Metabolism of Pyriproxyfen. 2. Comparison of *in Vivo* Metabolism between Rats and Mice

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The metabolic fate of pyriproxyfen [4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy]propyl ether, Sumilarv) was examined in rats and mice given single oral doses of [*pyridyl*-2,6-^{14}C]- or [*phenoxyphenyl*-^{14}C]-pyriproxyfen at doses of 2 and 1000 mg/kg. The carbon-14 was excreted almost completely into urine and feces within 7 days after dosing and fecal excretion of carbon-14 predominated in both animals. Excretion of carbon-14 into feces and urine was, respectively, 84–97% and 4–12% of the dose in rats and 64–91% and 9–38% in mice. Major metabolic reactions of pyriproxyfen were (*1*) hydroxylation at the 4-position of the terminal phenyl ring, (*2*) hydroxylation at the 2-position of the terminal phenyl ring, (*3*) hydroxylation at the 5-position of the pyridyl ring, (*4*) dephenylation, (*5*) cleavage of ether linkages, and (*6*) conjugation of the resultant phenols with sulfuric acid or glucuronic acid. Although there was generally no marked difference in the metabolic profile of pyriproxyfen between the two species, significant sex-related differences were found in metabolic reactions *1*, *3*, and *6* in the rat but not in the mouse.

Keywords: *Metabolism; pyriproxyfen; excretion; biotransformation; rat; mouse*

INTRODUCTION

Pyriproxyfen [4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy)-propyl ether], newly synthesized in our company, has a high juvenile hormone mimic activity. The high efficacy of pyriproxyfen as a control agent for flies, mosquitoes, and cockroaches has recently been reported (Estrada and Mulla, 1986; Hatakoshi et al., 1987; Kawada et al., 1987, 1988, and 1989). This compound is now in the advanced developmental stage and has been undergoing toxicological evaluation, including studies of metabolism.

One metabolic study of pyriproxyfen in rats, using [*phenoxyphenyl*-^{14}C]pyriproxyfen, indicates the following metabolic fate (Matsunaga et al., 1995): (*1*) excretion is rapid and complete; (*2*) tissue residues are small; and (*3*) major metabolic reactions are hydroxylation at the 4- or 2-position of the terminal phenyl ring and the 5-position of the pyridyl ring, cleavage of the ether linkages, and conjugation of the resultant phenols with sulfuric acid.

The purpose of the present study was to investigate the metabolism of pyriproxyfen in rats in more detail and to compare it with that in mice. To this end, metabolism studies of pyriproxyfen were conducted in rats using [*pyridyl*-2,6-^{14}C]pyriproxyfen and in mice using [*phenoxyphenyl*-^{14}C]pyriproxyfen.

MATERIALS AND METHODS

Chemicals. [*phenoxyphenyl*-^{14}C]Pyriproxyfen and [*pyridyl*-2,6-^{14}C]pyriproxyfen were prepared at the Environmental Health Science Laboratory, Sumitomo Chemical Company, Ltd., Hyogo, Japan. [*phenoxyphenyl*-^{14}C]Pyriproxyfen was uniformly labeled with carbon-14 at the phenoxyphenyl ring with a specific activity of 2.73 GBq/mmol. [*pyridyl*-2,6-^{14}C]-Pyriproxyfen was labeled at the 2 and 6 positions of the pyridyl ring with a specific activity of 4.27 GBq/mmol (Figure 1). These chemicals had a purity of >98% as shown by thin-layer chromatography (TLC) in two different solvent systems (*n*-hexane:acetone, 4:1; and chloroform:ethyl acetate 7:3).

The following authentic standards are used: (*RS*)-5-hydroxy 2-{1-methyl-2-(4-phenoxyphenoxy)}ethoxypyridine (5″-OH-pyr) was synthesized at the Pesticide Laboratory, Takarazuka Research Center, Sumitomo Chemical Company, Ltd. (Hyogo, Japan). 4-(4-Hydroxyphenoxy)phenyl (*RS*)-2-(2-pyridyloxy)-propyl ether (4′-OH-pyr), 4-(2-hydroxyphenoxy)phenyl (*RS*)-2-(2-pyridyloxy)propyl ether (2′-OH-pyr), 4-hydroxyphenyl (*RS*)-2-(2-pyridyloxy)propyl ether (DPH-pyr), and (*RS*)-2-(2-pyridyloxy)propionic acid (PYPAC) were synthesized at Sumika Chemical Company, Ltd. (Osaka, Japan). 4-(4-Hydroxyphenoxyphenyl 2-(5-hydroxypyridyl-2-oxy)propyl ether (5″,4′-OH-pyr) was isolated from the feces of rats given [*phenoxyphenyl*-^{14}C]pyriproxyfen and identified by spectroanalysis (Matsunaga et al., 1995). Analytical data for 5″-OH-pyr and PYPAC are as follows. 5″-OH-pyr: ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.45 (3H, d), 4.09 (2H, m), 5.40 (1H, m), 6.66 (1H, d), 7.06–6.88 (8H, m), 7.17 (1H, dd), 7.32 (1H, t), 7.76 (1H, d); EI-MS (*m*/*z*, %) 337 (M^+). PYPAC: ^{1}H NMR (270 MHz, $CDCl_3$) δ 1.64 (3H, d), 5.33 (1H, q), 6.85 (1H, d), 6.92 (1H, m), 7.63 (1H, m), 8.11 (1H, m); EI-MS (*m*/*z*, %) 167 (M^+). Analytical data for the other standards are described in a previous report (Matsunaga et al., 1995).

