Metabolism of Pyriproxyfen in Rats. 1. Absorption, Disposition, Excretion, and Biotransformation Studies with [*phenoxyphenyl*-^{14}C]Pyriproxyfen

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Male and female rats were given a single oral dose of [*phenoxyphenyl*-^{14}C]pyriproxyfen [4-phenoxyphenyl (*R,S*)-2-(2-pyridyloxy)propyl ether] at 2 (low dose) or 1000 (high dose) mg/kg. ^{14}C was rapidly excreted into feces and urine, with the former route predominating (about 90% of the dose). Peak ^{14}C concentrations in blood, kidney, liver, and other tissues except for fat occurred 2–8 h after administration, being 0.4, 0.4, 2.5, and <0.2 μg of pyriproxyfen equivalents/g of tissue (ppm), respectively. Peak ^{14}C concentration in fat occurred 12–24 h after administration, being 0.3–0.5 ppm. ^{14}C tissue residues on the seventh day were below 0.02 and 10 ppm for the low and high doses, respectively. The major metabolic reactions were hydroxylation at the 2- or 4-position of the terminal phenyl ring, hydroxylation at the 5-position of the pyridyl ring, cleavage of the ether linkages, and conjugation of the resultant phenols with sulfuric acid. No marked sex-related differences were observed for ^{14}C excretion or ^{14}C tissue residues. However, a slight sex-related variation was found for the extent of metabolic reactions.

Keywords: *Metabolism; pyriproxyfen; rat; absorption; disposition; excretion; biotransformation*

INTRODUCTION

Pyriproxyfen [4-phenoxyphenyl (*R,S*)-2-(2-pyridyloxy)-propyl ether, Sumilarv] is a new insect growth regulator (IGR) with insecticidal activities against houseflies, mosquitoes, and cockroaches (Hatakoshi *et al.*, 1987; Kawada *et al.*, 1987).

IGRs have an effect on insect-specific phenomena, so that selectivity between insects and mammals is highly expected. IGRs are divided into two groups by their mode of action: chitin synthesis inhibitors, which disrupt molting of insects, and juvenile hormone analogs (JHAs), which interfere with metamorphic changes. Some of them are presently being used to control pest species in many fields (Miyamoto *et al.*, 1993). Pyriproxyfen is classified as a JHA.

Ecological effects and metabolism in aquatic organisms of pyriproxyfen were investigated and reported by Miyamoto *et al.* (1993). The present paper deals with metabolism studies of pyriproxyfen in rats carried out along with toxicological studies for safety evaluation using pyriproxyfen labeled with ^{14}C at the phenoxyphenyl group ([*phenoxyphenyl*-^{14}C]pyriproxyfen).

MATERIALS AND METHODS

Chemicals. [*phenoxyphenyl*-^{14}C]Pyriproxyfen ([^{14}C]pyriproxyfen) with a specific activity of 1.57 GBq/mmol was prepared in the Environmental Health Science Laboratory, Sumitumo Chemical Co., Ltd. The radiochemical purity was more than 99% on thin-layer chromatography (TLC).

Synthetic (unlabeled) standards, 4-phenoxyphenol (POP) and 4-phenoxyphenyl (*R,S*)-2-hydroxypropyl ether (POPA), were synthesized in the Pesticide Laboratory, Takarazuka Research Center, Sumitomo Chemical Co., Ltd. 4,4′-Oxydiphenol (4′-OH-POP) was purchased from Kodak. 4-(4-Hydroxyphenoxy)phenyl (*R,S*)-2-(2-pyridyloxy)propyl ether (4′-OH-pyr), 4-(2-hydroxyphenoxy)phenyl (*R,S*)-2-(2-pyridyloxy)-propyl ether (2′-OH-pyr), and 4-(4-hydroxyphenoxy)phenyl (*R,S*)-2-hydroxypropyl ether (4′-OH-POPA) were prepared by Sumika Chemical Analysis Service Ltd., Osaka, Japan. Mass (MS) and nuclear magnetic resonance (NMR) spectral data of these synthetic standards were as follows: POP, ^{1}H NMR (90 MHz, $CDCl_3$) δ 6.82–7.20 (9H, m); EI-MS *m/z* 186 (M^+). POPA, ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.20 (3H, d), 3.88 (2H, m), 4.10 (1H, m), 6.81–7.18 (9H, m); EI-MS *m/z* 244 (M^+), 186. 4′-OH-POP, ^{1}H NMR (90 MHz, DMSO-d_6) δ 6.72 (8H, s); EI-MS *m/z* 202 (M^+). 4′-OH-pyr, ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.51 (3H, d), 4.12 (2H, m), 5.56–5.59 (1H, m), 6.81–8.14 (12H, m), EI-MS *m/z* 337 (M^+), 136. 2′-OH-pyr, ^{1}H NMR (90 MHz, $CDCl_3$) δ 1.46 (3H, d), 4.10 (2H, m), 5.56 (1H, m), 6.81–8.14 (12H, m); EI-MS *m/z* 337 (M^+), 136. 4′-OH-POPA, ^{1}H NMR (90 MHz, DMSO-d_6) δ 1.19 (3H, d), 3.71–3.97 (4H, m), 6.78 (4H, m), 6.84 (4H, s); EI-MS *m/z* 260 (M^+), 202.

