0090-9556/09/3712-2284–2289$20.00
DRUG METABOLISM AND DISPOSITION
Vol. 37, No. 12
28878/3535913
DMD 37:2284–2289, 2009
Printed in U.S.A.

Metabolism of Pyridalyl in Rats

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Received June 8, 2009; accepted September 16, 2009

ABSTRACT:

Metabolism of pyridalyl [2,6-dichloro-4-(3,3-dichloroallyloxy)phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy]propyl ether] was examined in male and female Sprague-Dawley rats. After a single oral administration of [dichlorophenyl-^{14}C]pyridalyl at 5 or 500 mg/kg, the ^{14}C concentration in blood reached maxima at 2 to 10 h and then decreased rapidly with a biological half-life of approximately 11 to 12 h. ^{14}C concentrations in liver, fat, adrenal gland, and spleen were relatively high at a low dose, reaching 2.3 to 2.7, 1.9 to 2.3, 1.1 to 1.9, and 1.4 ppm, respectively, in these tissues at 2 to 24 h after administration. Although ^{14}C elimination from fat and hair and skin was relatively slow compared with that from other tissues, the total residue on the 7th day was low, in the range of 1.3 to 2.3% of the dose. The ^{14}C distribution in tissues with a high dose, as examined by whole-body autoradiography, was similar to that observed for the low dose. Results revealed that more than 88% of the dosed radiocarbon was excreted within 1 day after administration, with cumulative ^{14}C excretion into urine and feces 7 days after administration of 1.7 to 2.6 and 98.7 to 101.7%, respectively. One urinary and fecal major metabolite (resulting from *O*-dealkylation) and two minor metabolites were identified by NMR and mass spectrometry. Residual ^{14}C in fat was extracted, and analysis by thin-layer chromatography showed it to be due to pyridalyl itself. No marked sex-related differences were observed in ^{14}C elimination, ^{14}C distribution, and metabolites.

Pyridalyl [2,6-dichloro-4-(3,3-dichloroallyloxy)phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy]propyl ether; S-1812] is a new class of insecticide for *Lepidoptera* and *Thysanoptera* (Sakamoto et al., 2004; Isayama et al., 2005). It has dichloropropenyl, phenyl and pyridyl groups in its structure but does not share structural similarity with other insecticides. Toxicity studies, including acute, chronic, oncogenicity, developmental, mutagenicity, and reproductive studies, have all been conducted previously with low acute toxicity, no oncogenicity and mutagenicity, and no teratogenicity observed (US Environmental Protection Agency, 2008).

In the present study, metabolism of [dichlorophenyl-^{14}C]pyridalyl in rats was investigated in conjunction with toxicological studies for safety evaluation. ^{14}C excretion, ^{14}C tissue distribution, and metabolites were investigated in support of rodent toxicology studies. The present report deals with metabolism (^{14}C excretion into feces, urine, and expired air, ^{14}C concentrations in tissues, and amounts of metabolites in excreta) of pyridalyl in rats.

Materials and Methods

Chemicals. [Dichlorophenyl-^{14}C]pyridalyl was prepared at the Environmental Health Science Laboratory of Sumitomo Chemical Co., Ltd. (Osaka, Japan) with a specific activity of 4.37 GBq/mmol. Labeled compound was purified with thin-layer chromatography (TLC) before use, and the radiochemical purity was >96.2%. Unlabeled pyridalyl (purity 98.4%) was also obtained from our company. Pyridalyl was analyzed by NMR spectrometry: ^{1}H NMR (CD_3OD, 270 MHz, ppm) δ6.29 (1H, t, J = 6.2 Hz), 4.66 (2H, m), 6.97 (2H, m), 4.15 (2H, t, J = 5.6 Hz), 2.30 (2H, m), 4.66 (2H, m), 6.97 (1H, m), 7.94 (1H, dd, J = 8.5, 2.3 Hz), 8.49 (1H, d, J = 2.3 Hz); ^{13}C NMR (CD_3OD, 67.5 MHz) δ126, 64, 117, 131, 71, 31, 67, 112, 137, 146 ppm. FAB-MS showed a molecular ion peak at m/z 490[M + H]$^+$. Other chemicals were reagent grade.

TLC Analysis. The solvent systems were as follows: A, hexane-diethyl ether (10:1, v/v); B, hexane-toluene-acetic acid (3:15:2, v/v/v, developed twice); and C, ethyl acetate-ethanol-water (4:2:1, v/v/v). Unlabeled pyridalyl on TLC plates was detected by viewing under UV light (254 nm), and radioactive metabolites were detected by autoradiography using films developed with a model M6B processor (Eastman Kodak, Rochester, NY) or imaging plates processed with a BAS2000 Bio-image Analyzer (Fuji Photo Film, Kanagawa, Japan).

High-Performance Liquid Chromatography Analysis. Analysis of samples was conducted using an L-6200 type intelligent pump (Hitachi, Tokyo, Japan), an L-4000 UV detector (Hitachi), an LB 507A high-performance liquid chromatography (HPLC) Radioactivity Monitor (Berthold, Germany), and an 805 data station (Japan Millipore Limited, Tokyo, Japan). The wavelength of the UV detector was set at 254 nm. Preparative isolation was achieved on an YMC Pack column (ODS, 20 mm i.d. × 25 cm; YMC, Kyoto, Japan) and a guard column (Waters Guard-Pak, μBondapak C18; Millipore Corporation, Billerica, MA) with mobile phases of methanol-water (85:15 and 80:20 for analytical systems A and B, respectively). The flow rate was 3 ml/min.

