

Metabolism of Pyridalyl in Rats

*Hirohisa Nagahori**, Koichi Saito, Yoshitaka Tomigahara, Naohiko Isobe and Hideo Kaneko

Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., 1-98, 3-Chome, Kasugade-
Naka, Konohana-Ku, Osaka 554-8558, Japan

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Corresponding author: Hirohisa Nagahori,

Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd., 1-98, 3-Chome, Kasugade-Naka, Konohana-Ku, Osaka 554-8558, Japan

Tel.: 81-6-6466-5321. Fax: 81-6-6466-5442. E-mail: nagahori@sc.sumitomo-chem.co.jp.

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Abbreviations used are: SD, Sprague-Dawley; NMR, nuclear magnetic resonance; MS, mass spectrometry; TLC, thin-layer chromatography; FAB, fast atom bombardment; HPLC, high performance liquid chromatography; LSC, liquid scintillation counting; AUC, area under the curve; CO₂, carbon dioxide; C_{max}, maximum plasma concentration; T_{max}, time to reach maximum concentration; WBA, whole-body autoradiography; EI, electron ionization; DDT, dichlorodiphenyltrichloroethane; DDE, 1,1-dichloro-2,2 bis (*p*-chlorophenyl) ethylene; PCB, polychlorinated biphenyl

DMD28878

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 SD rats. After single oral administration of [dichlorophenyl- ^{14}C]pyridalyl at 5 or 500 mg/kg, the ^{14}C -concentration in blood reached maxima at 2-10 hours and then decreased rapidly with a biological half-life of about 11 - 12 hours. ^{14}C -Concentrations in liver, fat, adrenal and spleen were relatively high at low dose, reaching 2.3 - 2.7 ppm, 1.9 - 2.3 ppm, 1.1 - 1.9 ppm and 1.4 ppm, respectively in these tissues at 2 to 24 hours after administration. Though ^{14}C -elimination from fat and hair & skin were relatively slow as compared to other tissues, the total residue on the 7th day was low, in the range of 1.3 - 2.3% of the dose. The ^{14}C -distribution in tissues with high dose, as examined by whole-body autoradiography, was similar to that observed for the low dose. Results revealed that over 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 - 2.6% and 98.7 - 101.4%, respectively. One urinary and fecal major metabolite (resulting from *O*-dealkylation) and two minor metabolites were identified by NMR and MS spectrometry. Residual ^{14}C in fat was extracted and analysis by TLC 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.

INTRODUCTION

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 previously conducted with low acute toxicity, no oncogenicity and mutagenicity, and no teratogenicity observed (USEPA, 2008).

In the present study, metabolism of [dichlorophenyl-¹⁴C]pyridalyl in rats was investigated in conjunction with toxicological studies for safety evaluation. ¹⁴C-Excretion, the ¹⁴C-tissue distribution and metabolites were investigated in support of rodent toxicology studies.

The present report thus deals with metabolism (¹⁴C-excretion into feces, urine, expired air, ¹⁴C-concentrations in tissues, and amounts of metabolites in excreta) of pyridalyl in rats.

METHODS

Chemicals. [dichlorophenyl-¹⁴C]pyridalyl was prepared at the Environmental Health Science Laboratory of Sumitomo Chemical, Co., Ltd. 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; ¹H-NMR (CD₃OD, 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); ¹³C-NMR (CD₃OD, 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.

Thin layer chromatography (TLC) analysis. The solvent systems were: 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 by autoradiography using films developed with a Model M6B processor (Kodak, NY) or imaging plates processed with a BAS2000 Bio-image Analyzer (Fuji Photo Film, Kanagawa, Japan).

HPLC 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 HPLC Radioactivity Monitor (Berthold,

Germany), and an 805 data station (Japan Millipore Limited, Tokyo). The wavelength of the UV detector was set at 254 nm. Preparative isolation was achieved on an YMC Packed column (ODS, 20 mm i.d. x 25 cm, YMC, Kyoto, Japan) and a guard column (Waters Guard-Pak, μ Bondapak C₁₈, Millipore Corporation, USA) 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 (dpm) by the external standard method. Samples (100-300 mg) of fecal homogenates, unextractable fecal residues and tissues were combusted with a sample oxidizer (Packard) prior to LSC after air-drying (combustion-LSC method). Quantification of radiocarbon on TLC plates was conducted by scraping methods.