Table 1. TLC R_f Values of Authentic Standards

compound	R_f value with solvent system[a]				
	A	B	C	D	E
pyriproxyfen	0.74	0.73	0.55	0.65	0.48
2′-OH-pyr	0.66	0.66	0.41	0.58	0.22
4′-OH-pyr	0.52	0.47	0.32	0.46	0.15
POPA	0.40	0.56	0.32		
4′-OH-POP	0.34	0.20	0.25	0.32	0.06
5″,4′-OH-pyr	0.26	0.19	0.17	0.21	0.05
4′-OH-POPA	0.22	0.25	0.16	0.22	0.05

[a] Solvent systems: (A) toluene–diethyl ether (3:2); (B) chloroform–methanol (95:5); (C) *n*-hexane-ethyl acetate–acetic acid (10:4:1); (D) chloroform–ethyl acetate (7:3); (E) *n*-hexane–acetone (4:1).

Radioanalysis. Radioanalysis was carried out according to the methods of Yoshino *et al.* (1993b). Radioactivity in fecal extracts, urine, and silica gel regions scraped from TLC plates was quantified by liquid scintillation counting (LSC) with a Tri-Carb 460CD spectrometer (Packard). Radioactivity in fecal homogenates, unextractable fecal residues, and tissue samples was quantified by combustion analysis using a Tri-Carb 306 sample oxidizer (Packard) prior to LSC. Eight milliliters of Oxisorb-CO_2 and 12 mL of Oxiprep-2 (New England Nuclear)

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were used as $^{14}CO_2$ absorbent and scintillator, respectively. ^{14}C recovery was 95% or more for the combustion.

Thin-Layer Chromatography (TLC). TLC analysis was conducted in a manner similar to those reported by Saito *et al.* (1991) and Yoshino *et al.* (1993a). Precoated silica gel 60 F_{254} chromatoplates (20 × 20 cm, 0.25 mm layer thickness, Merck, Darmstadt, Germany) were used for TLC analysis and purification. R_f values for authentic standards and solvent systems used are shown in Table 1. Unlabeled standards were detected under UV light. Radioactive metabolites were detected by autoradiography using Kodak SB-5 films.

High-Performance Liquid Chromatography (HPLC). HPLC was conducted in the same manner as reported by Yoshino *et al.* (1993a) with a system composed of a 638-50 HPLC pump (Hitachi, Japan), a 635M LC UV detector (Hitachi), an LB-503 radioactivity monitor (Berthold, Germany), and a semipreparative column, S-343-15 (ODS, 15 μm, 20 mm i.d. × 250 mm, YMC, Japan). The mobile phase was 80% methanol in water. The flow rate was 5 mL/min.

Spectroscopy. EI mass spectra were obtained with a Hitachi M-80B mass spectrometer (Hitachi) with an ionizing energy of 70 eV. The ^1H NMR spectra were obtained with JEOL JNM FX-90 (90 MHz) or FX-100 (100 MHz) spectrometers (JEOL Ltd., Tokyo). ^{13}C NMR spectra were obtained with a JEOL JNM GSX-270 spectrometer (JEOL) at 67.5 MHz.