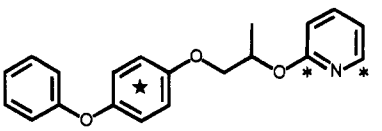

Figure 1. Position of carbon-14 label on [*phenoxyphenyl*-^{14}C] (★) and [*pyridyl*-2,6-^{14}C] (*) pyriproxyfen.

The chemical names and abbreviations of the other metabolites are as follows: 4-phenoxyphenol (POP); 4-phenoxyphenyl (*RS*)-2-hydroxypropyl ether (POPA); 4′-oxydiphenol (4′-OH-POP); and 4-(4-hydroxyphenoxy)phenyl (*RS*)-2-hydroxypropyl ether (4′-OH-POPA). The analytical data of these chemicals are described in a previous report (Matsunaga et al., 1995).

TLC. TLC was carried out according to the methods reported previously (Saito et al., 1991) with precoated silica gel 60 F_{254} chromatoplates (20 × 20 cm, 0.25-mm layer thickness, Merck, Germany). The solvent systems used were as follows: A, toluene:diethyl ether (3:2); B, chloroform:methanol (95:1); C, toluene:ethyl formate:formic acid (5:7:1); D, chloroform:methanol (9:1); and E, ethyl acetate:acetone:formic acid (8:2:1), two developments. The R_f values for authentic standards are shown in Table 1.

High-Performance Liquid Chromatography. The high-performance liquid chromatography (HPLC) was performed

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Table 1. TLC R_f Values of Authentic Standards

compound	R_f value with solvent system[a]				
	A	B	C	D	E
pyriproxyfen	0.92	0.87	0.66	0.86	—[b]
2′-OH-pyr	0.71	0.68	—	—	—
4′-OH-pyr	0.56	0.64	0.38	0.56	—
5″-OH-pyr	0.51	—	0.62	—	—
5″,4′-OH-pyr	0.26	0.19	—	—	—
DPH-pyr	0.52	0.52	0.46	0.49	—
PYPAC	0.16	—	0.39	—	0.87

[a] Solvent systems: (A) toluene:diethyl ether (3:2); (B) chloroform:methanol (95:5); (C) toluene:ethyl formate:formic acid (5:7:1); (D) chloroform:methanol (9:1); (E) ethyl acetate:acetone:formic acid (8:2:1), two developments. [b] —, not determined.

with a system composed of an Hitachi L-6200 intelligent pump, a Hitachi L-4200 UV–vis detector set at 272 nm, and a Berthold LB-507A radioactivity monitor. Analytical conditions were as follows: column, Cosmosil packed column (5C18, 10 μm, 4.6 mm i.d. × 25 cm; Nacarai Tesque Inc., Japan) and Guard-Pak (μBondapak C_{18}, Millipore Corp., Bedford, MA); flow rate, 1 mL/min; mobile phase, (I) 10 min isocratic with 25% methanol:75% water followed by a 35-min linear gradient to 100% methanol and (II) 10 min isocratic with 1% acetonitrile:99% water containing 1% acetic acid followed by a 25-min linear gradient to 100% acetonitrile containing 1% acetic acid.

Radioanalysis. Liquid scintillation counting (LSC), combustion analysis, and TLC autoradiography were carried out in the same manner as described by Yoshino et al. (1993a) and Matsunaga et al. (1995). Radioactivity in the expired air trapped in 10% NaOH solution was quantified by LSC using HIONIC-FLUOR (Packard, Downers Grove, IL) as a scintillator. All of the radioanalyses were conducted in duplicate for each sample.