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

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 - 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 5 or 500 mg/kg dose of [dichlorophenyl-¹⁴C]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 hours 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 - 72 hours and 10 - 72 hours 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 5 mg/kg dose of [dichlorophenyl-¹⁴C]pyridalyl (1.48 MBq/mg) in corn oil at 5 mL/kg

DMD28878

and housed in aluminum cages or glass metabolism cages (only for the 168 hr group). Rats were euthanized with collection of blood from the abdominal artery at 1, 2, 24 and 168 hours 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 hours 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 (CM-3600, Lyca, Germany) and placed in contact with imaging plates for 1-5 days. The plates were then processed with a BAS2000 Bio-image Analyzer.

Metabolism studies. The animals of the 168 hr 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 three 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 post-extracted 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 co-chromatography 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, 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).

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 5 male rats at a constant volume of 5 ml/kg for 5 consecutive days once daily. Feces were collected from the first administration to 2 days after the last administration. All collected feces 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, Germany) column chromatography (25 mm I.D. \times 300 mm). Eluents were sequentially separated into 9 fractions of about 200 ml. Used solvents 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 an 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 an 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

1. ^{14}C -Concentrations in blood

Data for ^{14}C -concentrations in blood of male and female rats after single oral administration of [dichlorophenyl- ^{14}C]pyridalyl at 5 or 500 mg/kg are shown in Figure 1. Calculated kinetic parameters are summarized in Table 1. ^{14}C -concentration in blood rapidly increased after administration. In the low dose group, a maximum was reached at 2 hours with C_{\max} of 0.31 and 0.40 μg eq. of pyridalyl / g blood (ppm) in males and females, respectively. In the high dose group, T_{\max} was later, with ^{14}C -concentration maxima at 7.33 and 10 hours in males and females, respectively, and C_{\max} values of 18.4 ppm and 22.7 ppm. The biological half-life was about 11 - 12 hours with both low and high doses. The area under the curve (AUC) for the high dose was about 100 times higher than for the low dose

group, which corresponds to the dose ratio. No marked sex-related differences in ^{14}C -concentration in blood were observed.

2. ^{14}C -Concentrations in tissues

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

3. Whole-body autoradiography (WBA)

^{14}C -Concentrations in tissues at 2 hours, 1 day and 7 days after administration of the ^{14}C -labeled compound at 500 mg/kg are illustrated in Figure 2. Stomach content and liver showed high radioactivity relative to other tissues at 2 hours, 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 7days, except in fat and skin.

4. ^{14}C -Excretion

Data for cumulative ^{14}C -excretion into feces, urine and expired air of male and female rats 7 days after single oral administration of [dichlorophenyl- ^{14}C]pyridalyl at 5 mg/kg are shown in Figure 3. ^{14}C was rapidly and almost completely excreted into urine and feces in rats. Over 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 were 101.3% (feces: 98.7% and urine: 2.6%) in male rats, and 103.3% (feces: 101.4% and urine: 1.7%) in female rats. No marked sex-related differences were observed in the rate of ^{14}C -elimination.

5. Metabolites in feces, urine and fat

Table 4 shows amounts (% 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

identified metabolites, 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.