Isolation and Identification of the Major Fecal Metabolite. Acetone extracts of feces of Sprague-Dawley (SD) male rats given daily oral doses of [^{14}C]pyriproxyfen at 1000 mg/kg were subjected to silica gel open column chromatography (silica gel 60, 70–230 mesh, Merck; 46 mm i.d. × 45 cm) and eluted into fractions with a 7:3 *n*-hexane–acetone solvent system. The fractions containing the major metabolite with an R_f value of about 0.3 on TLC developed with a 3:2 toluene–diethyl ether solvent system were combined and subjected to preparative TLC, using the solvent systems 1:1 toluene–diethyl ether and 2:1 toluene–ethyl acetate. Finally, the metabolite was further isolated by HPLC. It was identified by spectroanalysis (MS and NMR) as follows: 4-(4-hydroxyphenoxy)phenyl 2-(5-hydroxypyridyl)-2-oxy)propyl ether (5″,4′-OH-pyr), ^1H NMR (methanol-d_4) δ 1.38 (3H, d, 6.4 Hz), 3 (2H, m), 5.26 (1H, m), 6.64 (1H, dd, J = 0.5, 8.8 Hz), 6.7–6.9 (8H), 7.17 (1H, dd, J = 3.0, 8.8 Hz), 7.65 (1H, dd, J = 0.5, 3.0 Hz); ^{13}C NMR (chloroform-*d*) δ 17.0, 69.6, 71.2, 111.9, 115.7, 116.2, 119.5, 119.70, 127.5, 132.7, 146.9, 151.1, 151.7, 154.7, 157.7; EI-MS *m/z* 353 (M^+), 242, 152 (base peak).

This metabolite was used in this study as an authentic standard for 5″,4′-OH-pyr.

Animal Treatment and Sample Collection. SD male and female rats were purchased at 6 weeks of age from Charles River Japan Inc. (Atsugi, Japan) and acclimatized for 1 week. Water and pelleted diet (CE-2, Clea Japan Inc., Tokyo) were provided *ad libitum*.

For ^{14}C excretion and ^{14}C tissue residue studies, groups of five male and five female rats were given a single oral dose of [^{14}C]pyriproxyfen at 2 (low dose) or 1000 mg/kg (high dose). Corn oil was used as a vehicle and given at 5 mL/kg. Rats dosed with [^{14}C]pyriproxyfen were housed individually in Metabolica CO_2 cages (Sugiyamagen Iriki Co., Ltd., Tokyo), and urine and feces were collected for 7 days. On the seventh day, rats were sacrificed by collection of blood from the abdominal aorta under light anesthesia with diethyl ether, and 19–20 major tissues were removed.

For the ^{14}C bile excretion, groups of three male and three female bile duct cannulated rats were orally dosed with [^{14}C]-pyriproxyfen at 2 mg/kg. The bile duct cannulation was carried out as described previously (Isobe *et al.*, 1987). The rats were individually housed in a Bollman cage (Sugiyamagen Iriki), after administration, and feces, urine, and bile were collected for 2 days.

For the ^{14}C distribution study, male and female SD rats were orally dosed with [^{14}C]pyriproxyfen at 2 mg/kg. Groups of three rats of each sex were sacrificed 2, 4, 8, 12, 24, 48, and 72 h after administration, and 10–11 tissues were dissected out. The amounts of ^{14}C distributed into these tissues were measured by combustion analysis.

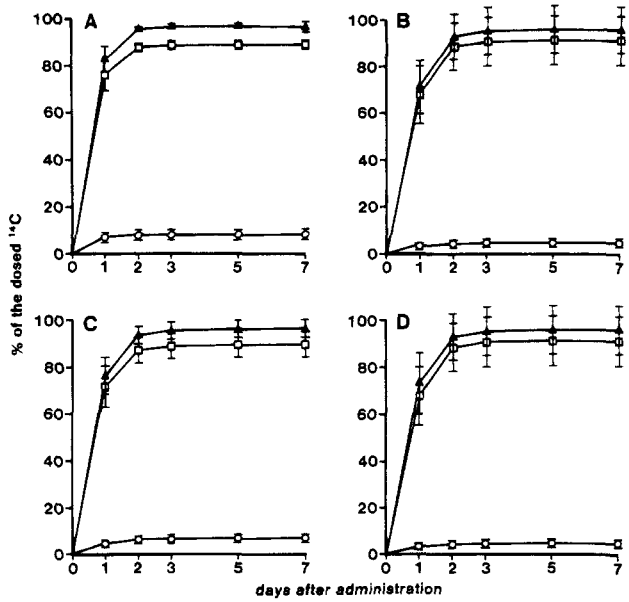

Figure 1. Cumulative ^{14}C excretion in feces and urine of rats after a single oral dose of [*phenoxyphenyl*-^{14}C]pyriproxyfen: (A) male, at a dose of 2 mg/kg; (B) female, at a dose of 2 mg/kg; (C) male, at a dose of 1000 mg/kg; (C) female, at a dose of 1000 mg/kg. Key: (□) feces; (○) urine; (▲) total. Results are mean ± SD values (n = 5).

