91.1	1180 ± 176.0	NE
liver	130 ± 20.3	207 ± 45.7	21 ± 2.6	18 ± 3.5	1.1 ± 0.21
lung	48 ± 5.3	76 ± 21.7	2 ± 0.3	2 ± 0.3	0.3 ± 0.11
muscle	27 ± 4.9	45 ± 12.9	1 ± 0.2	1 ± 0.1	0.3 ± 0.07
ovary	51 ± 13.6	76 ± 23.6	2 ± 0.5	1 ± 0.0	0.3 ± 0.07
skin	30 ± 7.6	48 ± 16.1	6 ± 2.9	3 ± 0.9	0.6 ± 0.42
small intestine	78 ± 14.7	243 ± 11.3	29 ± 13.5	56 ± 57.2	0.2 ± 0.10
small intestine content	240 ± 40.3	534 ± 140.9	266 ± 98.7	284 ± 292.3	NE
spleen	33 ± 5.3	181 ± 205.9	2 ± 0.5	2 ± 1.2	0.3 ± 0.08
stomach	177 ± 54.5	433 ± 188.4	10 ± 3.6	6 ± 0.8	0.1 ± 0.07
stomach content	5010 ± 1485	5300 ± 5770	125 ± 94.4	18 ± 11.3	NE
thyroid	38 ± 2.9	333 ± 171.4	8 ± 8.7	3 ± 0.3	<0.8
uterus	27 ± 8.3	49 ± 11.0	2 ± 0.5	1 ± 0.1	0.1 ± 0.03

[a] Data are the mean value ± SD of values for three rats. SD, standard deviation; NE, not examined.

8.1%. Other identified metabolites in urine were <5.0%. Ten glucuronides were detected in urine, identified to be conjugates of DM-Fur-OH, DM-Fur-HK-CH_2OH, Fur-OH, DM-Fur-CH_2OH, or unidentified metabolites, accounting for <2.1%.

Metabolites in Blood, Kidney, and Liver. Concentrations of metabolites in blood, kidney, and liver of rats after a single oral administration of [*phenyl*-^{14}C]-furametpyr at low dose are presented in Tables 4, 5, and 6, respectively.

The concentrations of unchanged furametpyr in blood, kidney, and liver were highest at first sampling time, 0.5 h after administration, and then decreased rapidly. The highest concentrations of unchanged furametpyr in blood, kidney, and liver accounted for 7, 29, and 327 ng/g in males, respectively, and 8, 42, and 247 ng/g in females, respectively. The percentages of furametpyr with respect to the total distribution of ^{14}C were 1.7−1.8% in blood, 2.4−3.5% in kidney, and 5.8−8.6% in liver at the peak concentrations, indicating that the compound was rapidly metabolized after absorption.

Major metabolites in blood, kidney, and liver were DM-Fur, DM-Fur-OH, and DM-Fur-CH_2OH. The concentrations of these metabolites were generally highest at first sampling time, 0.5 h after administration, and DM-Fur decreased more rapidly than DM-Fur-OH and DM-Fur-CH_2OH.

Bile Study. The ^{14}C excretion rates within 2 days after a single oral administration of [*phenyl*-^{14}C]-furametpyr at a dose of 1 mg/kg (low dose) are shown in Table 7.

^{14}C excretion was rapid in both males and females, with average rates for three animals within 2 days after administration of 95.7% in males [feces, 1.2%; urine, 40.2%; gastrointestinal contents, less than the limit of detection (LOD); and bile, 54.2%] and 95.3% in females (feces, 1.5%; urine, 41.2%; gastrointestinal contents, <LOD; and bile, 52.5%).

The results suggested that a major proportion of ^{14}C in feces was excreted from bile after the absorption of furametpyr, because the sum of ^{14}C excreted into gastrointestinal contents, feces, and bile (54.1−55.4%)