Treatment of Animals. Sprague Dawley (SD) rats and ICR mice were purchased at 6 weeks of age from Charles River Japan Inc. (Japan) and acclimatized for 1 week. The animals were given free access to pelleted diet (CRF-1, Oriental Yeast Inc., Japan) and water *ad libitum* during all treatments. [*pyridyl*-2,6-^{14}C] and [*phenoxyphenyl*-^{14}C]pyriproxyfen were dissolved or suspended in corn oil at 2 (low dose) or 1000 (high dose) mg/5 mL and administered orally to rats (five animals/dose/sex) and mice (three animals/dose/sex) at a dose of 5 mL/kg of body weight using a glass syringe equipped with a stainless steel gastric probe (1 mm in diameter and 80 mm in length for rats; 0.8 mm in diameter and 70 mm in length for mice). The dosed amount of carbon-14 was adjusted to 250 μCi/kg for all groups. After administration of ^{14}C-labeled compounds, rats (individually) and the mice (three of each different group) were placed in Metabolica CO_2 cages (Sugiyamagen Iriki Company, Ltd., Japan) to allow separate collection of urine and feces. The urine was directly radioassayed by LSC. The 0–2-day feces were extracted with acetone in the same manner as described for the previous study (Matsunaga et al., 1995). The extracts were directly radioassayed by LSC. The residues and 3–7-day feces homogenized in water were combusted for the radioassay. The expired air was trapped in 10% NaOH solution during the first 2 days for rats only. On the 7th day, animals under slight anesthesia with diethyl ether were sacrificed by collection of blood from the abdominal artery. A total of 15–16 organs or tissues were removed from rats and combusted for the radioassay.

Analysis of Metabolites in Excreta. The 0–2-day fecal extracts and urine samples of rats in the high-dose group were pooled, and nonradioactive crude material was roughly removed with Sep-Pak cartridges (Millipore) and then fractionated into samples of each metabolite by HPLC with mobile phases I (feces) and II (urine). The nonpolar fecal metabolites that could not be separated by HPLC were isolated by preparative TLC with solvent system A. Conjugates that were inseparable by HPLC were isolated by TLC with solvent system E and subjected to enzymatic hydrolysis with aryl sulfatase (Limpets, Type VII, Sigma Chemical Company, St. Louis, MO) or β-glucuronidase (Type B-1, Sigma) in 0.1 M acetate buffer (pH 5.0) or 0.1 M phosphate buffer (pH 5.0) at 37 °C overnight as previously described (Matsunaga et al., 1995; Yoshino et al., 1993b). The sulfatase activity is inhibited in the phosphate buffer. The same experiments were made with saccharo-1,4-lactone (Sigma), an inhibitor of β-glucuronidase, added to the incubation mixture. The released aglycons were extracted with diethyl ether and identified by two-dimensional TLC cochromatography and HPLC coinjection with appropriate standards. Metabolites in mice were identified by TLC cochromatography with the rat samples obtained in the previous study (Matsunaga et al., 1995).

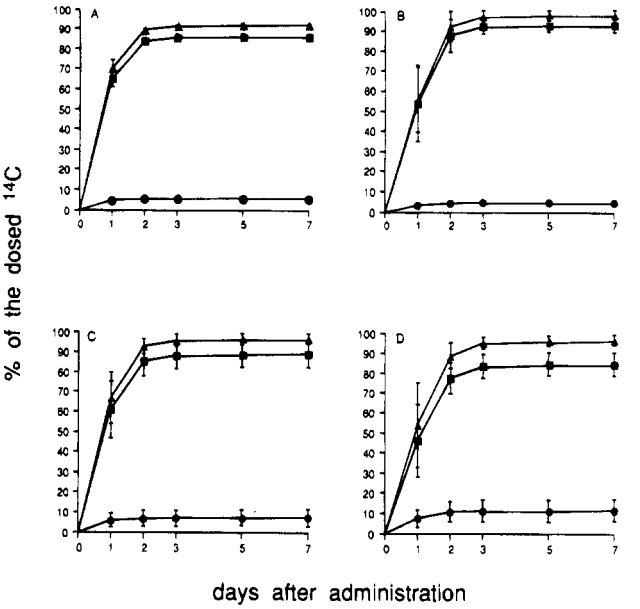

Figure 2. Cumulative carbon-14 excretion into feces and urine of rats after a single oral dose of [*pyridyl*-2,6-^{14}C]-pyriproxyfen: (A) male, at a dose of 2 mg/kg; (B) female, at a dose of 2 mg/kg; (C) male, at a dose of 1000 mg/kg; (D) female, at a dose of 1000 mg/kg. Key: (■) feces; (●) urine; (▲) total. Results are mean ± SD values (n = 5).