Analysis of Metabolites in Excreta, Bile, and Tissues. Feces of individual rats on each collection day were homogenized in a 3-fold volume of distilled water with a Waring blender (Nihonseiki Co., Tokyo) or a Multi Blender Mill (Nihonseiki Co.). The 0–2 day fecal homogenates were mixed with acetone, shaken, and centrifuged at 5000*g* for 10 min. The precipitates were further extracted twice or three times with acetone. The collected extracts were concentrated by evaporation.

Metabolites in the 0–2 day fecal extracts, urine, and bile samples were tentatively identified by TLC cochromatography with authentic standards. Polar metabolites were isolated and treated with aryl sulfatase (limpets, type IV, Sigma Chemical Co.) 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. The sulfatase activity is inhibited in the phosphate buffer. The same experiments were made with saccharo-1,4-lactone, an inhibitor of β-glucuronidase, added to the incubation mixture. The released aglycons were extracted with diethyl ether and analyzed by TLC.

For the ^{14}C distribution study, blood, kidney, and liver samples from each collection time were homogenized in a 5-fold volume of acetone using a Polytron (Kinematica) and the homogenates centrifuged at 1500*g* for 10 min with a Hitachi 05PR-2 refrigerated centrifuge. Each precipitate was further extracted twice with acetone. These acetone extracts of blood, kidney, and liver were subjected to TLC analysis.

^{14}C in fat samples that showed maximal ^{14}C concentrations were extracted with acetone, followed by partition between *n*-hexane and acetonitrile. The acetonitrile phase was subjected to TLC analysis.

The quantification of each metabolite was conducted as follows: urine, bile, and extracts of feces or tissues were subjected to TLC with a 3:2 toluene–diethylether solvent system. The fraction at the origin was scraped off, extracted with methanol, and subsequently analyzed by TLC with a 6:1:1 *n*-butanol–acetic acid–water solvent system.

RESULTS

^{14}C Excretion. Data for cumulative ^{14}C excretion in feces and urine over 7 days after single oral administration of [^{14}C]pyriproxyfen at low- and high-dose levels are shown in Figure 1. The total ^{14}C recovery within

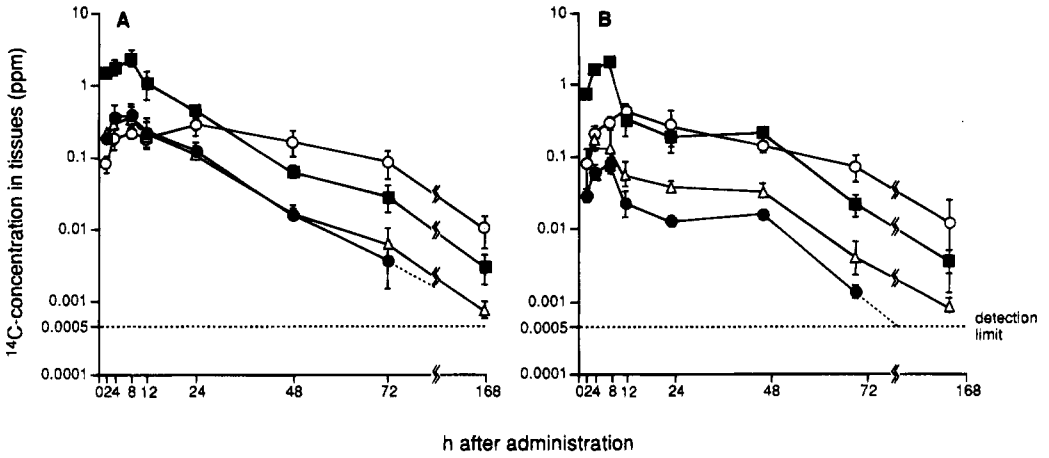

Figure 2. ^{14}C concentration in the blood (●), fat (○), kidney (△), and liver (■) of rats after single oral administration of [*phenoxyphenyl*-^{14}C]pyriproxyfen at 2 mg/kg: (A) male; (B) female. Results are mean ± SD values (n = 5 for the 168 h time point, and n = 3 for others).