Table 3. Amounts of Metabolites in Urine and Feces within 2 Days after a Single Oral Administration of [*phenyl*-^{14}C]Furametpyr to Rats at Low (1 mg/kg) or High (300 mg/kg for Males and 200 mg/kg for Females) Doses

	amount (% of the dosed ^{14}C)			
	low dose		high dose	
metabolite	male	female	male	female
Feces				
DM-Fur	0.9	1.5	0.6	1.0
furametpyr	0.1	0.1	0.5	0.3
DM-Fur-OH	2.8	2.1	3.7	2.1
DM-Fur-HK-OH	1.4	0.3	2.0	1.2
Fur-OH	0.8	0.5	1.5	0.5
3-CH_2OH-DM-Fur-OH	1.3	2.6	2.2	1.7
DM-Fur-COOH	4.3	1.5	3.3	1.5
DM-Fur-HK-CH_2OH	1.0	0.9	2.5	1.1
DM-Fur-CH_2OH	4.2	3.5	1.5	1.3
DM-Fur-CH_2OH-OH	0.9	0.7	0.9	0.5
Fur-COOH	1.6	2.8	3.9	3.4
3-CH_2OH-DM-Fur-CH_2OH	2.8	3.3	0.4	0.4
unidentified metabolites[a]	17.2	20.2	22.3	21.0
unextractable ^{14}C	8.1	5.0	7.7	5.5
total	47.4	45.1	52.9	41.3
Urine				
DM-Fur	0.1	0.2	0.1	1.2
DM-Fur-OH	0.6	0.6	0.6	0.7
DM-Fur-HK-OH	2.0	1.0	4.4	2.8
3-CH_2OH-DM-Fur-COOH	0.3	0.2	ND[d]	ND
3-CH_2OH-DM-Fur-OH	0.7	1.7	1.1	0.8
DM-Fur-COOH	5.6	2.3	3.5	2.2
DM-Fur-HK-CH_2OH	7.8	3.3	8.1	7.6
DM-Fur-CH_2OH	1.7	5.0	0.7	2.5
DM-Fur-CH_2OH-OH	1.0	0.9	0.8	1.1
Fur-COOH	4.2	4.7	2.9	4.5
3-CH_2OH-DM-Fur-CH_2OH	2.1	7.6	1.2	2.3
glucuronides-1[b]	1.0	0.8	0.1	1.3
glucuronides-2[b]	1.3	0.8	1.3	2.1
glucuronides-3[b]	1.1	1.0	1.7	1.2
glucuronide-4[b]	1.7	0.9	2.0	1.8
unidentified metabolites[c]	21.3	22.8	15.5	18.9
total	52.4	53.6	43.9	51.0
total	99.8	98.7	96.8	92.4

[a] At least 34 unidentified metabolites. [b] Glucuronides 1: glucuronides of two unidentified metabolites. Glucuronides-2: glucuronides of DM-Fur-OH, DM-Fur-HK-CH_2OH, and an unidentified metabolite. Glucuronides-3: glucuronides of Fur-OH, DM-Fur-CH_2OH, and two unidentified metabolites. Glucuronide-4: glucuronide of an unidentified metabolite. [c] At least 22 unidentified metabolites. [d] ND, not detected.

Table 4. Concentration of Metabolites in the Blood of Rats after a Single Oral Dose of [*phenyl*-^{14}C]Furametpyr at Low Dose (1 mg/kg)[a]

	ng equiv of furametpyr/g of wet tissue (ppb) at			
metabolite	0.5 h postdose	4 h postdose	8 h postdose	24 h postdose
Male				
DM-Fur	78	8	2.0	ND[e]
furametpyr	7	0	0.4	ND
DM-Fur-HK	4	0	0.0	ND
DM-Fur-OH	12	4	1.6	ND
Fur-HK	9	1	0.1	ND
DM-Fur-HK-OH	5	2	0.9	ND
DM-Fur-COOH	1	ND	ND	ND
DM-Fur-HK-CH_2OH	7	6	0.7	0.1
DM-Fur-CH_2OH	13	11	2.6	0.1
Fur-COOH	20	6	2.0	ND
3-CH_2OH-DM-Fur-CH_2OH	2	2	0.4	ND
glucuronides-1[b]	3	4	1.1	ND
glucuronides-2[b]	8	4	1.6	ND
glucuronide-3[b]	7	6	1.4	ND
unidentified metabolites[c]	188	64	23.3	3.6
unextractable ^{14}C	13	14	10.5	5.4
total	377	132	48.6	9.2
Female				
DM-Fur	173	27	2.3	0.3
furametpyr	8	ND	ND	ND
DM-Fur-HK	4	1	ND	ND
DM-Fur-OH	29	18	4.1	ND
Fur-HK	9	0	ND	ND
DM-Fur-HK-OH	3	5	1.1	ND
DM-Fur-COOH	3	1	ND	ND
DM-Fur-HK-CH_2OH	3	5	0.7	0.2
DM-Fur-CH_2OH	36	38	7.2	1.0
Fur-COOH	9	2	1.5	0.3
3-CH_2OH-DM-Fur-CH_2OH	6	7	1.4	0.1
glucuronides-1	1	5	1.4	0.3
glucuronides-2	4	2	0.1	ND
glucuronide-3	7	3	2.0	0.4
unidentified metabolites[d]	158	78	38.6	9.4
unextractable ^{14}C	6	14	11.8	6.8
total	458	206	72.1	18.8