Radioanalysis. Radioactivity in organosoluble fractions or urine was quantified by liquid scintillation counting (LSC) giving disintegrations per minute by the external standard method. Samples (100–300 mg) of fecal homogenates, unextractable fecal residues, and tissues were combusted with a sample

Article, publication date, and citation information can be found at http://dmd.aspetjournals.org.
doi:10.1124/dmd.109.028878.

ABBREVIATIONS: S-1812, 2,6-dichloro-4-(3,3-dichloroallyloxy)phenyl 3-[5-(trifluoromethyl)-2-pyridyloxy]propyl ether; TLC, thin-layer chromatography; FAB, fast atom bombardment; MS, mass spectrometry; HPLC, high-performance liquid chromatography; LSC, liquid scintillation counting; EI, electron ionization; SD, Sprague-Dawley; AUC, area under the curve; T_{max}, time to reach maximum concentration; C_{max}, maximum plasma concentration; DDT, dichlorodiphenyltrichloroethane; DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; PCB, polychlorinated biphenyl; DCHM, 2-6-dichloro-4-(3,3-dichloroprop-2-enyloxy)phenol; S-1812-Py-OH, 2-(3-(2,6-dichloro-4-(3,3-dichloroprop-2-enyloxy)phenoxy)propoxy)-3-hydroxy-5-(trifluoromethyl)pyridine; S-1812-DP, 3,5-dichloro-4-(3-(5-trifluoromethyl-2-pyridyloxy)propoxy)phenol.

oxidizer (PerkinElmer Life and Analytical Sciences, Waltham, MA) before LSC after air-drying (combustion-LSC method). Radiocarbon on TLC plates was quantified by scraping methods.

Spectroscopic and Spectrometric Analysis. Chemical structures of purified metabolites were determined by NMR and mass spectrometry. ^{1}H NMR and ^{13}C NMR spectra were obtained with a JEOL GSX-270 spectrometer (JEOL Ltd., Tokyo, Japan) with methanol-d_4 as the solvent. FAB-MS or EI-MS spectra were obtained with a DF/GC/MS M-80B spectrometer (Hitachi, Tokyo, Japan).

Kinetic Studies. Three male and female Crj:CD(SD) rats at the age of 6 weeks were purchased from Charles River Japan Inc. (Kanagawa, Japan). All animal experiments were conducted in accordance with the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan). Animals that showed normal weight gain and no abnormal clinical symptoms during 7 days of quarantine and acclimation were selected for dosing. The in-life portion of the study was conducted under the following environmental conditions: room temperature, 20 to 26°C; relative humidity, 55 ± 10%; ventilation, 10 air exchanges per hour; and artificial lighting from 8:00 AM to 8:00 PM. Animals had free access to pelleted diet and water through the study. Rats were orally dosed with a 5 or 500 mg/kg dose of [dichlorophenyl-^{14}C]pyridalyl (1.48 MBq/mg or 14.8 kBq/mg, respectively) in corn oil at 5 ml/kg. Blood was collected from an orbital vein into heparinized capillaries at 1, 2, 4, 6, 10, 24, 72, and 168 h after administration. The radioactivity in blood was determined by the combustion-LSC method. AUCs were generated by the trapezoidal rule. $t_{1/2}$ was calculated by linear regression analysis from data of 2 to 72 h and 10 to 72 h for low- and high-dose groups, respectively.

Tissue Distribution Studies. Four groups of three male and female Crj:CD(SD) rats at the age of 6 weeks were used. The environmental conditions were the same as for the kinetic studies. Rats were orally dosed with a 5 mg/kg dose of [dichlorophenyl-^{14}C]pyridalyl (1.48 MBq/mg) in corn oil at 5 ml/kg and housed in aluminum cages or glass metabolism cages (only for the 168-h group). Rats were euthanized with collection of blood from the abdominal artery at 1, 2, 24, and 168 h after administration. Their tissues and organs were dissected out and plasma and blood cells were separated by centrifugation (2000*g*, 10 min). The amounts of ^{14}C distributed to tissues were measured by a combustion-LSC method.

Whole-Body Autoradiography. Three groups of three male Crj:CD(SD) rats at the age of 6 weeks were used. The environmental conditions were the same as for the kinetic studies. Rats were orally dosed with a 500 mg/kg dose of [dichlorophenyl-^{14}C]pyridalyl (18.5 kBq/mg) in corn oil at 5 ml/kg and housed in aluminum cages. After euthanasia at 2, 24, and 168 h after administration, the rats in 6% carboxymethyl cellulose aqueous solution were frozen in acetone refrigerated by dry ice. Slices (30 μm) was prepared with a cryostat microtome (CM3600; Leica, Wetzlar, Germany) and placed in contact with imaging plates for 1 to 5 days. The plates were then processed with a BAS2000 Bio-image Analyzer.