6. Identification of metabolites

M1 was analyzed by NMR spectrometry; $^1\text{H-NMR}$ (CD_3OD , 270 MHz, ppm) δ 6.25 (1H, t, $J = 5.9$ Hz), 4.58 (2H, d, $J = 5.9$ Hz), 6.91 (2H, s); $^{13}\text{C-NMR}$ (CD_3OD , 67.5 MHz) δ 127, 65, 116, 124 ppm. The $^1\text{H-NMR}$ spectrum of M1 showed 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 $^{13}\text{C-NMR}$ 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; $^1\text{H-NMR}$ (CD_3OD , 270 MHz, ppm) δ 6.30 (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); $^{13}\text{C-NMR}$ (CD_3OD , 67.5 MHz) δ 128, 65, 117, 131, 72, 31, 67, 119, 135 ppm. The $^1\text{H-NMR}$ 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 a oxidation at the position-3 of the pyridyl ring, and the protons at the positions-4 and -6 of the pyridyl ring were considered to undergo meta coupling. The $^{13}\text{C-NMR}$ spectrum of M2 showed the signal for 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 a fragment ion peak at m/z 486[M-F] $^+$ and 220[C₅H₂N(CF₃)(OH)OCH₂CH₂CH₂] $^+$. Based on these results, this metabolite was considered to be received hydroxylation at the position-3 of the pyridyl ring of pyridalyl and identified as 2-(3-(2,6-dichloro-4-(3,3-dichloroprop-2-enyloxy)phenoxy)propoxy)-3-hydroxy-5-(trifluoromethyl)pyridine (S-1812-Py-OH).

DMD28878

M3 was analyzed by NMR spectrometry; $^1\text{H-NMR}$ (CD_3OD , 270 MHz, ppm) δ 6.78 (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); $^{13}\text{C-NMR}$ (CD_3OD , 67.5 MHz) δ 117, 131, 71, 31, 65, 112, 137, 146 ppm. The $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra of M3 showed disappearance of signals for the dichloropropenyl group at 6.29 and 4.66 ppm, and 126 and 64 ppm, respectively. FAB-MS showed a molecular ion peak at m/z 382 $[\text{M}+\text{H}]^+$ with a chlorine isotope peak. EI-MS showed a fragment ion peak at m/z 362 $[\text{M}-\text{F}]^+$ and 204 $[\text{C}_5\text{H}_3\text{N}(\text{CF}_3)\text{OCH}_2\text{CH}_2\text{CH}_2]^+$. Based on these results, the metabolite was considered to be 3,5-dichloro-4-(3-(5-trifluoromethyl-2-pyridyloxy)propoxy)phenol (S-1812-DP), formed by 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 - 10 hours after administration. In the low dose group, relatively high levels were observed in liver, fat, adrenals and spleen. Then, ^{14}C was rapidly excreted into feces and urine, at 99 - 101% and 2 - 3%, respectively, with total ^{14}C recoveries of 101 - 103%. Total ^{14}C -residues in tissues at 7 days were below 1.3 - 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, the ^{14}C -distribution and metabolite profile.

Absorbed ^{14}C was rapidly eliminated into feces as S-1812-DP (M3, resulting from *O*-dealkylation of pyridalyl). Based on the WBA 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 excreted into bile, since 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 metabolism study using bile duct-cannulated rats (unpublished observation).

^{14}C -Concentrations were here found to be relatively high in liver, fat, adrenal and spleen and that in fat was solely due to pyridalyl itself. Further, ^{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. Though 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

DMD28878

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 (Wayland, 1974; Mühlebach et al., 1991; Matthews and Tuey, 1980; Abraham et al., 1988). The difference may depend on metabolic stability. Pyridalyl has an ether bond which is easily cleaved, while 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 about 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 increase of dose to 500 mg/kg. Though 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 below 500 mg/kg.

The elimination of ^{14}C from blood was rapid, with a $T_{1/2}$ of about 11 - 12 hours calculated by linear regression analysis (from data of 2 - 72 hours and 10 - 72 hours for low and high dose groups, respectively). So that concentrations in blood at 72 hours after administration were about 0.006 ppm and 0.5 ppm, with the low and high doses, respectively. This indicates that pyridalyl is readily metabolized and excreted from the body. The fact that elimination from the fat was relatively slow, might also 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 frequently occur in mammals, independent of the species. Further investigations are required to clarify species-related differences in rates of *O*-dealkylations of the allyl and alkyl ethers, and determinations 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 Figure 4.

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FIGURE LEGENDS

Figure 1. ^{14}C -Concentrations in blood after single oral administration of [dichlorophenyl- ^{14}C]pyridalyl to male and female rats at 5 mg/kg (Low dose).