[a] Analysis was conducted for samples pooled from three rats. [b] Glucuronides-1: glucuronides of two unidentified metabolites. Glucuronides-2: glucuronides of DM-Fur-OH, DM-Fur-HK-CH_2OH, Fur-OH, DM-Fur-CH_2OH, and three unidentified metabolites. Glucuronide-3: glucuronide of an unidentified metabolite. [c] At least 25 unidentified metabolites. [d] At least 21 unidentified metabolites. [e] ND, not detected.

was almost the same as that excreted in feces (45.5−47.6%) of normal rats at the 1 mg/kg low dose. The absorption ratio was calculated to be >93.7% in rat at 1 mg/kg by the addition of the excretion rates for ^{14}C in urine and bile.

Amounts of metabolites (percent of the dose) identified in bile and urine within 2 days after a single oral administration of [*phenyl*-^{14}C]furametpyr are shown in Table 8.

A total of 26 metabolites was detected in bile and urine, and 5 metabolites and 4 conjugates were identified. No unchanged furametpyr was detected in either bile or urine. Major metabolites in bile were glucuronic acid conjugates of hydroxyl derivatives (DM-Fur-OH, DM-Fur-HK-CH_2OH, Fur-OH, and DM-Fur-CH_2OH), accounting for totally 34.8−37.2% of the dose. Other identified metabolites made up 0.2−2.0% of the dose. Major metabolites in urine were DM-Fur-COOH, DM-Fur-HK-CH_2OH, Fur-COOH, and 3-CH_2OH-DM-Fur-CH_2OH, accounting for 0.5−3.7%. Ten glucuronides were detected in urine, conjugates of DM-Fur-OH, DM-Fur-HK-CH_2OH, Fur-OH, DM-Fur-CH_2OH,or unidentified metabolites, accounting for <2.9%.

Extents of Metabolic Reactions. The extents of metabolic reactions were calculated by summing the amounts of metabolites in excreta corresponding to each metabolic reaction, and the results are summarized in Table 9. The *N*-demethylation and oxidation of the methyl group at C1 of the 1,3-dihydroisobenzofuran ring predominated, accounting for 33.3−44.1 and 28.5−37.5% of the dose, respectively. Other metabolic reactions were minor, accounting for <17.1% of the dose.

DISCUSSION

The present study revealed that, with a single oral administration of [*phenyl*-^{14}C]furametpyr to male and female rats, the radiocarbon was rapidly and completely excreted into the feces and urine at 44.1−53.8 and 45.5−53.3%, respectively, with total ^{14}C recoveries of 97−100%. ^{14}C excretion in the high-dose group took longer for completion than in the low-dose group, presumably because of the delay of test compound uptake across the gastrointestinal tract and saturation of hepatic enzyme systems (Sue et al., 1992). ^{14}C excretion in female rats

Table 5. Concentration of Metabolites in the Kidneys of Rats after a Single Oral Dose of [*phenyl*-^{14}C]Furametpyr at Low Dose (1 mg/kg)[a]

