Original Article

Species differences in the developmental toxicity of procymidone —Remarkable variation in pharmacokinetics, metabolism, and excretion—

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There are species differences regarding the developmental toxicity of procymidone (Sumilex®), a fungicide with a weak anti-androgenic activity. To clarify key factors of these species differences, pharmacokinetic and excretion studies in rats, rabbits, and monkeys were conducted using ¹⁴C-labeled procymidone. One hydroxylated metabolite of procymidone (Hydroxylated-PCM: very weak anti-androgen) was found to exist longer and at a much higher concentration in rat plasma than in rabbit and monkey plasma. In rabbits and monkeys, Hydroxylated-PCM was transformed into a glucuronic acid conjugate (Hydroxylated-PCM-glucuronide: non-anti-androgen) and rapidly excreted into urine as a major metabolite. On the other hand, it was a minor metabolite in rat urine. The results of biliary excretion studies indicated that these species differences were caused by the species differences in the biliary excretion of Hydroxylated-PCM-glucuronide; this variation in biliary excretion rate was concluded to be related to species differences in developmental toxicity. © Pesticide Science Society of Japan

Keywords: species differences, developmental toxicity, pharmacokinetics, metabolism, biliary excretion, enterohepatic circulation.

Electronic supplementary materials: The online version of this article contains supplementary materials (Supplemental Tables S1-S10), which are available at <http://www.jstage.jst.go.jp/browse/jpestics/>.

Introduction

Procymidone (Sumilex®) is a fungicide with both protective and curative activities used to control plant diseases, such as fruit rot; gray mold on fruits, vines, and vegetables; and Sclerotinia rot of kidney beans and vegetable crops.¹⁻³⁾ The metabolic pathways of procymidone in mammals are shown in Fig. 1.⁴⁻⁶⁾ Because the imide (cyclic) compounds (procymidone, PCM-CH₂OH, and PCM-COOH) and the corresponding imide linkage-cleaved metabolites (PCM-NH-COOH, PA-CH₂OH, and PA-COOH, respectively) are in equilibrium, also shown in Fig. 1,⁷⁾ the sum of both components is considered to be an appropriate index for comparing the amounts (concentrations) of metabolites. Therefore, in this report, the following abbreviations are used for procymidone metabolites: PCM (sum of

procymidone and PCM-NH-COOH), Hydroxylated-PCM (sum of PCM-CH₂OH and PA-CH₂OH), Carboxylated-PCM (sum of PCM-COOH and PA-COOH), and Hydroxylated-PCM-glucuronide (sum of PCM-CH₂OH-glucuronide and PA-CH₂OH-glucuronide). Procymidone is hydroxylated at the methyl group of the imide ring. Hydroxylated-PCM is then metabolized in one of two ways: it is either oxidized at the hydroxyl group to form Carboxylated-PCM or transformed into the glucuronic acid conjugate, Hydroxylated-PCM-glucuronide. Carboxylated-PCM and Hydroxylated-PCM-glucuronide are more hydrophilic and thus more readily excreted than Hydroxylated-PCM.^{4,5)}

Many toxicity studies have been conducted to evaluate the safety of procymidone. Procymidone is anti-androgenic *in vitro*, although its activity is much lower (20–70 times lower) than that of hydroxyflutamide, which is a representative anti-androgenic agent.^{8,9)} When rats were dosed with procymidone at 125 mg/kg/day in teratogenicity studies (repeated oral administration for 14 days), hypospadias was observed in the vast majority of male pups in all litters. The lowest teratogenic dose level was 37.5 mg/kg/day in rats. In contrast, no offspring with hypospa-

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Fig. 1. Proposed main metabolic pathways of procymidone in mammals.

Table 1. Summary of the results for teratogenicity in rats, rabbits and monkeys

Species	Dose (mg/kg/day)	Effects on external genitalia (e.g. hypospadias)	
		LOAELs (mg/kg/day)	NOAELs (mg/kg/day)
Rats	3.5–500	37.5	12.5
Rabbits	125	Negative	125
Monkeys	62.5, 125	Negative	125

dias were noted in rabbits or monkeys, even when treated at 125 mg/kg/day, the highest dose tested (Table 1). These results suggest that rats are more sensitive to procymidone exposure than rabbits and monkeys. Thus, a series of mechanistic studies was conducted to determine whether there are species differences related to developmental toxicity in metabolism, excretion, placental transfer, or protein binding among rats, rabbits, monkeys, and humans. The present report pertains to species differences in metabolism and excretion. To clarify key factors of these species differences, pharmacokinetic and excretion studies in rats, rabbits, and monkeys were conducted using ¹⁴C-labeled procymidone. The results of other studies, including those with human relevance, will be described elsewhere.