RESULTS

Carbon-14 Excretion. Data for cumulative carbon-14 excretion (percent of the dosed carbon-14) into feces, urine, and expired air in rats within the 7-day period after single oral administration of [*pyridyl*-2,6-^{14}C]-pyriproxyfen are shown in Figure 2. The total carbon-14 recovery was 92–99%. The fecal carbon-14 excretion was 84–94%. The urinary carbon-14 excretion was 5–12%. The carbon-14 excretion into expired air was 0.5% or less.

Data for cumulative carbon-14 excretion into feces and urine in mice within the 7-day period after single oral administration of [*phenoxyphenyl*-^{14}C]pyriproxyfen are shown in Figure 3. The total carbon recoveries were 100.0% (feces, 90.3%; urine, 9.7%) in males and 104.8% (feces, 78.1%; urine, 26.7%) in females for the low-dose group and 101.6% (feces, 64.4%; urine, 37.2%) in males and 100.0% (feces, 64.7%; urine, 35.3%) in females for the high-dose group.

Carbon-14 Tissue Residues. Tissue residues of carbon-14 in rats on the 7th day after single oral administration of [*pyridyl*-2,6-^{14}C]pyriproxyfen are shown in Table 2. The carbon-14 residue level in fat, 0.014–0.015 μg equivalents pyriproxyfen/g wet tissue (ppm) for the low-dose group and 6.0–6.3 ppm for the high-dose group, was the highest. In the other tissues, carbon-14 residue levels were 0.009 ppm or less for the low-dose group and 4.5 ppm or less for the high-dose group.

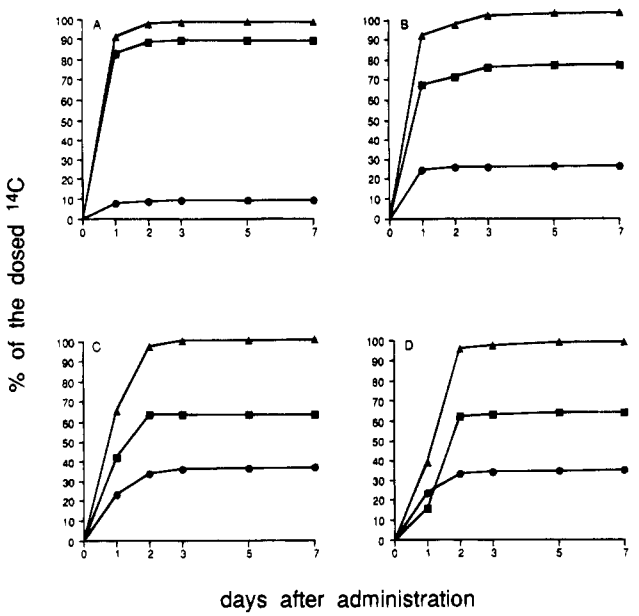

Figure 3. Cumulative carbon-14 excretion into feces and urine of mice after a single oral dose of [*phenoxyphenyl*-^{14}C]pyriproxyfen: (A) male, at a dose of 2 mg/kg; (B) female, at a dose of 2 mg/kg; (C) male, at a dose of 1000 mg/kg; (D) female, at a dose of 1000 mg/kg. Key: (■) feces; (●) urine; (▲) total. Analyses were conducted on pooled samples from three mice.

Table 2. Tissue Residues of Carbon-14 in Rats on the Seventh Day after Single Oral Administration of [*pyridyl*-2,6-^{14}C]Pyriproxyfen at 2 (Low Dose) or 1000 (High Dose) mg/kg[a]

	μg or ng equivalents of pyriproxyfen/g of tissue			
	low dose (ppb)		high dose (ppm)	
tissue	male	female	male	female
blood	2 ± 0.7[b]	2 ± 0.2[b]	1.9 ± 0.52	1.2 ± 0.17
bone	<1	<1	0.4 ± 0.09	0.3 ± 0.09[d]
brain	<1	<1	<0.2	0.3[c]
carcass	2 ± 0.6[b]	1 ± 0.2[b]	1.7 ± 1.07	1.8 ± 0.87
fat	14 ± 5.7	15 ± 4.5[b]	6.3 ± 3.77	6.0 ± 3.96
heart	1[c]	<1	0.5 ± 0.09[b]	0.4 ± 0.08
kidney	6 ± 2.0	5 ± 1.6	3.1 ± 0.72	2.7 ± 0.75
liver	9 ± 2.5	7 ± 1.4	4.5 ± 1.20	3.3 ± 0.76
lung	2 ± 0.4[d]	2 ± 0.2[d]	0.9 ± 0.31	0.6 ± 0.08
muscle	1 ± 0.1[e]	<1	0.3 ± 0.00[d]	0.3 ± 0.08[d]
ovary	—[f]	<4	—	0.8 ± 0.24
spleen	2 ± 0.3[d]	2 ± 0.4[e]	0.8 ± 0.18	0.6 ± 0.12
testis	<1	—	0.4 ± 0.10	—
uterus	—	2[c]	—	0.4 ± 0.08