Metabolism Studies. The animals of the 168-h group in the tissue distribution study were used for metabolism studies. Urine and feces were separately collected for 7 days. Expired air was passed through an alkaline trap containing 10% NaOH solution for 3 days after administration to collect expired CO_2 gas. Rats were euthanized by bleeding at 7 days after administration. The metabolites in feces collected within 2 days after administration were extracted three times with acetone, and radioactivity in supernatants and postextracted solids was analyzed. Feces collected from 3 to 7 days were homogenized with water and combusted for radioanalysis. Aliquots of the sodium hydroxide solution in which expired CO_2 was trapped were analyzed by LSC.

Metabolites in urine and fecal extracts within 1 day after administration were identified by TLC cochromatography with using solvent systems A, B, and C with pyridalyl metabolites previously isolated from rat feces and identified as described below. Subsequently, urine and fecal extracts were subjected to TLC using solvent systems C and A, respectively, for quantification analysis. The metabolites were quantified by the scraping method. Other areas were also scraped, and the included radioactivity was summed up as "others."

Fat samples were homogenized with a Polytron (Kinematica, Littau-Lucerne, Switzerland) and shaken twice in chloroform-methanol (2:1, v/v), and supernatants were obtained after centrifugation at 2000*g* for 10 min. Metabolites were then identified by two-dimensional TLC cochromatography with standards using solvent systems A (first) and B (second).

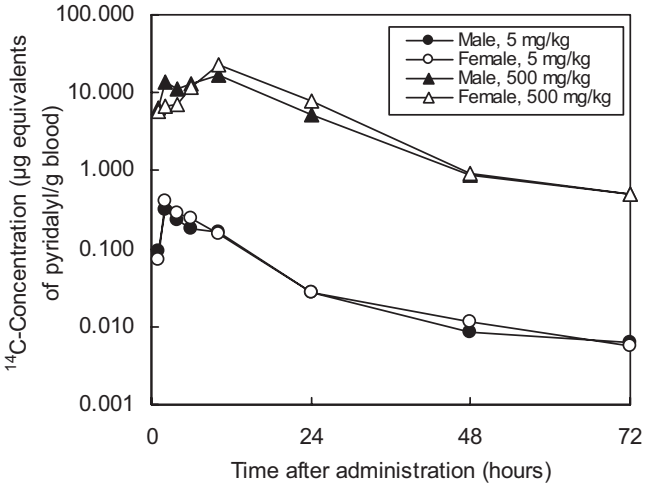

FIG. 1. ^{14}C concentrations in blood after a single oral administration of [dichlorophenyl-^{14}C]pyridalyl to male and female rats at 5 (low dose) and 500 (high dose) mg/kg.

Isolation and Purification of Metabolites. Five male Crj:CD(SD) rats were used for dosing at the age of 7 weeks. The environmental conditions were as described for the in vivo metabolism study. After ^{14}C-labeled compound was diluted to a concentration of approximately 37 kBq/mg specific activity using unlabeled compound, it was suspended in aqueous 10% Tween 80 at 500 mg/5 ml dosing solution. Oral administration of ^{14}C-labeled compound was carried out by gavage to five male rats at a constant volume of 5 ml/kg once daily for 5 consecutive days. Feces were collected from the first administration to 2 days after the last administration. All feces collected were combined and homogenized with a 3-fold volume of acetone using a Waring blender, and the homogenates were centrifuged at 2000*g* for 10 min. The supernatants were obtained by decantation, and the precipitates were further extracted twice with acetone. All fecal acetone extracts were combined, concentrated in vacuo (fecal extract), and subjected to silica gel (Kieselgel 60, 70–230 mesh; Merck, Darmstadt, Germany) column chromatography (25 mm i.d. × 300 mm). Eluents were sequentially separated into nine fractions of approximately 200 ml. Solvents used were 600 ml of hexane-diethyl ether (50:1, v/v) (fractions 1–3), 200 ml of hexane-diethyl ether (20:1, v/v) (fraction 4), 400 ml of hexane-diethyl ether (5:1, v/v) (fractions 5 and 6), and 200 ml each of diethyl ether, ethyl acetate, and ethanol (fractions 7–9). Fractions 3 and 4 were combined and subjected to HPLC using analytical system A. Eluates corresponding to the peaks at retention time (t_R) = 6.08 min and at t_R = 14.63 min were collected separately. The main metabolites in the two eluates were designated as M1 and M2, respectively. Fraction 5 was concentrated and subjected to HPLC using analytical system B. Eluates corresponding to the peaks at t_R = 10.95 min were collected. The main metabolites in the eluates were designated as M3. Collected metabolites, M1, M2, and M3, were further purified by HPLC and applied to the spectroanalyses. The metabolites were then used as standards.