Figure 2. Whole-body autoradiography (central axis section) of a male rat 2 hours (Top), 1 day (Middle) and 7 days (Bottom) after administration of [dichlorophenyl- ^{14}C]pyridalyl at 500 mg/kg.

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

Figure 4. Proposed metabolic pathways for pyridalyl in rats.

DMD28878

TABLES

Table 1 Maximum times (T_{\max}), maximum concentrations (C_{\max}) and $AUC_{0-168hr}$ 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.

		T_{\max} (hr)	C_{\max} (ppm)	$AUC_{0-168hr}$ ($\mu g \text{ eq.} \cdot \text{ hr/g}$)
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

Data are the mean values \pm standard deviation for three animals.

DMD28878

Table 2 ^{14}C -Concentrations in tissues after single oral administration of [dichlorophenyl- ^{14}C]pyridalyl to male rats at 5 mg/kg.

Tissue	μg equivalents of pyridalyl/g wet tissue (ppm)			
	Time after administration (hr)			
	1	2	24	168
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
Caecum	----	----	----	0.055±0.0312
Carcass	----	----	----	0.142±0.0493
Fat	0.051±0.0122	0.301±0.1143	2.275±0.2040	1.922±0.4978
Hair & 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	----	----	----	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

DMD28878

Pituitary	<0.08	0.199±0.0269	0.314±0.4745	0.022±0.0310
Small intestine	----	----	----	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	----	----	----	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

Data are the mean values ± standard deviation for three animals.

DMD28878

Table 3 ¹⁴C-Concentrations in tissues after single oral administration of [dichlorophenyl-¹⁴C]pyridalyl to female rats at 5 mg/kg.

Tissue	µg equivalents of pyridalyl/g wet tissue (ppm)			
	Time after administration (hr)			
	1	2	24	168
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	0.0680.0686±	0.009±0.0028
Brain	0.006±0.0014	0.023±0.0033	0.026±0.0159	<0.002
Caecum	----	----	----	0.120±0.1778
Carcass	----	----	----	0.080±0.0117
Fat	0.051±0.0173	0.248±0.0804	1.904±0.2378	0.802±0.2069
Hair & 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	----	----	----	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

DMD28878

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	----	----	----	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	----	----	----	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

Data are the mean values ± standard deviation for three animals.

DMD28878

Table 4 Amounts of metabolites in urine and feces of rats within 1 day after single oral administration of [dichlorophenyl-¹⁴C]pyridalyl to male and female rats at 5 mg/kg.

Metabolite	% of dosed ¹⁴ C					
	Male			Female		
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	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

Data are the mean values ± standard deviation for three rats.

a) Others in feces includes trace amount of DCHM (M1) and S-1812-Py-OH (M2).