metabolite	ng equiv of furametpyr/g of wet tissue (ppb) at 0.5 h postdose	4 h postdose	8 h postdose	24 h postdose
	Male			
DM-Fur	238	24	12	ND[e]
furametpyr	29	0	ND	ND
DM-Fur-HK	58	ND	ND	ND
DM-Fur-OH	76	36	ND	ND
Fur-HK	12	1	ND	ND
DM-Fur-HK-OH	3	ND	ND	ND
3-CH_2OH-DM-Fur-OH	2	ND	ND	ND
DM-Fur-COOH	68	ND	ND	ND
DM-Fur-CH_2OH	43	18	3	ND
Fur-COOH	72	23	ND	ND
3-CH_2OH-DM-Fur-CH_2OH	15	0	14	ND
glucuronides-1[b]	13	23	3	ND
glucuronides-2[b]	20	18	ND	ND
glucuronide-3[b]	24	20	ND	ND
unidentified metabolites[c]	498	360	177	14
unextractable ^{14}C	25	11	11	5
total	1200	533	220	19
	Female			
DM-Fur	494	62	ND	ND
furametpyr	42	6	ND	ND
DM-Fur-HK	12	ND	ND	ND
DM-Fur-OH	71	60	6	ND
Fur-HK	1	2	ND	ND
DM-Fur-HK-OH	ND	ND	ND	ND
3-CH_2OH-DM-Fur-OH	ND	ND	ND	ND
DM-Fur-COOH	ND	ND	ND	ND
DM-Fur-CH_2OH	158	85	17	ND
Fur-COOH	38	22	7	ND
3-CH_2OH-DM-Fur-CH_2OH	24	38	3	ND
glucuronides-1	5	24	8	ND
glucuronides-2	ND	ND	ND	ND
glucuronide-3	8	18	ND	ND
unidentified metabolites[d]	348	343	124	78
unextractable ^{14}C	4	7	4	7
total	1210	666	169	85

[a] Analysis was conducted for samples pooled from three rats. [b] Glucuronides-1: glucuronides of two unidentified metabolites. Glucuronides-2: glucuronides of DM-Fur-OH, DM-Fur-HK-CH_2OH, Fur-OH, DM-Fur-CH_2OH, and three unidentified metabolites. Glucuronide-3: glucuronide of an unidentified metabolite. [c] At least 24 unidentified metabolites. [d] At least 10 unidentified metabolites. [e] ND, not detected.

Table 6. Concentration of Metabolites in the Livers of Rats after a Single Oral Dose of [*phenyl*-^{14}C]Furametpyr at Low Dose (1 mg/kg)[a]

netabolite	ng equiv of furametpyr/g of wet tissue (ppb) at 0.5 h postdose	4 h postdose	8 h postdose	24 h postdose
	Male			
DM-Fur	1330	166	64	2
furametpyr	327	18	5	ND[e]
DM-Fur-HK	236	26	8	ND
DM-Fur-OH	315	113	37	1
Fur-HK	7	2	ND	ND
DM-Fur-HK-OH	11	14	5	ND
3-CH_2OH-DM-Fur-OH	26	ND	ND	ND
DM-Fur-COOH	46	13	7	ND
DM-Fur-HK-CH_2OH	18	45	6	ND
DM-Fur-CH_2OH	159	122	46	4
Fur-COOH	30	ND	ND	ND
3-CH_2OH-DM-Fur-CH_2OH	17	9	7	ND
glucuronides-1[b]	13	18	8	ND
glucuronides-2[b]	46	11	ND	ND
glucuronide-3[b]	78	30	10	ND
unidentified metabolites[c]	926	491	303	50
unextractable ^{14}C	220	135	112	35
total	3800	1210	618	91
	Female			
DM-Fur	2230	299	95	ND
furametpyr	247	1	1	ND
DM-Fur-HK	57	15	2	ND
DM-Fur-OH	273	195	91	6
Fur-HK	7	2	ND	ND
DM-Fur-HK-OH	ND	13	ND	ND
3-CH_2OH-DM-Fur-OH	10	6	ND	ND
DM-Fur-COOH	ND	ND	ND	ND
DM-Fur-HK-CH_2OH	ND	56	6	ND
DM-Fur-CH_2OH	463	565	184	17
Fur-COOH	34	ND	ND	ND
3-CH_2OH-DM-Fur-CH_2OH	31	1	16	2
glucuronides-1	20	67	18	2
glucuronides-2	14	21	3	ND
glucuronide-3	47	41	10	ND
unidentified metabolites[d]	642	713	385	106
unextractable ^{14}C	153	199	125	35
total	4230	2200	937	169

[a] Analysis was conducted for samples pooled from three rats. [b] Glucuronides-1: glucuronides of two unidentified metabolites. Glucuronides-2: glucuronides of DM-Fur-OH, DM-Fur-HK-CH_2OH, Fur-OH, DM-Fur-CH_2OH, and three unidentified metabolites. Glucuronide-3: glucuronide of an unidentified metabolite. [c] At least 35 unidentified metabolites. [d] At least 25 unidentified metabolites. [e] ND, not detected.

was also found to be slower than that in male rats. Sex-related differences have often been reported in rats, because of variation in profiles of P450 isoforms between males and females (Mugford et al., 1998). In most case, male rats metabolize chemicals more rapidly than females, as shown with diazepam (Okuyama et al., 1997) and amitriptyline (Masubuchi et al., 1996) *N*-demethylation. The extents of *N*-demethylation, oxidation at C1 of the 1,3-dihydroisobenzofuran ring, and hydroxylation in female rats were less than those in male rats as shown in Table 9.