Results

^{14}C Concentrations in Blood. Data for ^{14}C concentrations in blood of male and female rats after a single oral administration of [dichlorophenyl-^{14}C]pyridalyl at 5 or 500 mg/kg are shown in Fig. 1. Calculated kinetic parameters are summarized in Table 1. The ^{14}C concentration in blood increased rapidly after administration. In the low-dose group, a maximum was reached at 2 h with a C_{max} of 0.31 and 0.40 μg Eq of pyridalyl/g of blood (parts per million) in males and females, respectively. In the high-dose group, T_{max} was later, with ^{14}C concentration maxima at 7.33 and 10 h in males and females, respectively, and C_{max} values of 18.4 and 22.7 ppm. The biological half-life was approximately 11 to 12 h with both low and high doses. The AUC

TABLE 1

T_{max}, C_{max}, and $AUC_{0-168\ h}$ values for ^{14}C in blood after single oral administration of [dichlorophenyl-^{14}C]pyridalyl to male and female rats at 5 (low dose) and 500 (high dose) mg/kg

Data are the mean values ± S.D. for three animals.

	T_{max}	C_{max}	$AUC_{0-168\ h}$
	h	*ppm*	*μg Eq, h/g*
Male			
Low dose	2.00 ± 0.000	0.31 ± 0.063	4.21 ± 0.602
High dose	7.33 ± 4.619	18.4 ± 3.89	402.4 ± 98.25
Female			
Low dose	2.00 ± 0.000	0.40 ± 0.082	4.63 ± 1.365
High dose	10.00 ± 0.000	22.7 ± 4.27	477.5 ± 147.88

for the high dose was approximately 100 times higher than that for the low-dose group, which corresponds to the dose ratio. No marked sex-related differences in ^{14}C concentration in blood were observed.

^{14}C Concentrations in Tissues. Data for ^{14}C concentrations in tissues of rats 1, 2, 24, and 168 h after administration of the ^{14}C-labeled compound at 5 mg/kg are shown in Tables 2 and 3, respectively. In most tissues the highest levels were reached after 2 h, but the T_{max} for fat, hair and skin, brain, pituitary, spinal cord, testis, thymus, and thyroid was 24 h. ^{14}C concentrations were found to be relatively high in liver, fat, adrenal gland, and spleen at 2.3 to 2.7, 1.9 to 2.3, 1.1 to 1.9, and 1.4 ppm, respectively. The ^{14}C concentration decreased after T_{max} in the same manner as with blood. However, the ^{14}C concentrations in fat and hair and skin decreased more slowly, with a biological half-life of >2 days. Sex-related variations in ^{14}C concentrations in tissue were not observed. The total ^{14}C tissue residue on the 7th day after administration was 1.3 to 2.3% of the dose.

Whole-Body Autoradiography. ^{14}C concentrations in tissues at 2 h, 1 day, and 7 days after administration of the ^{14}C-labeled compound at 500 mg/kg are illustrated in Fig. 2. Stomach content and liver showed high radioactivity relative to that of other tissues at 2 h, intestinal content, fat, adrenal, liver, and skin at 1 day, and intestinal content, fat, and skin at 7 days. The results are consistent with the values for tissues, with the low dose shown in Tables 2 and 3. ^{14}C concentrations in most tissues decreased rapidly within 7 days, except in fat and skin.

^{14}C Excretion. Data for cumulative ^{14}C excretion into feces, urine, and expired air of male and female rats 7 days after a single oral administration of [dichlorophenyl-^{14}C]pyridalyl at 5 mg/kg are shown in Fig. 3. ^{14}C was rapidly and almost completely excreted into urine and feces in rats. More than 88% of the dosed ^{14}C was excreted within 1 day. ^{14}C excretion into expired air was not detected. ^{14}C excretion within 7 days after administration was 101.3% (feces 98.7% and urine 2.6%) in male rats and 103.3% (feces 101.7% and urine 1.7%) in female rats. No marked sex-related differences were observed in the rate of ^{14}C-elimination.

Metabolites in Feces, Urine, and Fat. Table 4 shows amounts (percentage of the dosed ^{14}C) of fecal and urinary metabolites in rats. The one major metabolite (M3) in both feces and urine was identified as S-1812-DP, as detailed below. Other metabolites identified, M1 and M2, were detected as minor metabolites (<3%). The parent compound was detected only in feces, and this accounted for 54.1 to 55.4% of the dose. Marked sex-related differences were not observed in the metabolism of pyridalyl in rats. The compound in fat was also analyzed by two-dimensional TLC analysis, and only the parent pyridalyl was found.

Identification of Metabolites. M1 was analyzed by NMR spectrometry: ^1H NMR (CD_3OD, 270 MHz, ppm) δ6.25 (1H, t, J = 5.9 Hz), 4.58 (2H, d, J = 5.9 Hz), 6.91 (2H, s); ^{13}C NMR (CD_3OD, 67.5 MHz) δ127, 65, 116, 124 ppm. The ^1H NMR spectrum of M1 showed

TABLE 2

^{14}C concentrations in tissues after single oral administration of [dichlorophenyl-^{14}C]pyridalyl to male rats at 5 mg/kg

Data are the mean values ± S.D. for three animals.