Materials and Methods

1. Compounds

Procymidone labeled uniformly in the phenyl group, [phenyl-¹⁴C]procymidone (specific radioactivity: 4.48 GBq/mmol, radiochemical purity: >99%, chemical purity: >99%, Fig. 1), and in the carbonyl group, [carbonyl-¹⁴C]procymidone, (specific radio-

activity: 2.28 GBq/mmol, radiochemical purity: >99%, chemical purity: >99%, Fig. 1), was synthesized by Sumitomo Chemical Co., Ltd., Japan. Unlabeled procymidone (chemical purity: 99.2%) was synthesized by Sumitomo Chemical Co., Ltd. Unlabeled authentic standards, PCM-NH-COOH (chemical purity: 98.6%), PCM-CH₂OH (chemical purity: 99.9%), PCM-COOH (chemical purity: 94.7%), PA-CH₂OH (chemical purity: 98.8%), and PA-COOH (chemical purity: 96.9%), were also synthesized by Sumitomo Chemical Co., Ltd. and were used for the identification of the metabolites. The ¹⁴C-labeled procymidone was diluted with unlabeled procymidone to achieve the appropriate specific radioactivity. The labeled and unlabeled test substances were dissolved in corn oil (rats) or suspended in a 0.5% methylcellulose solution (rabbits and monkeys) (5 mL/kg body weight). In an earlier study, it was demonstrated that these different vehicles caused no effects on pharmacokinetics or excretion.

2. Animal husbandry

Crj: CD (SD strain) IGS female rats aged 6 weeks were purchased from Charles River Japan Inc.; Kbs: NZW female rab-

bits aged 12 weeks from Kitayama Labes Co., Ltd., Japan; and female cynomolgus monkeys aged 4–6 years from the Guangxi Research Center of Primate Laboratory Animals, China. The animals were maintained in an air-conditioned room at 22–26°C (rats), 22–25°C (rabbits), and 22–28°C (monkeys) with an alternating 12-hr light and 12-hr dark cycle. For rats and rabbits, water and pelleted diet (CRF-1 (rats), RC4 (rabbits), Oriental Yeast Co., Ltd., Japan) were provided *ad libitum*. For monkeys, water was provided *ad libitum* and approximately 108 g of solid food (Teklad Global Certified 25% Protein Primate Diet, Harlan Sprague Dawley Inc., USA) was provided to the animals once a day. All studies were approved by the committee concerning animal protection at each laboratory and were performed in accordance with their established ethical standards.

3. Animal treatment

Because all metabolites could be detected and quantitated with either of the labeled preparations ([phenyl-¹⁴C]procymidone or [carbonyl-¹⁴C]procymidone), [phenyl-¹⁴C]procymidone was used in the pharmacokinetic and excretion studies (single dose and repeated dose), and [carbonyl-¹⁴C]procymidone was employed for the biliary excretion studies. Hereafter, [phenyl-¹⁴C]procymidone and [carbonyl-¹⁴C]procymidone are both designated as ¹⁴C-procymidone.

3.1. Single-administration pharmacokinetic and excretion studies

After quarantine (at least 1 week), ¹⁴C-procymidone was administered orally at dosages of 37.5 (rats only), 62.5, 125, 250, and 500 mg/kg to animals in two groups (one group for the pharmacokinetic study and the other for the excretion study: rats, 4 animals/group; rabbits and monkeys, 3 animals/group). The radiation dose was set at about 3.70 MBq/kg. For the pharmacokinetic studies, blood was collected from the caudal vein (rats), an auricular vein (rabbits), a femoral vein (monkeys), or the abdominal aorta (at 120 hr only for rats and rabbits) 1, 2, 4, 6 (rats only), 8, 10, 12, 24, 48, 72, and 120 hr after administration. For the excretion studies, after administration, animals were housed individually in metabolic cages to allow separate collection of urine and feces. Urine and feces from each animal were collected 6 (urine only), 24, 48, 72, and 120 hr after administration. After the final samples had been taken, the rats were sacrificed by exsanguination *via* the abdominal aorta, and the carcasses were obtained.