Figure 1

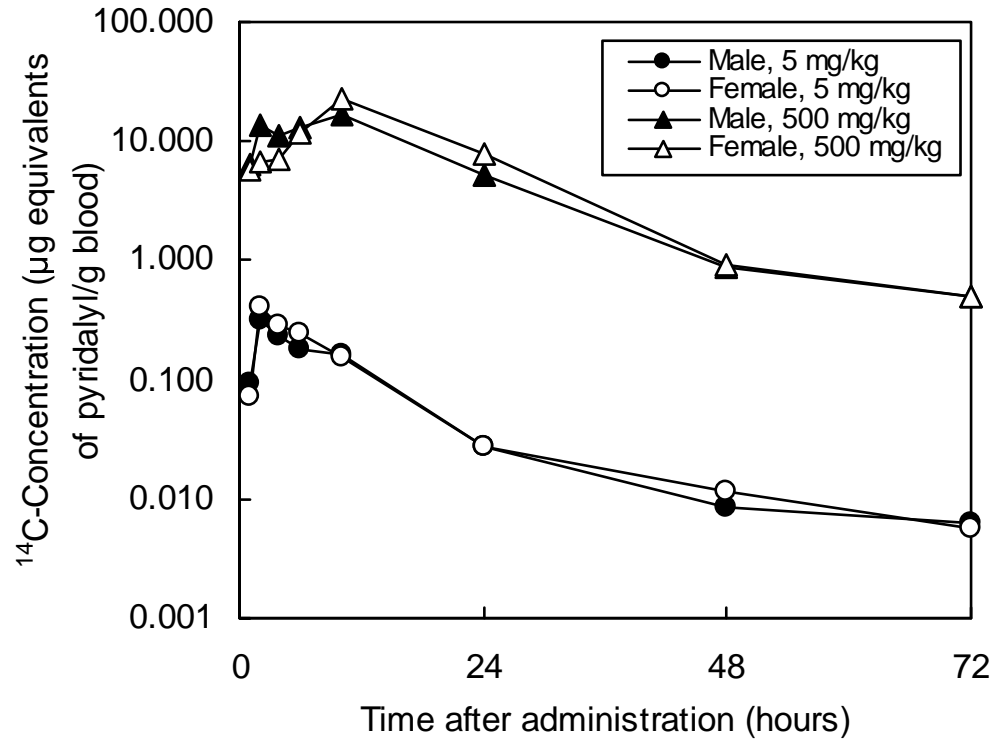


Figure 2

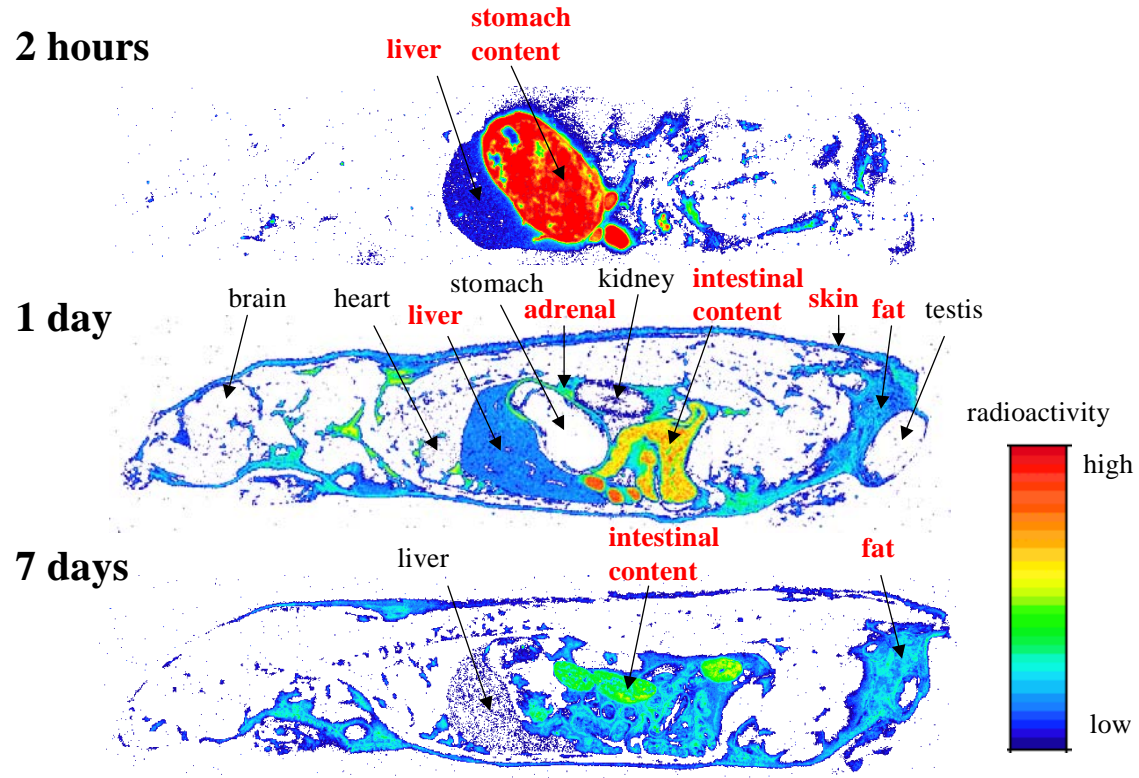