Tissue	Time after Administration			
	1 h	2 h	24 h	168 h
	μg equivalents of pyridalyl/g wet tissue (ppm)			
Adrenal	0.222 ± 0.0338	1.127 ± 0.0809	0.177 ± 0.0835	0.087 ± 0.0563
Blood	0.131 ± 0.0382	0.412 ± 0.1146	0.017 ± 0.0037	0.004 ± 0.0047
Blood cells	0.046 ± 0.0160	0.113 ± 0.0400	0.008 ± 0.0026	<0.002
Plasma	0.189 ± 0.0612	0.645 ± 0.2090	0.020 ± 0.0045	<0.002
Bone	0.014 ± 0.0021	0.054 ± 0.0159	0.014 ± 0.0068	<0.002
Bone marrow	0.039 ± 0.0065	0.172 ± 0.0291	0.044 ± 0.0303	0.013 ± 0.0166
Brain	0.010 ± 0.0035	0.021 ± 0.0038	0.025 ± 0.0081	<0.002
Cecum	NA	NA	NA	0.055 ± 0.0312
Carcass	NA	NA	NA	0.142 ± 0.0493
Fat	0.051 ± 0.0122	0.301 ± 0.1143	2.275 ± 0.2040	1.922 ± 0.4978
Hair and skin	0.035 ± 0.0124	0.103 ± 0.0201	0.306 ± 0.0091	0.059 ± 0.0143
Heart	0.153 ± 0.0428	0.548 ± 0.0634	0.037 ± 0.0012	0.005 ± 0.0002
Kidney	0.088 ± 0.0185	0.301 ± 0.0331	0.180 ± 0.0552	0.011 ± 0.0023
Large intestine	NA	NA	NA	0.014 ± 0.0076
Liver	0.433 ± 0.0640	2.282 ± 0.6599	0.685 ± 0.1670	0.045 ± 0.0151
Lung	0.182 ± 0.0691	0.505 ± 0.0790	0.113 ± 0.0476	0.018 ± 0.0159
Mandibular gland	0.027 ± 0.0051	0.126 ± 0.0176	0.052 ± 0.0026	0.005 ± 0.0007
Muscle	0.016 ± 0.0034	0.070 ± 0.0089	0.037 ± 0.0135	0.009 ± 0.0070
Pancreas	0.093 ± 0.0688	0.272 ± 0.0600	0.122 ± 0.0479	0.112 ± 0.1193
Pituitary	<0.083	0.199 ± 0.0269	0.314 ± 0.4745	0.022 ± 0.0310
Small intestine	NA	NA	NA	0.051 ± 0.0486
Spinal cord	0.011 ± 0.0006	0.021 ± 0.0064	0.026 ± 0.0067	<0.002
Spleen	0.171 ± 0.0529	1.434 ± 0.4373	0.024 ± 0.0017	0.006 ± 0.0038
Stomach	NA	NA	NA	0.014 ± 0.0003
Testis	0.005 ± 0.0014	0.017 ± 0.0025	0.025 ± 0.0026	<0.002
Thymus	0.015 ± 0.0001	0.063 ± 0.0107	0.065 ± 0.0231	0.020 ± 0.0147
Thyroid	0.344 ± 0.3817	0.328 ± 0.1362	0.395 ± 0.1310	0.042 ± 0.0191

NA, not analyzed.

TABLE 3

^{14}C concentrations in tissues after single oral administration of [dichlorophenyl-^{14}C]pyridalyl to female rats at 5 mg/kg

Data are the mean values ± S.D. for three animals.

Tissue	Time after Administration			
	1 h	2 h	24 h	168 h
	μg equivalents of pyridalyl/g wet tissue (ppm)			
Adrenal	0.239 ± 0.0716	1.871 ± 1.3293	0.364 ± 0.4026	0.035 ± 0.0099
Blood	0.122 ± 0.0262	0.250 ± 0.0514	0.032 ± 0.0333	0.003 ± 0.0012
Blood cells	0.052 ± 0.0031	0.090 ± 0.0331	0.017 ± 0.0176	<0.002
Plasma	0.188 ± 0.0398	0.420 ± 0.0793	0.050 ± 0.0527	<0.002
Bone	0.014 ± 0.0024	0.061 ± 0.0106	0.035 ± 0.0303	0.004 ± 0.0029
Bone marrow	0.047 ± 0.0087	0.184 ± 0.0415	0.068 ± 0.0686	0.009 ± 0.0028
Brain	0.006 ± 0.0014	0.023 ± 0.0033	0.026 ± 0.0159	<0.002
Cecum	NA	NA	NA	0.120 ± 0.1778
Carcass	NA	NA	NA	0.080 ± 0.0117
Fat	0.051 ± 0.0173	0.248 ± 0.0804	1.904 ± 0.2378	0.802 ± 0.2069
Hair and skin	0.043 ± 0.0112	0.102 ± 0.0163	0.275 ± 0.0239	0.100 ± 0.1020
Heart	0.208 ± 0.0738	1.042 ± 0.2117	0.093 ± 0.1056	0.006 ± 0.0026
Kidney	0.090 ± 0.0210	0.327 ± 0.0494	0.181 ± 0.1312	0.010 ± 0.0030
Large intestine	NA	NA	NA	0.008 ± 0.0034
Liver	0.611 ± 0.1140	2.678 ± 0.3560	1.189 ± 1.0307	0.026 ± 0.0089
Lung	0.148 ± 0.0463	0.503 ± 0.0238	0.143 ± 0.1173	0.011 ± 0.0057
Mandibular gland	0.032 ± 0.0133	0.150 ± 0.0489	0.071 ± 0.0698	0.006 ± 0.0029
Muscle	0.021 ± 0.0038	0.091 ± 0.0228	0.060 ± 0.0437	0.004 ± 0.0026
Ovary	0.078 ± 0.0226	0.512 ± 0.2162	0.406 ± 0.2996	0.037 ± 0.0022
Pancreas	0.080 ± 0.0169	0.541 ± 0.1801	0.179 ± 0.0445	0.042 ± 0.0221
Pituitary	0.122 ± 0.0889	0.267 ± 0.2064	0.099 ± 0.0820	<0.037
Small intestine	NA	NA	NA	0.037 ± 0.0139
Spinal cord	0.011 ± 0.0005	0.023 ± 0.0063	0.032 ± 0.0144	<0.002
Spleen	0.210 ± 0.0885	1.426 ± 0.3243	0.058 ± 0.0638	0.004 ± 0.0017
Stomach	NA	NA	NA	0.018 ± 0.0032
Thymus	0.020 ± 0.0068	0.078 ± 0.0327	0.073 ± 0.0549	0.009 ± 0.0016
Thyroid	0.204 ± 0.2299	0.258 ± 0.0313	0.602 ± 0.2025	0.031 ± 0.0100
Uterus	0.027 ± 0.0144	0.080 ± 0.0435	0.044 ± 0.0372	0.008 ± 0.0085