[a] Data are the mean ± SD values for five rats, but data below the detection limit were excluded from the calculations of these mean values. [b] Mean value for four rats. [c] Value for one rat. [d] Mean value for three rats. [e] Mean value for two rats. [f] —, not determined.

Metabolites in Excreta. Amounts (percent of the dosed carbon-14) of identified metabolites in the 0–2-day feces and urine of rats are shown in Table 3. The fecal metabolite in the largest amount was 4′-OH-pyr, which accounted for 23.3–38.4% in males and 46.4–47.2% in females. The other major fecal metabolites for the low-dose group were 5″,4′-OH-pyr and 2′-OH-pyr, accounting for 7.2 and 1.8%, respectively, in males, and 1.2 and 2.8%, respectively, in females. The other major fecal metabolites for the high-dose group were DPH-pyr and a sulfate of 4′-OH-pyr (4′-OH-pyr-sul), accounting for 1.2–1.6%. The amount of parent compound, pyriproxyfen, was 32.5–34.8% in males and 21.2–21.9% in females. The major urinary metabolite was PYPAC, accounting for 1.0–4.9%.

Amounts of identified metabolites in the 0–2-day feces and urine of the mice are shown in Table 4. As with rats, 4′-OH-pyr was the fecal metabolite found in the largest amount, which accounted for 35.8–38.2% and 12.6–15.2% in the low- and high-dose groups, respectively. The other major fecal metabolites were DPH-pyr and POPA, accounting for 2.6–3.1% for the low-dose group and 1.4–2.7% for the high-dose group. The amounts of pyriproxyfen were 12.2–19.8% and 22.5–25.4% for the low- and high-dose groups, respectively. The major urinary metabolites were 4′-OH-pyr, a sulfate of POPA (POPA-sul), and a glucuronide of 4′-OH-pyr (4′-OH-pyr-glu). Amounts of 4′-OH-pyr and POPA-sul were 0.3–4.6% and 2.6–5.9%, respectively. The amounts of 4′-OH-pyr-glu were 2.9% in males and 12.9% in females for the low-dose group, and 27.7% in males and 17.8% in females for the high-dose group.

DISCUSSION

In this study, when [*pyridyl*-2,6-^{14}C]pyriproxyfen was orally administered to rats at 2 (low dose) or 1000 (high dose) mg/kg, carbon-14 was rapidly and completely excreted into the feces, urine, and expired air. The major route of carbon-14 excretion was in the feces (85–93% of the dose). There were no significant sex- or dose-related differences in the carbon-14 excretion pattern, which was almost the same for both [*pyridyl*-2,6-^{14}C] and [*phenoxyphenyl*-^{14}C]pyriproxyfen (Matsunaga et al., 1995). Carbon-14 excretion in mice given [*phenoxyphenyl*-^{14}C]pyriproxyfen orally was also rapid and complete, and the major route of carbon-14 excretion was in the feces (64–90%). The urinary excretion tended to be larger in the high-dose group than in the low-dose one, especially for males and in mice compared with in rats.

Tissue residues of carbon-14 in rats given an oral dose of [*pyridyl*-2,6-^{14}C]pyriproxyfen were generally very low and almost the same as those in rats given [*phenoxyphenyl*-^{14}C]pyriproxyfen (Matsunaga et al., 1995). Tissue residue levels of carbon-14 were higher for the high-dose group than for the low-dose group, whereas the increase ratio was not in proportion to that of the dose and in fact rather smaller. Although the level of carbon-14 residues was higher in fat than in the other tissues, according to a previous study (Matsunaga et al., 1995), most of the carbon-14 distributed in fat was attributed to pyriproxyfen and was gradually eliminated.