Table 2. Cumulative ^{14}C Excretion in Bile, Feces, and Urine of Bile Duct Cannulated Rats Given a Single Oral Dose of [*phenoxyphenyl*-^{14}C]Pyriproxyfen at 2 mg/kg[a]

		% of dosed ^{14}C	
sex	fraction	24 days after administration	48 days after administration
male	bile	28 ± 7	34 ± 7
	feces	24 ± 17	43 ± 8
	urine	2 ± 1	3 ± 2
	total	57 ± 12	82 ± 10
female	bile	34 ± 15	37 ± 15
	feces	48 ± 20	52 ± 15
	urine	1 ± 0	2 ± 1
	total	83 ± 18	92 ± 11

[a] Data are the mean ± SD values for three rats.

the 7-day period was 96–98% of the dosed ^{14}C. The fecal ^{14}C excretion was 89–92%, while the urinary ^{14}C excretion was 8% or less. Most of the dosed ^{14}C (93–96%) was excreted within 2 days after the administration.

Data for cumulative ^{14}C excretion in feces, urine, and bile of bile duct cannulated rats given a single oral dose of [^{14}C]pyriproxyfen are summarized in Table 2. ^{14}C excreted in feces, urine, and bile was 43, 3, and 34%, respectively, of the dosed ^{14}C for males and 52, 2, and 37%, respectively, of the dosed ^{14}C for females.

^{14}C Tissue Residues. Table 3 shows ^{14}C tissue residue levels on the seventh day after administration. The ^{14}C residue level in the fat was the highest, being 0.010–0.013 and 8.0–9.5 μg of pyriproxyfen equivalents/g of tissue (ppm) for the low- and high-dose groups, respectively. In the other tissues, ^{14}C residue levels were 0.001 ppm or less for the low-dose group and 2.6 ppm or less for the high-dose group. The sum of the residual ^{14}C in the body was 0.3% or less of the dosed ^{14}C.

^{14}C Concentrations in Tissues. ^{14}C concentrations in blood, fat, kidney, and liver are shown in Figure 2. Peak ^{14}C concentrations in blood, kidney, and liver occurred 4–8 h after administration, and ^{14}C concentrations in these tissues decreased thereafter, being below 0.03 ppm at 72 h after administration. The maximum ^{14}C concentrations in blood, kidney, and liver were 0.4, 0.4, and 2.5 ppm, respectively, for males and 0.1, 0.2, and 2.1 ppm, respectively, for females.

Peak ^{14}C concentrations in fat occurred 12–24 h after administration and decreased thereafter more slowly than in other tissues, being below 0.09 ppm at 72 h after administration. The maximum ^{14}C concentrations in fat were 0.3 ppm for males and 0.5 ppm for females.

Peak ^{14}C concentrations in other tissues occurred 2–8 h after administration and decreased rapidly thereafter. In all cases, the maximum concentration was less than 0.2 ppm.

Table 3. ^{14}C Tissue Residues in Rats on the Seventh Day after Single Oral Administration of [*phenoxyphenyl*-^{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	<1	<1	<0.3	<0.3
bone	1[b]	<1	<0.2	<0.2
brain	<1	<1	0.2[e]	0.3[e]
cecum	1[c]	1	0.5	0.5
carcass	1	1	2.6	2.3
fat	10	13	8.0	9.5
heart	<1	<1	<0.2	<0.2
kidney	1[c]	1	0.4	0.4
large intestine	1[d]	1	0.4	0.5
liver	3	4	1.7	1.5
lung	<1	<1	<0.2	<0.2
muscle	<1	<1	0.3[e]	<0.2
ovary		2		0.9
small intestine	1	1	0.6	0.5
spleen	1[b]	1[e]	0.2[e]	0.2[e]
stomach	1[b]	<1	0.3[e]	<0.3
testis	<1		<0.2	
uterus		<1		0.3[d]

[a] Data are the mean values for five rats, but data below the detection limit were excluded from the calculations of mean values. [b] Value for one rat. [c] Mean value for four rats. [d] Mean value for three rats. [e] Mean value for two rats.

Metabolites in Feces, Urine, and Bile. Amounts (expressed as percentage of the dosed ^{14}C) of fecal and urinary metabolites are summarized in Table 4. Parent compound was detected only in feces, accounting for 25.1–37.2%. The major metabolites were 4′-OH-pyr, 5″,4′-OH pyr, and 4′-OH-POPA. Amounts of 4′-OH-pyr were 24.5–35.2% for males and 43.3–48.3% for females. 5″,4′-OH-pyr accounted for 8.5% in males and 2.0% in females of the low-dose group and 1.0–1.5% in the high-dose group. Amounts of 4′-OH-POPA were in the range 1.3–3.3%. These metabolites were detected only in the feces. The sulfates of the above three metabolites were not detected in feces for the low-dose group but were for the high-dose group, accounting for 0.4–3.7%. The