3.2. Repeated-administration pharmacokinetic and excretion studies

After quarantine (at least 1 week), ¹⁴C-procymidone was administered orally once a day at doses of 37.5 (rats only), 62.5, 125, 250, and 500 (monkeys only) mg/kg for 14 days to rats and monkeys in each group (rats, 4 animals/group; monkeys, 3 animals/group). The dose of radiation was set at about 1.2 MBq/kg. After the first administration, animals were housed individually in metabolic cages to allow the separate collection of urine and feces. Blood was collected from the caudal vein (rats), a femoral vein (monkeys), or the abdominal aorta (rats only, 120 hr after

the 14th dosing) 2, 4, 8, and 24 hr after the 1st, 3rd, 7th, 10th, and 14th oral dosing, and 48, 72, and 120 hr after the 14th dosing. Urine and feces from each animal were collected 1, 2 (monkeys only), 3, 4, 5, 7, 9, 11, 13, 14, 16, and 19 days after the first administration. After the final samples had been taken, the rats were sacrificed by exsanguination *via* the abdominal aorta, and the carcasses were obtained.

3.3. Biliary excretion studies

After quarantine (at least 1 week), animals were anesthetized and laparotomized for the insertion of a cannula into the common bile duct. After the animals had awoken from the anesthesia, ¹⁴C-procymidone was administered orally at 3.5 (rats) and 62.5 (rats) and 125 (rabbits and monkeys) mg/kg (rats, 4 animals/group; rabbits and monkeys, 1 animal each). The dose level for rabbits and monkeys was different from that for rats, since the latter showed some toxicological effects, such as decreased spontaneous activity, ataxic gait, and/or a prone position at lower dose levels. The dose of radiation set at about 3.70 MBq/kg. After administration, the animals were housed individually in cages to allow for the separate collection of bile, urine, and feces at 6 (bile only), 24, and 48 hr after administration. After the final samples had been taken, animals were sacrificed by exsanguination, and the intestinal tract contents and the carcasses (rats only) were obtained.

4. Sample processing and radioanalysis

Blood of each animal was centrifuged (3000 rpm, 10 min, 4°C, CR5B2, Hitachi Koki Co., Ltd., Japan) to separate the plasma. The radioactivity in the urine, bile, and plasma of each animal was measured by conventional liquid scintillation counting (LSC). To analyze the metabolites, equal amounts of plasma (rats, 50–100 µL; rabbits, 0.8–1.2-mL; monkeys, 0.3–0.8-mL) at each time point were combined (pooled plasma). The pooled plasma samples were extracted 3 times with 4 times the volume of acetonitrile to obtain extracts and residues. Feces and intestinal contents were extracted several times with 3–5 times the volume of acetonitrile and/or water. Carcasses (rats only) were dissolved in 2 mol/L potassium hydroxide. The radioactivity in pooled plasma extracts, fecal extracts, intestinal content extracts, and carcass solutions (rats only) was measured by LSC. The radioactivity in pooled plasma residue, fecal residue, and intestinal content residue was measured by LSC after treatment with tissue solubilizer (Soluene-350, PerkinElmer, Inc., USA) or after combustion with a sample oxidizer (System 307, PerkinElmer, Inc.).

5. Analysis of metabolites

In a manner described previously,^{4–7)} qualitative and quantitative analysis of metabolites was conducted by TLC analysis. Silica-gel 60F₂₅₄ (thickness: 0.25 mm, E. Merck, Germany) plates were used as TLC plates, and an imaging analyzer (BAS-1800 II, Fuji Photo Film Co., Ltd., Japan) was used as a radio-detector. The solvent systems were as follows: (1) toluene/ethyl formate/formic acid (5:7:1, by vol.) and (2) butanol/acetic acid/water (6:1:1, by vol.). Qualitative and quantitative analyses of

metabolites were conducted with an imaging analyzer using the TLC developed by the solvent system (1). For qualitative analysis, TLC co-chromatography with procymidone and authentic standards was conducted to identify metabolites.

Qualitative analysis of rat bile, urine, and fecal extracts, and rabbit urine and fecal extracts in the pharmacokinetic and excretion studies (20–50 μ L) was conducted to verify individual differences in the metabolic profile of each animal. As a result, it was confirmed that the profiles of metabolites in these samples were not different individually. Accordingly, for quantitative analysis, equal proportions of urine (rats, 1/20; rabbits, 1/100), equal proportions of bile (rats only, 1/50), and equal proportions of fecal extracts (rats, 1/20; rabbits, 1/100) at each time point were mixed (pooled urine, pooled bile and pooled fecal extracts, respectively), and pooled plasma extracts, pooled urine, pooled bile, and pooled fecal extracts (20–50 μ L) were subjected to TLC analysis. Individual samples of bile, urine, fecal extracts, and intestinal content extracts of monkeys and bile, urine, fecal extracts, and intestinal content extracts of rabbit in the biliary excretion study (20–50 μ L) were used for both qualitative and quantitative analysis.