NA, not analyzed.

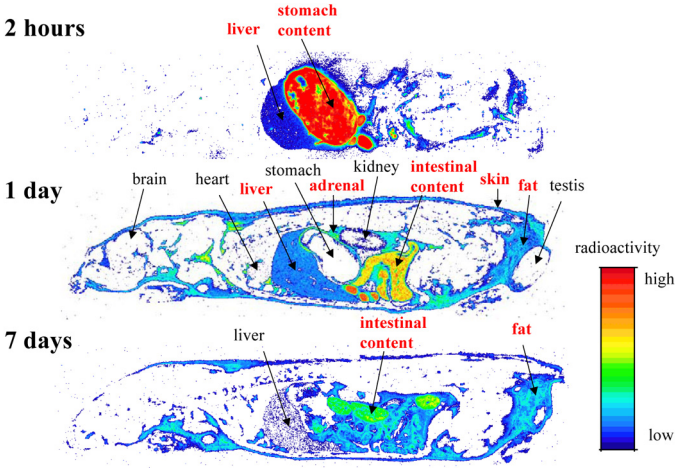

FIG. 2. Whole-body autoradiography (central axis section) of a male rat 2 h (top), 1 day (middle), and 7 days (bottom) after administration of [dichlorophenyl-^{14}C]pyridalyl at 500 mg/kg.

disappearance of signals for the pyridyl and trimethylene groups of pyridalyl by absence of signals at 6.97, 7.94, 8.48, 2.30, 4.15, and 4.66 ppm. The ^{13}C NMR spectrum also showed disappearance of signals for the pyridyl, trimethylene, and trifluoromethyl groups of pyridalyl by absence of signals at 71, 31, 67, 112, 137, and 146 ppm. EI-MS and FAB-MS showed a molecular ion peak at *m/z* 286 $[M]^+$. Based on these results, the metabolite was considered to be 2,6-dichloro-4-(3,3-dichloroprop-2-enyloxy)phenol (DCHM), formed by oxidative *O*-dealkylation by cytochrome P450 of pyridalyl between the trimethylene and dichlorophenylene groups.

M2 was analyzed by NMR spectrometry: ^{1}H NMR (CD_3OD, 270 MHz, ppm) δ6.30 (1H, t, J = 6.2 Hz), 4.69 (2H, m), 6.99 (2H, s), 4.20 (2H, t, J = 5.2 Hz), 2.36 (2H, m), 4.69 (2H, m), 7.26 (1H, d, J = 1.6 Hz), 7.95 (1H, d, J = 1.6 Hz); ^{13}C NMR (CD_3OD, 67.5 MHz) δ128, 65, 117, 131, 72, 31, 67, 119, 135 ppm. The ^{1}H NMR spectrum of M2 showed disappearance of one proton signal for the pyridyl ring of pyridalyl and existence of J = 1.6 Hz coupling of signals for the pyridyl ring at 7.26 and 7.95 ppm. M2 was considered to be received oxidation at position 3 of the pyridyl ring, and the protons at positions 4 and 6 of the pyridyl ring were considered to undergo meta coupling. The ^{13}C NMR spectrum of M2 showed that the signal for the 3-C atom of the pyridyl ring (112 ppm) disappeared. FAB-MS showed a molecular ion peak at *m/z* 506 $[M + H]^+$, which was 16 larger than the molecular ion peak of pyridalyl (*m/z* 490 $[M + H]^+$ by FAB-MS). EI-MS showed fragment ion peaks at *m/z* 486 $[M - F]^+$ and 220 $[C_5H_2N(CF_3)(OH)OCH_2CH_2CH_2]^+$. Based on these results, this metabolite was considered to be received hydroxylation at position 3 of the pyridyl ring of pyridalyl and was identified as S-1812-Py-OH.