In addition to the metabolites identified in the previous study (Matsunaga et al., 1995), 5″-OH-pyr, DPH-pyr, and 4′-OH-pyr-glu were identified for the first time in the present study. The metabolic pathways of pyriproxyfen in rats or mice proposed on the basis of the identified metabolites are shown in Figure 4. The extents of each metabolic reaction were calculated on the basis of amounts of identified metabolites and are summarized (expressed as percent of the dosed carbon-14) in Table 5. The major metabolic reactions of pyriproxyfen in rats and mice were hydroxylation at the 4-position of the terminal phenyl ring (4′-hydroxylation), hydroxylation at the 2-position of the terminal phenyl ring (2′-hydroxylation), hydroxylation at the 5-position of the pyridyl ring (5″-hydroxylation), dephenylation, cleavage of the ether linkages, and conjugation of the resultant phenols with sulfuric acid or glucuronic acid (sulfation or glucuronidation, respectively). There were no marked differences in the qualitative profile of metabolites between sexes, doses, or species, but some minor differences were found from the quantitative

Table 3. Amounts of Metabolites in the Urine and Feces within 2 Days after Single Oral Administrations of [*pyridyl*-2,6-^{14}C]Pyriproxyfen to Rats at 2 (Low Dose) or 1000 (High Dose) mg/kg[a]

	percentage of the dosed carbon-14			
	low dose		high dose	
metabolite	male	female	male	female
feces				
organosoluble fraction				
pyriproxyfen	34.8 ± 12.9	21.2 ± 11.2	32.5 ± 12.2	21.9 ± 5.8
5″-OH-pyr	0.3 ± 0.2	0.3 ± 0.3	0.1 ± 0.0	0.1 ± 0.1
2′-OH-pyr	1.8 ± 0.5	2.8 ± 0.9	0.2 ± 0.0	0.2 ± 0.0
4′-OH-pyr	23.3 ± 5.5	47.2 ± 8.1	38.4 ± 8.6	46.4 ± 5.7
5″,4′-OH-pyr	7.2 ± 3.8	1.2 ± 0.7	0.3 ± 0.2	0.4 ± 0.1
DPH-pyr	0.8 ± 0.3	1.1 ± 0.3	1.6 ± 0.8	1.2 ± 0.3
4′-OH-pyr-sul	0.4 ± 0.1	0.4 ± 0.2	1.6 ± 0.6	1.2 ± 0.6
4′-OH-pyr-glu	0.3 ± 0.1	0.2 ± 0.0	1.1 ± 0.8	1.1 ± 1.2
5″,4′-OH-pyr-sul	0.3 ± 0.2	0.2 ± 0.1	0.9 ± 0.3	0.3 ± 0.2
others	3.9 ± 2.4	2.6 ± 2.3	0.4 ± 2.4	0.5 ± 1.4
unextractable ^{14}C	10.4 ± 3.3	10.6 ± 3.4	8.4 ± 1.6	4.6 ± 1.1
total	83.8 ± 1.5	87.7 ± 8.5	85.6 ± 7.4	77.8 ± 7.9
urine				
pyriproxyfen	—[b]	—	1.3 ± 1.7	2.7 ± 2.3
4′-OH-pyr	—	—	1.1 ± 0.5	5.6 ± 2.6
PYPAC	1.0 ± 0.3	1.7 ± 0.6	3.0 ± 2.3	4.9 ± 1.6
4′-OH-pyr-sul	0.4 ± 0.3	0.3 ± 0.2	0.2 ± 0.2	0.8 ± 0.3
5″,4′-OH-pyr-sul	—	—	0.1 ± 0.1	0.2 ± 0.1
others	4.1 ± 1.0	3.0 ± 0.4	2.9 ± 1.4	2.4 ± 3.5
total	5.5 ± 1.1	4.9 ± 0.4	7.8 ± 4.0	12.7 ± 5.4

[a] Data are the mean ± SD values for five rats. [b] —, not determined.

Table 4. Amounts of Metabolites in the Urine and Feces within 2 Days after Single Oral Administrations of [*phenoxyphenyl*-^{14}C]Pyriproxyfen to Mice at 2 (Low Dose) or 1000 (High Dose) mg/kg[a]

	percentage of the dosed carbon-14			
	low dose		high dose	
metabolite	male	female	male	female
feces				
organosoluble fraction				
pyriproxyfen	19.8	12.2	25.4	22.5
5″-OH-pyr	1.3	2.1	0.7	0.7
2′-OH-pyr	0.4	0.5	0.3	0.3
POP	0.8	0.4	0.2	0.2
4′-OH-pyr	38.2	35.8	15.2	12.6
DPH-pyr	3.1	2.9	2.7	2.6
POPA	3.4	2.6	1.4	2.2
4′-OH-POP	0.6	0.3	0.2	0.2
5″,4′-OH-pyr	0.6	1.2	0.4	0.4
4′-OH-POPA	0.9	1.1	0.4	0.7
POPA-sul	3.8	0.5	1.1	0.8
4′-OH-pyr-gul	0.4	0.2	1.9	0.3
others	5.3	2.7	2.9	3.4
unextractable ^{14}C	10.7	9.6	11.1	15.8
total	89.3	72.1	64.0	62.8
urine				
pyriproxyfen	0.0	0.0	0.2	0.1
POP	0.1	0.1	0.0	0.6
4′-OH-pyr	0.7	2.6	0.3	4.6
DPH-pyr	0.2	0.1	0.1	1.8
POPA	0.2	0.1	0.0	0.2
5″,4′-OH-pyr	0.1	0.0	0.0	0.1
POPA-sul	3.1	5.9	3.9	2.6
4′-OH-pyr-glu	2.9	12.9	27.7	17.8
others	2.4	4.6	2.0	5.8
total	9.7	26.7	37.2	35.3