For the Hydroxylated-PCM-glucuronide, enzymatic hydrolysis was carried out with a representative urine sample. Urine samples were added to 0.5 mL of 0.2 mol/L acetate buffer (pH 5.0) and incubated with β -glucuronidase (containing sulfatase, Type H-1, Sigma-Aldrich Corporation, USA) at 37°C for approximately 16 hr for hydrolysis. Following incubation, the mixture was applied to TLC co-chromatography (solvent systems (1) and (2)) to identify aglycones.

6. Calculations

6.1. Calculation of pharmacokinetic parameters

The maximum plasma concentration ($C_{\max}$) and time to reach $C_{\max}$ ($T_{\max}$) were determined from the values obtained. The elimination half-life ($t_{1/2}$) was calculated by linear regression analysis. Areas under the plasma concentration time curves until 120 hr (AUC_{0-120}) for the single-dose studies and until 24 hr (AUC_{0-24}) for the repeated-dose studies were calculated using the trapezoidal approximation method. For the single-dose studies, AUC from time 0 to infinity ($AUC_{0-\infty}$) after administration was the sum of AUC_{0-t} and $AUC_{t-\infty}$.

6.2. Calculation of the amounts absorbed from the gastrointestinal tract

The oral absorption rate was estimated based on the cumulative urinary excretion rate only. The amounts absorbed from the gastrointestinal tract were calculated as follows: amount absorbed (mg/kg) = dose level (mg/kg) $\times$ oral absorption rate (%) / 100 = dose level (mg/kg) $\times$ urinary excretion rate (%) / 100.

Results

1. Single-administration pharmacokinetic and excretion studies

The $C_{\max}$ values for 14 C in rabbits administered with 14 C-procymidone at each dose were similar to those in rats, while those in monkeys were much lower (Table 2). PCM concentrations in rats were higher than those in rabbits and monkeys following a single oral administration. The $C_{\max}$ of PCM in monkeys at a non-teratogenic dose level of 125 mg/kg/day was lower than that in rats at a teratogenic dose level. However, the AUC_{0-72} and $AUC_{0-\infty}$ of PCM in monkey plasma at 125 mg/kg were similar to those in rats at 62.5 mg/kg (Table 3). Therefore, it was demon-

Table 2. Pharmacokinetics parameters of 14 C in plasma after single oral administration of 14 C-procymidone to female rats, rabbits and monkeys

Rats	Dose (mg/kg)				
	37.5	62.5	125	250	500
$C_{\max}$ (μ g/mL)	11.5	16.4	30.1	35.7	50.3
$T_{\max}$ (hr)	8	12	24	24	24
AUC_{0-120} (μ g·hr/mL)	450	574	1108	1389	2317
$AUC_{0-\infty}$ (μ g·hr/mL)	454	578	1117	1401	2331
Rabbits	Dose (mg/kg)				
	62.5	125	250	500	
$C_{\max}$ (μ g/mL)	19.4	30.0	37.2	50.4	
$T_{\max}$ (hr)	1	1	1	4	
AUC_{0-120} (μ g·hr/mL)	180	251	457	1244	
$AUC_{0-\infty}$ (μ g·hr/mL)	189	258	474	1260	
Monkeys	Dose (mg/kg)				
	62.5	125	250	500	
$C_{\max}$ (μ g/mL)	5.9	4.4	8.8	8.7	
$T_{\max}$ (hr)	10	6	10	4	
AUC_{0-120} (μ g·hr/mL)	295	378	563	522	
$AUC_{0-\infty}$ (μ g·hr/mL)	427	600	754	836	

Data were calculated from mean value at each time point.

Table 3. Pharmacokinetics parameters of PCM in plasma after single oral administration of ^{14}C -procymidone to female rats, rabbits and monkeys

Rats	Dose (mg/kg)				
	37.5	62.5	125	250	500
$C_{\max}$ ($\mu\text{g/mL}$)	3.72	6.80	12.84	12.01	20.27
$T_{\max}$ (hr)	6	4	8	10	6
AUC_{0-72} ($\mu\text{g}\cdot\text{hr/mL}$)	40.9	89.9	227.4	236.5	575.1
$AUC_{0-\infty}$ ($\mu\text{g}\cdot\text{hr/mL}$)	40.9	89.9	227.4	236.5	575.1