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

	percentage of the dosed ^{14}C			
	low dose		high dose	
metabolite	male	female	male	female
feces				
organosoluble fraction				
pyriproxyfen	37.2 ± 9.9	31.1 ± 17.4	31.1 ± 10.0	25.1 ± 9.9
2′-OH-pyr	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
4′-OH-pyr	24.5 ± 4.2	43.3 ± 13.1	35.2 ± 2.5	48.3 ± 6.7
4′-OH-pyr sulfate			3.7 ± 0.4	2.1 ± 1.0
POPA	0.2 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
4′-OH-POP	0.5 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0.3 ± 0.1
4′-OH-POP sulfate			0.5 ± 0.1	0.2 ± 0.1
5″,4′-OH-pyr	8.5 ± 2.4	2.0 ± 1.0	1.5 ± 0.6	1.0 ± 0.4
5″,4′-OH-pyr sulfate			1.3 ± 0.4	0.4 ± 0.3
4′-OH-POPA	3.3 ± 0.8	1.3 ± 0.7	1.4 ± 0.3	1.3 ± 0.3
4′-OH-POPA sulfate			2.6 ± 0.8	0.8 ± 0.1
others	7.1 ± 2.4	6.1 ± 2.1	5.0 ± 0.9	5.1 ± 1.4
unextractable ^{14}C	6.4 ± 1.1	5.3 ± 1.6	4.4 ± 1.5	3.9 ± 0.9
total	87.8 ± 1.8	89.9 ± 2.9	87.1 ± 5.4	88.7 ± 10.3
urine				
4′-OH-pyr sulfate	0.4 ± 0.2	1.0 ± 0.3	0.5 ± 0.2	1.0 ± 0.4
4′-OH-POP sulfate	3.1 ± 1.0	0.5 ± 0.2	1.6 ± 0.4	0.3 ± 0.1
others	4.4 ± 2.2	3.5 ± 0.9	4.3 ± 1.2	3.1 ± 1.2
total	7.9 ± 2.2	5.0 ± 1.5	6.1 ± 1.7	4.4 ± 1.7

[a] Data are the mean ± SD values for five rats.

Table 5. Amounts of Metabolites in the Blood, Kidneys, and Liver of Male Rats Given a Single Oral Dose (2 mg/kg) of [*phenoxyphenyl*-^{14}C]Pyriproxyfen at 2, 4, 8, 12, 24, 48, and 72 h after Administration[a]

	ng equivalents of pyriproxyfen/g of tissue (ppb)						
metabolites	2 h	4h	8 h	12 h	24 h	48 h	72 h
blood							
organosoluble fraction							
pyriproxyfen	8	nd[b]	nd	1	1	1	6
4′-OH-pyr sulfate	14	12	7	3	1	nd	nd
4′-OH-POPA sulfate	8	24	12	5	2	nd	nd
5″,4′-OH-pyr sulfate	166	358	279	170	81	7	2
4′-OH-POP sulfate	12	21	18	16	9	1	nd
others	12	48	33	21	11	5	2
unextractable ^{14}C	31	54	46	37	23	3	4
total	251	516	395	395	127	17	14
kidney							
organosoluble fraction							
pyriproxyfen	39	21	10	5	nd	nd	nd
4′-OH-pyr	7	9	6	nd	nd	nd	nd
4′-OH-pyr sulfate	47	80	77	43	22	5	4
4′-OH-POPA sulfate	18	31	34	8	7	nd	nd
5″,4′-OH-pyr sulfate	57	143	153	101	65	8	3
4′-OH-POP sulfate	10	26	50	30	15	nd	2
others	41	58	74	18	23	13	5
unextractable ^{14}C	22	62	94	33	27	8	7
total	241	430	496	254	158	34	22
liver							
organosoluble fraction							
pyriproxyfen	63	26	11	11	5	nd	4
4′-OH-pyr	61	68	59	15	8	nd	3
4′-OH-pyr sulfate	770	755	689	310	137	15	23
4′-OH-POPA	6	nd	11	7	4	nd	nd
4′-OH-POPA sulfate	85	109	138	68	35	8	4
5″,4′-OH-pyr	12	15	21	10	4	nd	nd
5″,4′-OH-pyr sulfate	272	398	735	311	113	8	9
4′-OH-POP sulfate	14	26	69	60	24	4	nd
others	128	150	214	99	45	13	11
unextractable ^{14}C	204	863	348	173	119	28	26
total	1610	2410	2300	1060	493	76	79

[a] Analyses were conducted after combination of samples from three rats. [b] Not detected on TLC.

identified urinary metabolites were sulfates of 4′-OH-pyr and 4′-OH-POP, accounting for 0.3–3.1%.