[a] Analyses were conducted on pooled samples from three mice.

point of view. For the rat, the predominant metabolic reaction was 4′-hydroxylation, the extent of which was significantly higher in females (49.3–53.6%) than in males (32.1–43.3%). On the other hand, 5″-hydroxylation showed a significantly higher extent in males (1.4–7.8%) than in females (0.9–1.7%). There were no significant sex-related differences in the other metabolic reactions. The extents of metabolic reactions were generally higher for the low-dose group than for the high-dose one, except for sulfation, which was significantly higher for the high-dose group (2.4–2.8%) than for the low-dose one (0.8–1.2%). Because sulfate conjugates were mainly detected in feces, it is considered that the higher extent of sulfation for the high-dose group might be due to overflow of enzymatic hydrolysis of the conjugates by gastrointestinal flora. All of the tendencies just mentioned were also observed in the previous study using [*phenoxyphenyl*-^{14}C]pyriproxyfen (Matsunaga et al., 1995). For the mouse, 4′-hydroxylation was the major metabolic reaction, its extent being 44.3–47.2% in males and 37.7–54.1% in females. There were no marked sex- or dose-related differences, except that glucuronidation, especially in males, tended to be more extensive in the high-dose group (30.7% of the dose) than in the low-dose group (3.3% of the dose). The amount of 4′-OH-pyr-glu in urine of the mouse was larger for the high-dose group than for the low-dose group. This result suggests that the biliary excretion of this metabolite could be saturated in the high-dose group; that is, 4′-OH-pyr-glu might be excreted into bile by active transportation. The presence of ATP-dependent transport of glucuronides in canalicular liver plasma membranes has been established (Vore, 1993).

The extent of glucuronidation was much higher in the mouse than in the rat. The only glucuronide identified in the present study, 4′-OH-pyr-glu, was detected mainly in the urine for the mouse, whereas it was detected only in the feces for the rat. This difference might have some relation to the higher urinary excretion of carbon-14 in mice than in rats. Glucuronidation is catalyzed by UDP-glucuronyltransferase that is distributed mainly in hepatic microsomes (Dutton et al., 1966; Gram et al., 1968). Therefore, it is possible that 4′-OH-pyr-glu might be formed in the liver and then excreted into the bile leading to the feces, or into the blood and eventually the urine. The apparent significant difference in extent of glucuronidation between rats and mice might not be due to any real difference in enzyme activity, but rather to differences in capacity