Rabbits	Dose (mg/kg)			
	62.5	125	250	500
$C_{\max}$ ($\mu\text{g/mL}$)	0.00	0.17	0.70	1.01
$T_{\max}$ (hr)	—	10	6	1
AUC_{0-72} ($\mu\text{g}\cdot\text{hr/mL}$)	0.0	1.8	3.5	5.0
$AUC_{0-\infty}$ ($\mu\text{g}\cdot\text{hr/mL}$)	0.0	1.8	3.5	5.0

Monkeys	Dose (mg/kg)			
	62.5	125	250	500
$C_{\max}$ ($\mu\text{g/mL}$)	2.90	2.47	3.71	3.73
$T_{\max}$ (hr)	10	1	10	4
AUC_{0-72} ($\mu\text{g}\cdot\text{hr/mL}$)	73.8	76.9	123.8	85.1
$AUC_{0-\infty}$ ($\mu\text{g}\cdot\text{hr/mL}$)	73.8	76.9	123.8	85.1

Data were calculated from mean value at each time point.

—: Not determined.

Table 4. Cumulative ^{14}C -recoveries in urine and feces within 120hr after single oral administration of ^{14}C -procymidone to female rats, rabbits and monkeys, and the amounts absorbed from the gastrointestinal tract

Rats	Dose (mg/kg)				
	37.5	62.5	125	250	500
Urine (% of dose)	83.4 $\pm$ 1.1	83.6 $\pm$ 1.6	81.2 $\pm$ 1.7	66.7 $\pm$ 1.8	46.5 $\pm$ 4.1
Feces (% of dose)	11.5 $\pm$ 1.1	11.6 $\pm$ 1.8	14.7 $\pm$ 1.2	28.6 $\pm$ 2.2	48.7 $\pm$ 4.9
Carcass (% of dose)	0.3 $\pm$ 0.0	0.5 $\pm$ 0.1	0.5 $\pm$ 0.2	0.5 $\pm$ 0.1	0.3 $\pm$ 0.1
Total	95.2 $\pm$ 0.5	95.4 $\pm$ 0.1	96.4 $\pm$ 0.7	95.7 $\pm$ 0.8	95.4 $\pm$ 1.1
Amounts absorbed (mg/kg)	31.3	52.3	101.5	166.8	232.5

Rabbits	Dose (mg/kg)			
	62.5	125	250	500
Urine (% of dose)	92.8 $\pm$ 2.0	81.2 $\pm$ 3.1	75.0 $\pm$ 7.1	64.1 $\pm$ 2.8
Feces (% of dose)	3.7 $\pm$ 3.7	15.8 $\pm$ 2.1	22.1 $\pm$ 7.1	34.8 $\pm$ 4.0
Total	96.6 $\pm$ 1.8	97.0 $\pm$ 1.9	97.1 $\pm$ 2.4	98.9 $\pm$ 1.3
Amounts absorbed (mg/kg)	58.0	101.5	187.5	320.5

Monkeys	Dose (mg/kg)			
	62.5	125	250	500
Urine (% of dose)	57.1 $\pm$ 11.0	36.3 $\pm$ 1.0	28.5 $\pm$ 3.4	11.7 $\pm$ 1.2
Feces (% of dose)	42.3 $\pm$ 7.8	66.0 $\pm$ 1.8	72.4 $\pm$ 8.3	87.3 $\pm$ 5.5
Total	99.4 $\pm$ 3.4	102.3 $\pm$ 1.0	100.9 $\pm$ 5.7	99.0 $\pm$ 6.6
Amounts absorbed (mg/kg)	35.7	45.4	71.3	58.5

Data represent mean value $\pm$ S.D. (rats: $n=4$, rabbits and monkeys: $n=3$).

Carcasses were not analyzed in rabbits and monkeys.

Table 5. Amounts of metabolites in urine and feces after single oral administration of ^{14}C -procymidone to female rats, rabbits, and monkeys at 125 mg/kg