Enterohepatic circulation often prolongs the toxicity of chemicals by maintaining their blood concentration (Shepard et al., 1989). About half of the dose of furametpyr was excreted into bile, but enterohepatic circulation of metabolites seemed to be limited. As shown in Tables 1 and 2, the ^{14}C concentrations in blood rapidly decreased after 0.5 and 24 h, respectively, with the low and high doses. ^{14}C recoveries in urine (40.3−41.2%) and bile (52.5−54.2%) in the bile study were almost the same as those for urine and feces in the excretion study, respectively, as shown in Table 7 and Figure 1. The metabolites in urine in the bile study (see Table 8) were also similar to those in the excretion study (Table 3). Conjugated metabolites in bile thus appeared to be fully hydrolyzed in the intestine, rather than being reabsorbed, and directly excreted into feces.

^{14}C concentrations in major tissues reached a peak within 0.5 h for the low dose and within 8−24 h for the high dose and thereafter decreased rapidly to extremely low levels on the seventh day. The major metabolites in blood, liver, and kidney were DM-Fur at 0.5 h after administration and DM-Fur-OH and DM-Fur-CH_2OH at 8 h after administration, pointing to rapid *N*-demethylation and then sequential oxidation at C1, C3, or C7 of the 1,3-isobenzofuran ring or at the C3 of the pyrazole ring. A large amount of furametpyr was apparently metabolized during the first pass.

Amounts of metabolites in excreta differed between the low- and high-dose groups (see Table 9) with extents of *N*-demethylation and methyl group oxidation greater in the former case. In contrast, hydroxylation was more predominant with the high dose. This might imply saturation of the *N*-demethylation reaction. The K_m of *N*-demethylation described in the previous paper using human recombinant P450 (∼20−50 μM; Nagahori et al.,

Table 7. ^{14}C Excretion into Urine, Bile, Feces, and Gastrointestinal Contents within 2 Days after a Single Oral Administration of [*phenyl*-^{14}C]Furametpyr to Bile-Duct Cannulated Rats at 1 mg/kg[a]

	% of the dosed ^{14}C at		
	0−1 day	1−2 days	total
	Male		
urine	34.0 ± 21.20	6.3 ± 3.52	40.3 ± 18.94
bile	45.0 ± 12.97	9.2 ± 8.01	54.2 ± 18.90
feces	0.3 ± 0.48	0.9 ± 0.78	1.2 ± 1.15
cont[b]	NE	0.0 ± 0.01	0.0 ± 0.01
total	79.3 ± 11.88	16.4 ± 10.61	95.7 ± 1.27
	Female		
urine	39.2 ± 30.58	2.0 ± 1.54	41.2 ± 32.06
bile	51.6 ± 35.24	0.9 ± 1.26	52.5 ± 33.98
feces	0.9 ± 0.17	0.7 ± 0.26	1.6 ± 0.42
cont	NE	0.0 ± 0.01	0.0 ± 0.01
total	91.7 ± 5.37	3.6 ± 2.61	95.3 ± 2.93

[a] Data are represented as the mean value ± SD of three rats. SD, standard deviation. NE, not examined. [b] Cont, gastrointestinal contents (cecum, large intestine, small intestine, and stomach contents).

Table 8. Amounts of Metabolites in Urine and Bile within 2 Days after a Single Oral Administration of [*phenyl*-^{14}C]Furametpyr to Bile-Duct Cannulated Rats at 1 mg/kg

	amount (% of the dosed ^{14}C)	
metabolite	male	female
	Bile	
DM-Fur	ND[d]	0.4
DM-Fur-HK-OH	0.7	0.2
DM-Fur-COOH	1.6	1.3
DM-Fur-HK-CH_2OH	0.3	ND
Fur-COOH	1.8	2.0
glucuronides-1[a]	7.3	7.5
glucuronides-2[a]	11.3	6.6
glucuronides-3[a]	12.5	16.7
glucuronide-4[a]	3.7	6.4
unidentified metabolites[b]	15.1	11.5
total	54.2	52.5
	Urine	
DM-Fur-COOH	3.1	1.9
DM-Fur-HK-CH_2OH	3.7	0.5
DM-Fur-CH_2OH	1.0	1.2
Fur-COOH	2.8	3.6
3-CH_2OH-DM-Fur-CH_2OH	0.7	3.1
glucuronides-1	0.8	0.5
glucuronides-2	2.6	1.3
glucuronides-3	2.6	1.6
glucuronide-4	2.4	2.9
unidentified metabolites[c]	20.6	24.8
total	40.2	41.2
total	94.4	93.7