Figure 3

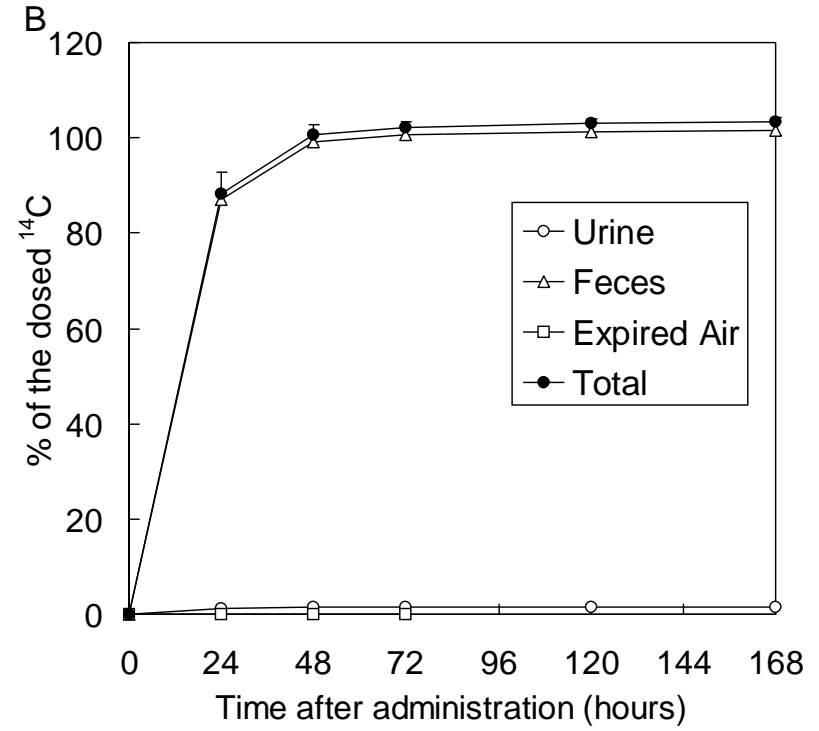
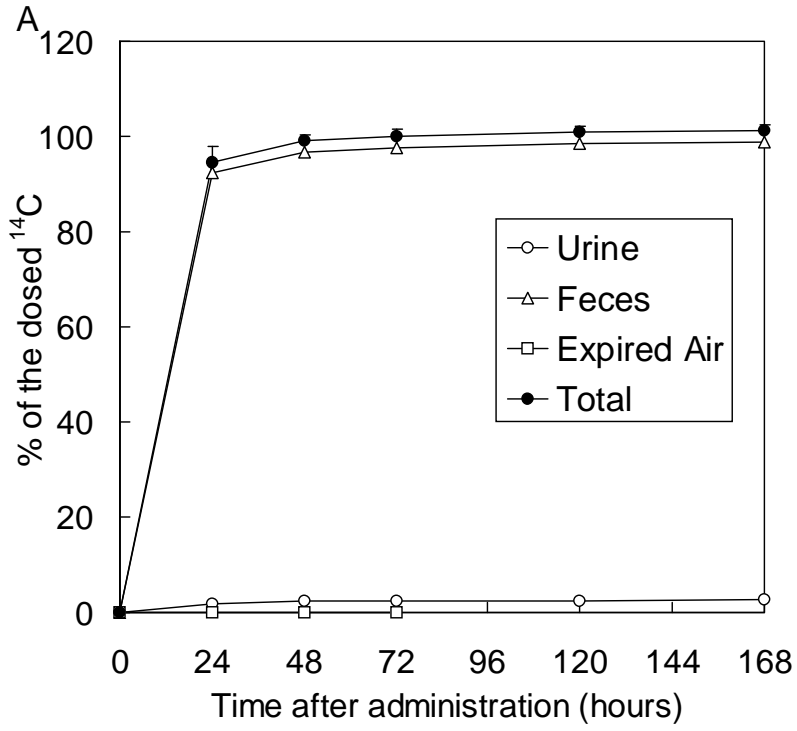


Figure 4