Metabolite	% of dose								
	Rat (0–48 hr)*			Rabbit (0–48 hr)*			Monkey (0–72 hr)**		
	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total
Procymidone	—	8.1	8.1	—	15.4	15.4	0.3	60.7	61.0
PCM-NH-COOH	—	—	—	—	—	—	0.1	0.2	0.4
PCM-COOH	5.3	0.1	5.4	—	—	—	3.3 ^{a)}	0.8 ^{a)}	4.0 ^{a)}
PCM-CH ₂ OH	—	3.6	3.6	—	0.1	0.1	—	—	—
PA-CH ₂ OH (1)	5.1	0.4	5.5	—	—	—	0.5 ^{b)}	0.2 ^{b)}	0.7 ^{b)}
PA-COOH (1)	49.4	—	49.4	8.5	—	8.5	—	—	—
PA-CH ₂ OH (2)	—	—	—	—	—	—	0.5	0.1	0.6
PA-COOH (2)	—	—	—	—	—	—	2.0	0.1	2.0
PCM-CH ₂ OH-glucuronide	5.5	—	5.5	71.7	—	71.7	25.6	—	25.6
Others	2.0	0.4	2.4	—	—	—	0.0	0.1	0.1
Unextractable	—	—	—	—	—	—	—	1.9	1.9
Total	67.2	12.6	79.8	80.2	15.5	95.7	32.2	64.1	96.3
PCM	0.0	8.1	8.1	0.0	15.4	15.4	0.4	60.9	61.3
Hydroxylated-PCM	5.1	4.0	9.1	0.0	0.1	0.1	4.3 ^{c)}	1.1 ^{c)}	5.4 ^{c)}
Carboxylated-PCM	54.7	0.1	54.8	8.5	0.0	8.5	5.8 ^{d)}	1.1 ^{d)}	6.8 ^{d)}
Hydroxylated-PCM-glucuronide	5.5	—	5.5	71.7	—	71.7	25.6	—	25.6

* Data were obtained from each pooled sample. ** Data represent mean value ($n=3$).

a) PCM-COOH and PCM-CH₂OH could not be separated. b) PA-CH₂OH (1) and PA-COOH (1) could not be separated. c) Data were produced by the following equation (as the worst scenario): $a+b+PA-CH_2OH(2)$. d) Data were produced by the following equation (as the worst scenario): $a+b+PA-COOH(2)$.

—: Not determined.

strated that the AUC of PCM in monkeys at a non-teratogenic dose level was larger than that in rats at the lowest teratogenic dose level (37.5 mg/kg).

The major components in the plasma of rats were PCM and Hydroxylated-PCM. Hydroxylated-PCM-glucuronide was a major component in rabbit plasma. In monkeys, the major component in plasma was PCM (Supplemental Tables S1–S3).

The amounts absorbed from the gastrointestinal tract in rabbits were similar to those in rats. In monkeys administered ^{14}C -procymidone at 125 mg/kg, the dose level used in a previous teratogenicity study, values were similar to those in rats administered ^{14}C -procymidone at 37.5 mg/kg (the lowest teratogenic dose) (Table 4).

In all species, the primary fecal component was PCM. In rats, Carboxylated-PCM was a major urinary component. In contrast, Hydroxylated-PCM-glucuronide was a major component in rabbit and monkey urine, but only a minor component in rat urine (Table 5, Supplemental Tables S4 and S5).

2. Repeated-administration pharmacokinetic and excretion studies

Hydroxylated-PCM was found to be the major component in the plasma of rats; however, the major component in the plasma of monkeys was PCM (Supplemental Tables S6–S8). In both species, the major fecal component was PCM. In rats, Carbox-

ylated-PCM was a major urinary metabolite. The metabolic profile in the rat repeated-administration study was similar to that in the single-administration study. In monkeys, Hydroxylated-PCM and Hydroxylated-PCM-glucuronide were major urinary metabolites (Table 6, Supplemental Tables S9 and S10). Although the metabolic profile in urine in the monkey repeated-administration study was partially different from that in the single-administration study, the difference observed in the repeated-administration study was related to Hydroxylated-PCM, which was produced *via* the deconjugation of Hydroxylated-PCM-glucuronide by contact with β -glucuronidase in intestinal bacteria due to insufficient washing of metabolic cages. Indeed, this tendency was observed remarkably in the lower-dose group. It was caused by the fact that absolute amounts of Hydroxylated-PCM-glucuronide excreted into urine in the lower-dose group were smaller than those in the higher-dose group, and, consequently, it seemed that glucuronides in the lower-dose group were easily deconjugated by β -glucuronidase in intestinal bacteria. Therefore, it was reasonable that the sum of the amounts of Hydroxylated-PCM and Hydroxylated-PCM-glucuronide should be used in comparison between repeated- and single-administration studies. Based on this comparison, no marked difference in metabolic profiles was observed between repeated- and single-administration studies in monkeys.