[a] Glucuronides-1: glucuronides of two unidentified metabolites. Glucuronides-2: glucuronides of DM-Fur-OH, DM-Fur-HK-CH_2OH, and an unidentified metabolite. Glucuronides-3: glucuronides of Fur-OH, DM-Fur-CH_2OH, and two unidentified metabolites. Glucuronide-4: glucuronide of an unidentified metabolite. [b] At least 17 unidentified metabolites. [c] At least 15 unidentified metabolites. [d] ND, not detected.

2000) was lower than the estimated maximum hepatic concentration of furametpyr (40−130 μM) at the high dose (200 or 300 mg/kg) in this study. Hepatic concentration for the high dose was estimated proportionally from maximum hepatic concentration for the low dose (1 mg/kg), accounting for 0.74−0.98 μM, and ^{14}C liver level in low and high doses, accounting for 3.8−4.2 and 207−559 ppm, respectively. The K_m of hydroxylation is usually higher than that of *N*-demethylation, as reported for diazepam (Ono et al., 1996), so no saturation of hydroxylation may occur.

Table 9. Extents of Metabolic Reactions of [*phenyl*-^{14}C]Furametpyr

	% of the dosed ^{14}C					
	intact rats				bile-duct cannulated rats[c]	
	low dose[a]		high dose[b]		low dose[a]	
metabolic reaction[d]	male	female	male	female	male	female
1	41.4	39.1	37.6	32.0	11.0	8.6
2	7.2	15.4	5.0	5.2	0.7	3.1
3	37.5	36.6	29.7	28.5	14.9	8.1
4	12.2	5.5	17.0	12.7	4.7	0.7
5	11.4	10.3	17.1	11.3	0.7	0.2
6	5.2	3.5	5.1	6.4	43.2	43.3

[a] Rats were dosed with [phenyl-^{14}C]furametpyr at low dose (1 mg/kg). [b] Rats were dosed with [phenyl-^{14}C]furametpyr at high dose (300 mg/kg for males and 200 mg/kg for females). [c] Bile-duct cunnulated rats were dosed with [phenyl-^{14}C]furametpyr at low dose (1 mg/kg). [d] Metabolic reactions: 1, *N*-demethylation; 2, oxidation of the methyl group at C3 of the pyrazole ring; 3, oxidation of the methyl groups at C1 of the 1,3-dihydroisobenzofuran ring; 4, hydroxylation at C3 of the 1,3-dihydroisobenzofuran ring; 5, hydroxylation at C7 of the 1,3-dihydroisobenzofuran ring; 6, glucuronidation.

A small amount of Fur-HK was detected in tissues but not in urine, feces, and bile, suggesting that this form is metabolized rapidly without forming a glucuronide. Other metabolites in tissues were the same as those isolated from excreta reported in the previous paper (Nagahori et al., 2000). In the previous paper, Fur-COOH and 3-CH_2OH-DM-Fur-COOH were identified as lactone metabolites with an artificial mechanism, conversion reaction between furancarboxylic acid and hydroxyl lactone, but we cannot know whether lactone or furancarboxylic acid was formed in vivo. Therefore, these metabolites are shown as furancarboxylic acid in the paper.

On the basis of the metabolites identified in this study, the major biotransformation reactions of furametpyr in rats can be concluded to be as follows: (1) *N*-demethylation; (2) oxidation of the methyl group at C3 of the pyrazole ring; (3) oxidation of the methyl group at C1 of the 1,3-dihydroisobenzofuran ring; (4) hydroxylation at C3 of the 1,3-dihydroisobenzofuran ring; (5) hydroxylation at C7 of the 1,3-dihydroisobenzofuran ring; and (6) glucuronide conjugation of hydroxyl groups generated by the aforementioned reactions.

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