In the bile of bile duct cannulated rats, sulfates of 4′-OH-pyr, 4′-OH-POPA, 4′-OH-POP, and 5″,4′-OH-pyr were identified. Parent compound was not detected in the bile.

Metabolites in Blood, Fat, Kidney, and Liver. Amounts of metabolites in the blood, kidneys, and liver of rats at different time points after administration of a single oral dose of pyriproxyfen are shown in Tables 5 and 6. Peak concentrations of parent compound in these tissues occurred 2 h after administration, with maxima of 8–10 ppb for blood, 28–39 ppb for kidneys, and 40–63 ppb for liver. Major metabolites in these tissues were sulfates of 4′-OH-pyr, 5″,4′-OH-pyr, and 4′-OH-POPA. Their peak concentrations occurred 2–4 h after administration. Peak concentrations of sulfate of 4′-OH-pyr in blood, kidney, and liver were 14, 80, and 770 ppb, respectively, for males and 1, 45, and 493 ppb, respectively, for females. Peak concentrations of sulfate of 5″,4′-OH-pyr in blood, kidney, and liver were 358, 153, and 735 ppb, respectively, for males and 37, 28, and 568 ppb, respectively, for females. Peak concentrations of sulfate of 4′-OH-POPA in blood, kidney, and liver were 24, 34, and 138 ppb, respectively, for males and 12, 30, and 162 ppb, respectively, for females. In the fat sample with the highest ^{14}C concentration, 89–93% of the total ^{14}C could be ascribed to the parent compound.

DISCUSSION

In the present study, the major excretion route of pyriproxyfen in rats was in the feces (about 90% of the dosed ^{14}C). No significant difference was observed in ^{14}C excretion profile, regardless of sex or dose.

In the biliary excretion study, unmetabolized pyriproxyfen was not detected in bile. Therefore, it is likely that the parent compound detected in the feces was excreted without absorption into the body. Accordingly, the extent of total absorption (percentage of the dosed ^{14}C) of pyriproxyfen can be calculated by substracting the amount of the parent compound in the feces from 100%, giving 63–75%.

The proposed metabolic pathways of pyriproxyfen in the rat are shown in Figure 3. On the basis of amounts of identified metabolites in excreta, the major metabolic

Table 6. Amounts of Metabolites in the Blood, Kidneys, and Liver of Female Rats Given a Single Oral Dose (2 mg/kg) of [*phenoxyphenyl*-^{14}C]Pyriproxyfen at 2, 4, 8, 12, 24, 48, and 72 h after Administration[a]

	ng equivalents of pyriproxyfen/g of tissue (ppb)						
metabolites	2 h	4 h	8 h	12 h	24 h	48 h	72 h
blood							
organosoluble fraction							
pyriproxyfen	10	12	9	11	12	3	2
4′-OH-pyr sulfate	nd[b]	nd	1	nd	nd	nd	nd
4′-OH-POPA sulfate	4	10	12	3	1	1	nd
5″,4′-OH-pyr sulfate	8	19	37	5	3	2	nd
4′-OH-POP sulfate	1	3	5	1	1	1	nd
others	8	19	21	9	7	4	3
unextractable ^{14}C	4	10	14	7	5	4	1
total	35	72	100	36	28	16	6
kidney							
organosoluble fraction							
pyriproxyfen	27	28	16	3	3	2	nd
4′-OH-pyr	4	12	8	nd	nd	nd	nd
4′-OH-pyr sulfate	11	43	45	13	5	6	nd
4′-OH-POPA sulfate	17	26	30	nd	nd	5	nd
5″,4′-OH-pyr sulfate	9	28	25	8	5	4	2
4′-OH-POP sulfate	nd	6	7	nd	nd	nd	nd
others	28	76	78	33	18	18	7
unextractable ^{14}C	14	48	47	16	12	15	4
total	110	256	257	71	42	50	12
liver							
organosoluble fractions							
pyriproxyfen	40	30	25	nd	nd	nd	nd
4′-OH-pyr	168	285	337	50	11	25	nd
4′-OH-pyr sulfate	221	427	493	62	38	44	11
4′-OH-POPA	nd	6	15	nd	2	nd	nd
4′-OH-POPA sulfate	43	124	162	35	10	18	nd
5″,4′-OH-pyr	nd	8	9	nd	nd	nd	nd
5″,4′-OH-pyr sulfate	134	381	568	46	28	27	5
4′-OH-POP sulfate	16	34	88	19	11	9	nd
others	173	318	377	102	37	51	8
unextractable ^{14}C	169	406	361	95	65	69	22
total	965	2020	2430	408	201	243	46

[a] Analyses were conducted after combination of samples from three rats. [b] Not detected on TLC.