In rats, the ^{14}C levels in plasma reached the steady-state level

Table 6. Amounts of metabolites in urine and feces after repeated oral administration of ^{14}C -procymidone to female rats and monkeys at 125 mg/kg/day

Metabolite	% of dose*					
	Rats**			Monkeys***		
	Urine	Feces	Total	Urine	Feces	Total
Procymidone	—	5.3	5.3	0.3	65.2	65.5
PCM-NH-COOH	—	—	—	0.3	0.5	0.8
PCM-COOH	14.8	0.5	15.3	4.5 ^{a)}	1.5 ^{a)}	6.0 ^{a)}
PCM-CH ₂ OH	—	0.6	0.6	—	—	—
PA-CH ₂ OH (1)	2.7	0.2	2.9	2.3 ^{b)}	0.4 ^{b)}	2.7 ^{b)}
PA-COOH (1)	51.8	—	51.8	—	—	—
PA-CH ₂ OH (2)	—	—	—	8.6	0.4	9.0
PA-COOH (2)	—	—	—	1.6	0.1	1.8
PCM-CH ₂ OH-glucuronide	4.6	—	4.6	7.7	0.2	7.9
Others	0.9	0.6	1.5	0.1	0.1	0.2
Unextractable	—	8.3	8.3	—	1.3	1.3
Total	74.7	15.5	90.2	25.3	69.8	95.1
PCM	0.0	5.3	5.3	0.5	65.7	66.2
Hydroxylated-PCM	2.7	0.8	3.5	15.4 ^{c)}	2.3 ^{c)}	17.7 ^{c)}
Carboxylated-PCM	66.6	0.5	67.1	8.4 ^{d)}	2.0 ^{d)}	10.5 ^{d)}
Hydroxylated-PCM-glucuronide	4.6	0.0	4.6	7.7	0.2	7.9

* Data were obtained from excreta within 24hr after 14th administration. ** Data were obtained from each pooled sample. *** Data represent mean value ($n=3$).

a) PCM-COOH and PCM-CH₂OH could not be separated. b) PA-CH₂OH (1) and PA-COOH (1) could not be separated. c) Data were produced by the following equation (as the worst scenario): $a+b+\text{PA-CH}_2\text{OH (2)}$. d) Data were produced by the following equation (as the worst scenario): $a+b+\text{PA-COOH (2)}$. —: Not determined.

Fig. 2. Concentrations of ^{14}C in plasma during and after repeated oral administration of ^{14}C -procymidone for 14 days to female rats and monkeys. Points and bars are mean and S.D. (Rats: $n=4$, Monkeys: $n=3$).

approximately 3 days after the first administration. In monkeys, the ^{14}C levels in plasma increased in direct proportion to the administration frequency (Fig. 2). The PCM concentration in rat plasma showed a decreasing trend with the number of administration (Fig. 3). In contrast, the internal exposure levels of PCM in monkeys remained relatively stable (Fig. 3). Therefore, following repeated administrations, PCM concentrations in monkeys at a non-teratogenic dose level of 125 mg/kg/day were

much higher than those in rats at teratogenic dose levels. Based on plasma concentrations, the $C_{\max}$ and AUC , the internal concentration of PCM was similar between monkeys and rats orally dosed at 125 mg/kg/day (Fig. 3). The $C_{\max}$ and AUC_{2-24} of Hydroxylated-PCM in rat plasma were 4–15 times and 6–16 times larger, respectively, than those in monkey plasma (Fig. 4). Also, concentrations of Hydroxylated-PCM were several times higher than those of PCM in rat plasma.

Fig. 3. $C_{\max}$ and AUC_{2-24} values for PCM in plasma during and after repeated oral administration of ^{14}C -procymidone for 14 days to female rats and monkeys.

Fig. 4. $C_{\max}$ and AUC_{2-24} values for Hydroxylated-PCM in plasma during and after repeated oral administration of ^{14}C -procymidone for 14 days to female rats and monkeys.

Table 7. Cumulative ^{14}C -recoveries in urine and feces within 120 hr (rats) or 72 hr (monkeys) after repeated oral administration of ^{14}C -procymidone to female rats and monkeys and the amounts absorbed from the gastrointestinal tract

Rats	Dose (mg/kg/day)			
	37.5	62.5	125	250
Urine (% of dose)	80.5±2.8	76.9±1.7	79.1±2.3	60.9±2.3
Feces (% of dose)	14.6±2.5	18.4±2.4	16.2±2.5	34.7±1.4
Carcass (% of dose)	0.3±0.1	0.2±0.1	0.2±0.1	0.1±0.1
Total	95.4±0.3	95.4±0.9	95.4±1.1	95.7±1.4
Amounts absorbed (mg/kg)	30.2	48.1	98.9	152.3
Monkeys	Dose (mg/kg/day)			
	62.5	125	250	500
Urine (% of dose)	38.6±0.5	25.3±3.4	19.1±2.6	17.8±1.6
Feces (% of dose)	55.0±3.0	69.8±1.6	72.1±4.8	78.1±1.8
Total	93.6±3.5	95.1±1.8	91.2±2.6	96.0±2.9
Amounts absorbed (mg/kg)	24.1	31.7	47.8	89.2

Data represent mean value±S.D. (rats: $n=4$, monkeys: $n=3$).