M3 was analyzed by NMR spectrometry: ^{1}H NMR (CD_3OD, 270 MHz, ppm) δ6.78 (2H, s), 4.10 (2H, t, J = 6.1 Hz), 2.26 (2H, m), 4.65 (2H, t, J = 6.1 Hz), 6.96 (1H, d, J = 9.3 Hz), 7.94 (1H, dd, J = 9.3, 2.0 Hz), 8.48 (1H, d, J = 2.0 Hz); ^{13}C NMR (CD_3OD, 67.5 MHz) δ117, 131, 71, 31, 65, 112, 137, 146 ppm. The ^{1}H NMR and ^{13}C NMR spectra of M3 showed disappearance of signals for the dichloropropenyl group at 6.29 and 4.66 and 126 and 64 ppm, respectively. FAB-MS showed a molecular ion peak at *m/z* 382 $[M + H]^+$ with a chlorine isotope peak. EI-MS showed a fragment ion peak at *m/z* 362 $[M - F]^+$ and 204 $[C_5H_3N(CF_3)OCH_2CH_2CH_2]^+$. Based on these results, the metabolite was considered to be S-1812-DP, formed by

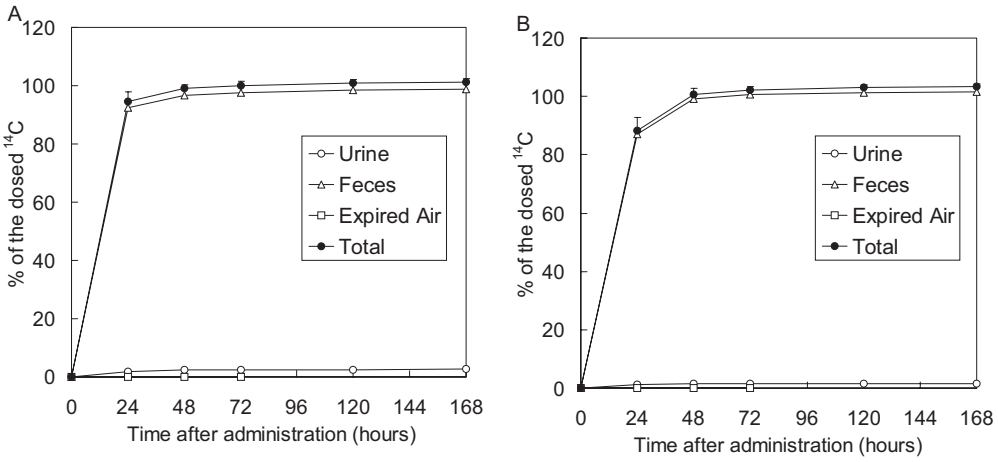

FIG. 3. Cumulative ^{14}C excretion into urine, feces, and expired air within 7 days after a single oral administration of [dichlorophenyl-^{14}C]pyridalyl to male (A) and female (B) rats at 5 mg/kg.

TABLE 4

Amounts of metabolites in urine and feces of rats within 1 day after single oral administration of [dichlorophenyl-^{14}C]pyridalyl to male and female rats at 5 mg/kg

Data are the mean values ± S.D. for three rats.

Metabolite	Male	Female
	% of dosed ^{14}C	
Urine		
S-1812-DP (M3)	0.1 ± 0.04	0.3 ± 0.16
Unknown U1	0.2 ± 0.06	0.1 ± 0.02
Unknown U2	0.2 ± 0.06	0.2 ± 0.11
Unknown U3	0.2 ± 0.04	0.1 ± 0.05
Unknown U4	1.1 ± 0.35	0.3 ± 0.17
Others	0.3 ± 0.04	0.3 ± 0.11
Subtotal	2.0 ± 0.52	1.2 ± 0.58
Feces		
Pyridalyl	54.1 ± 12.21	55.4 ± 11.56
S-1812-DP (M3)	21.7 ± 4.59	17.9 ± 6.23
Others[a]	3.1 ± 0.45	2.5 ± 1.01
Unextractable	13.6 ± 1.28	11.3 ± 3.03
Subtotal	92.6 ± 6.26	87.1 ± 8.29
Total	94.5 ± 5.88	88.3 ± 8.15

[a] Others in feces include trace amounts of DCHM (M1) and S-1812-Py-OH (M2).

oxidative *O*-dealkylation of the dichloropropenyl group of pyridalyl by cytochrome P450.

Discussion

The present study revealed that, on single oral administration of [dichlorophenyl-^{14}C]pyridalyl to male and female rats at 5 or 500 mg/kg, the radiocarbon was rapidly absorbed. ^{14}C concentrations in blood thus reached maxima at 2 to 10 h after administration. In the low-dose group, relatively high levels were observed in liver, fat, adrenal glands, and spleen. Then ^{14}C was rapidly excreted into feces and urine at 99 to 102 and 2 to 3%, respectively, with total ^{14}C recoveries of 101 to 103%. Total ^{14}C residues in tissues at 7 days were below 1.3 to 2.3% of the dose. S-1812-DP (M3) was the major metabolite in feces and urine. No marked sex-related differences were observed in ^{14}C elimination, ^{14}C distribution, or metabolite profile.