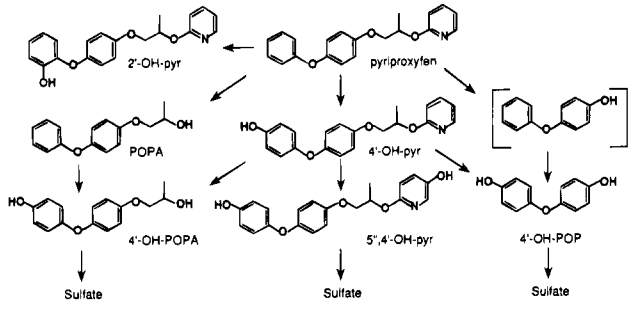

Figure 3. Proposed metabolic pathways of pyriproxyfen in rats.

reactions were concluded to be as follows: (1) hydroxylation at the 4-position of the terminal phenyl ring (4′-hydroxylation), (2) hydroxylation at the 2-position of the terminal phenyl ring (2′-hydroxylation), (3) hydroxylation at the 5-position of the pyridyl ring (5″-hydroxylation), (4) cleavage of the propyl phenyl ether, (5) cleavage of the propyl pyridyl ether, and (6) conjugation of the resultant phenols with sulfuric acid (sulfation). The extent (expressed as percentage of the dosed ^{14}C) of the metabolic reactions 1, 2, 3, 4, 5, and 6 were calculated to be 40, 0.2, 9, 4, 3, and 4%, respectively, for males and 49, 0.2, 2, 1, 2, and 2%, respectively, for females in the low-dose group. The extent of sulfation in the high-dose group was 10% for males and 5% for females, being significantly larger in the high-dose group than in the low-dose group for both sexes ($p < 0.01$, by t-test). As sulfates in feces were detected only in the high-dose group and their amounts in the urine were not different between the dose groups, it is considered that the larger amount of sulfates in the high-dose group is due to overflow of enzymatic hydrolysis of sulfates by gastrointestinal flora. No significant sex-related difference was found for 2′-hydroxylation. However, the extents of 5″-hydroxylation, ether cleavages, and sulfation were significantly greater in males than in females ($p < 0.01$ by t-test). The extent of 4′-hydroxylation tended to be greater in females than in males, but the difference was not significant. The above reactions 1–5 are oxidative ones, and it is considered that the cytocrome P-450 monooxygenases are involved (Gibson and Skett, 1986). The existence of sex-specific cytochrome P-450s in rat liver microsomes is well established (Kato and Yamazoe, 1990; Imaoka *et al.*, 1991), and therefore it is strongly implied that sex-related differences in metabolism of pyriproxyfen might have some relation to the sex-specific cytochrome P-450s. In the previous studies by Miyamoto *et al.* (1993), 4′-hydroxylation and cleavage of propyl pyridyl ether were the major metabolic pathways also in carp, dragonfly, mudge, and mosquito larvae. However, 5″-hydroxylation was not found in these aquatic organisms.

Peak ^{14}C concentrations in tissues except for fat occurred 2–8 h after administration and then decreased rapidly so that ^{14}C tissue residues on the seventh day after administration were generally very low in both sexes given either dose. While relatively higher ^{14}C concentrations were observed for fat than the other tissues, the major form involved was unmetabolized pyriproxyfen, and ^{14}C distributed to fat was gradually eliminated.

The fact that ^{14}C concentration in the blood was significantly higher in males than in females within 24 h after administration requires interpretation. The major metabolite in male blood was the sulfate of 5″,4′-OH-pyr, the concentration of which was at least 8 times higher than in female blood. Therefore, this difference could have been responsible and suggests a further relevance for the sex-related variation in 5′-hydroxylation activity mentioned above.

From the above points, it can be concluded that pyriproxyfen orally administered to rats is excreted rapidly and completely from the body in both males and females and that it does not persist in any tissue in either sex. Slight sex-related differences in some metabolic reactions were demonstrated, and the details of *in vitro* metabolism of pyriproxyfen are therefore now being investigated using rat liver microsomes.

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