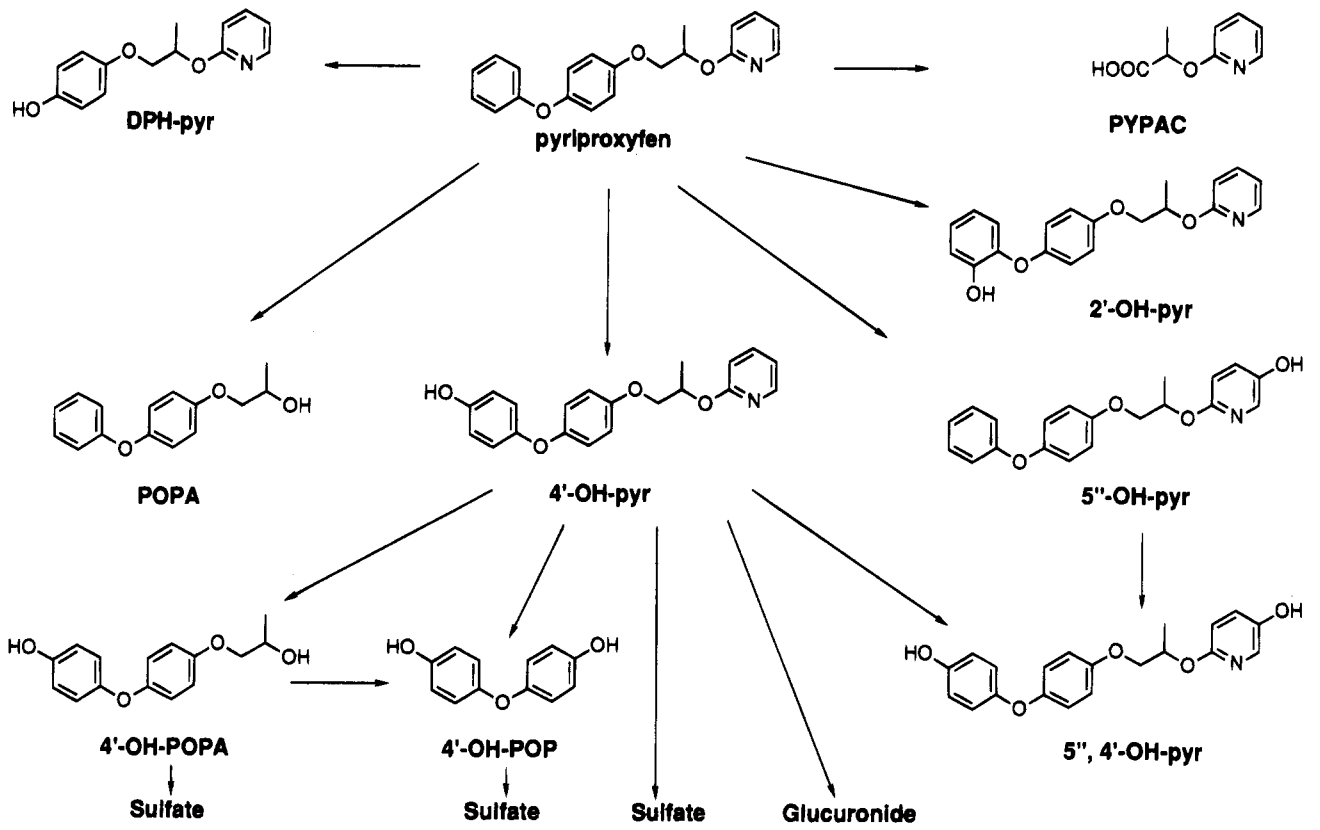

Figure 4. Metabolic pathways of pyriproxyfen in rats and mice proposed on the basis of results obtained in the previous (Matsunaga et al., 1995) and present studies.

Table 5. Extents[a] of Metabolic Reactions of Pyriproxyfen in Rats[b] and Mice[c]

	percent of the dosed carbon-14							
	rat				mouse			
	low dose		high dose		low dose		high dose	
reactions	male	female	male	female	male	female	male	female
5″-hydroxylation[d]	7.8	1.7	1.4	0.9	2.0	3.3	1.1	1.3
2′-hydroxylation[e]	1.8	2.8	0.2	0.2	0.4	0.5	0.3	0.3
4′-hydroxylation[f]	32.1	49.3	43.3	53.6	44.3	54.1	47.2	37.7
dephenylation	0.8	1.1	1.6	1.2	3.3	3.1	2.8	4.2
sulfation	1.2	0.8	2.8	2.4	7.0	6.3	4.8	3.5
glucuronidation	0.3	0.2	1.1	1.1	3.3	13.8	30.7	18.9
ether cleavage	1.0	1.7	3.0	4.9	12.9	11.0	7.3	7.7

[a] The values were calculated on the basis of amounts of identified metabolites in feces and urine. [b] Data are the mean values of five rats. [c] Data are obtained from pooled samples of three mice. [d] Hydroxylation at the 5-position of the pyridyl ring. [e] Hydroxylation at the 2-position of the terminal phenyl ring. [f] Hydroxylation at the 4-position of the terminal phenyl ring.

for biliary excretion of 4′-OH-pyr-glu because the glucuronides excreted into feces through bile may be hydrolyzed by gastrointestial flora and therefore detected as aglycones. A similar species difference in glucuronidation was reported for indobufen, a platelet aggregation inhibitor (Grubb et al., 1993a,b). The acyl glucuronide of indobufen was detected in urine for mice but in bile (not urine) for rats. In the present study, however, analysis of metabolites in bile of rats was not conducted.

The previous (Matsunaga et al., 1995) and present studies have shown that there are significant sex-related differences in metabolic reactions of pyriproxyfen; that is, 4′-hydroxylation, 5″-hydroxylation, and ether cleavage in the rat, but not in the mouse. Such differences should be examined in more detail by a metabolism study of pyriproxyfen using hepatic microsomes of both species. The results will be reported elsewhere.

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