Absorbed ^{14}C was rapidly eliminated into feces as S-1812-DP (M3, resulting from *O*-dealkylation of pyridalyl). Based on the whole-body autoradiography experiments, significant biliary excretion of metabolites is occurring (activity in intestinal contents). Double peaks in the ^{14}C concentrations in blood suggest enterohepatic circulation of metabolites. The hydroxyl group in S-1812-DP can be conjugated to form glucuronide or sulfate in liver after absorption. There is a threshold molecular weight of 325 ± 50 for appreciable biliary excretion of anions in rat (Hirom et al., 1972). Conjugated S-1812-DP is large enough to be excreted into bile, because S-1812-DP has a molecular weight of 381. Therefore, S-1812-DP formed in liver would be readily excreted into feces via the bile. Biliary excretion of pyridalyl was not observed in a metabolism study using bile duct-cannulated rats (H. Nagahori, unpublished observation).

^{14}C concentrations were here found to be relatively high in liver, fat, adrenal gland, and spleen and the concentration in fat was solely due to pyridalyl itself. Furthermore, ^{14}C concentrations decreased relatively slowly in fat and skin, which might have been caused by the lipophilic nature of pyridalyl (log P = 8.1). Lipophilic compounds such as dieldrin, DDT (DDE), PCB, and dioxin are also known to become distributed to fat and eliminated slowly. Although we see bioaccumulation of such lipophilic compounds as a problem, pyridalyl has a different nature. Residual ^{14}C in fat 7 days after administration of pyridalyl was 2% of the dose, but that of lipophilic compounds such as dieldrin, DDT (DDE), PCB, and dioxin was found to be >6, 53.03, 50 to 70, and 10 to 20%, respectively (Hayes, 1974; Matthews and Tuey, 1980; Abraham et al., 1988; Mühlebach et al., 1991). The difference may depend on metabolic stability. Pyridalyl has an ether bond that is easily cleaved, whereas dieldrin, DDT (DDE), and PCB are more resistant to degradation. Pyridalyl is rapidly metabolized to form S-1812-DP (M3) in liver after administration and then is excreted rapidly without distribution to tissues.

The AUC ratio for the low dose to high dose was approximately 100, and the ^{14}C distribution pattern with both doses was similar. The results indicated that the absorption ratio, metabolism, distribution, and excretion of pyridalyl were not affected by the increase in the dose to 500 mg/kg. Although the absorption was a little delayed at the high dose, the delay did not significantly affect the absorption ratio of pyridalyl. Metabolism and excretion can be considered to be unsaturated with doses at and less than 500 mg/kg.

The elimination of ^{14}C from blood was rapid, with a $t_{1/2}$ of approximately 11 to 12 h calculated by linear regression analysis (from data of 2 to 72 h and 10 to 72 h for low- and high-dose groups, respectively) so that concentrations in blood at 72 h after administration were approximately 0.006 and 0.5 ppm, with the low and high doses, respectively. This finding indicates that pyridalyl is readily metabolized and excreted from the body. Elimination from fat was relatively slow and may be partly dependent on flow or diffusion-limited kinetics.

S-1812-DP (M3) was the major metabolite. *O*-Dealkylation of allyl-alkyl ethers is considered to be catalyzed by cytochrome P450 in liver, and this reaction is considered to occur frequently in mammals,

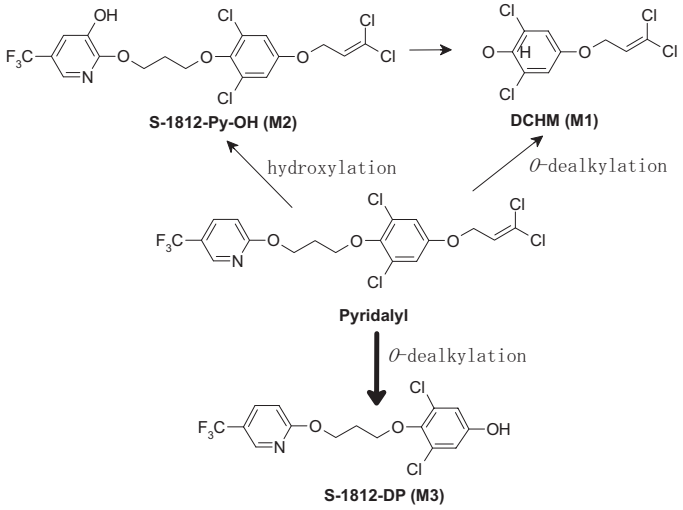

FIG. 4. Proposed metabolic pathways for pyridalyl in rats.

independent of the species. Further investigations are required to clarify species-related differences in rates of *O*-dealkylation of the allyl and alkyl ethers and determination of the various cytochrome P450 enzymes involved in the metabolism of pyridalyl. The identification of additional metabolites will also be the focus of further investigation, particularly as it relates to the fate of the dichloropropenyl group of pyridalyl.

On the basis of identification of the metabolite in this study, the biotransformation reaction in rats is proposed to be cleavage of the ether linkage between the dichloropropenyl group and the dichlorophenyl group to form S-1812-DP (M3). The proposed metabolic pathway of pyridalyl is shown in Fig. 4.

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