Carcasses were not analyzed in monkeys.

Table 8. Cumulative ^{14}C -excretion in bile, urine and feces and ^{14}C in gastrointestinal contents and carcass after single oral administration of ^{14}C -procymidone to female bile-duct cannulated rats, rabbit and monkey (0–48 hr)

Matrix	% of dose			
	Rats*		Rabbit**	Monkey**
	3.5 mg/kg	62.5 mg/kg	125 mg/kg	125 mg/kg
Bile	12.0±5.9	19.3±7.9	1.2	6.0
Urine	70.6±7.7	58.6±5.8	24.2	15.5
Feces	10.1±2.8	6.7±4.3	3.5	0.1
Gastrointestinal contents	0.6±0.9	3.7±4.9	49.3	53.2
Carcass	2.6±1.4	7.1±1.8	—	—
Total	95.9±1.6	95.3±0.3	78.2	74.7

* Data represent mean value±S.D. ($n=4$). ** Data were obtained from one animal. —: Not analyzed.

Table 9. Amounts of metabolites in bile, urine and feces after single oral administration of ^{14}C -procymidone to female rats, rabbit, and monkey (0–48 hr)

Metabolite	% of dose															
	Rats, 3.5 mg/kg*				Rats, 62.5 mg/kg*				Rabbit, 125 mg/kg**				Monkey, 125 mg/kg**			
	Bile	Urine	Feces	Total	Bile	Urine	Feces	Total	Bile	Urine	Feces	Total	Bile	Urine	Feces	Total
Procymidone	—	—	4.4	4.4	—	—	3.8	3.8	0.0	0.0	0.1	0.1	0.0	0.0	0.0	0.0
PCM-NH-COOH	—	—	—	0.0	—	—	—	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PCM-COOH	—	36.9	0.2	37.1	—	28.4	0.1	28.5	0.0 ^{a)}	2.2 ^{a)}	1.3 ^{a)}	3.5 ^{a)}	0.1 ^{a)}	1.1 ^{a)}	0.0 ^{a)}	1.2 ^{a)}
PCM-CH ₂ OH	—	—	1.3	1.3	—	—	1.1	1.1	—	—	—	—	—	—	—	—
PA-CH ₂ OH (1)	—	2.4	—	2.4	—	3.0	—	3.0	0.0 ^{b)}	0.5 ^{b)}	0.0 ^{b)}	0.6 ^{b)}	0.0 ^{b)}	0.1 ^{b)}	0.0 ^{b)}	0.1 ^{b)}
PA-COOH (1)	0.4	25.0	—	25.4	0.5	20.9	—	21.4	—	—	—	—	—	—	—	—
PA-CH ₂ OH (2)	—	—	—	0.0	—	—	—	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.1
PA-COOH (2)	—	—	—	0.0	—	—	—	0.0	0.0	1.4	0.0	1.4	0.1	0.4	0.0	0.4
PCM-CH ₂ OH-glucuronide	11.6	3.7	—	15.3	18.8	4.0	—	22.8	1.1	20.0	0.3	21.4	5.7	13.8	0.0	19.5
Others	—	2.5	—	2.5	—	2.4	—	2.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Unextractable	—	—	4.2	4.2	—	—	1.6	1.6	—	—	1.7	1.7	—	—	0.1	0.1
Total	12.0	70.6	10.1	92.7	19.3	58.6	6.7	84.6	1.2	24.2	3.5	28.8	6.0	15.5	0.1	21.5
PCM	0.0	0.0	4.4	4.4	0.0	0.0	3.8	3.8	0.0	0.1	0.1	0.2	0.0	0.0	0.0	0.1
Hydroxylated-PCM	0.0	2.4	1.3	3.7	0.0	3.0	1.1	4.1	0.1 ^{c)}	2.7 ^{c)}	1.4 ^{c)}	4.2 ^{c)}	0.2 ^{c)}	1.2 ^{c)}	0.0 ^{c)}	1.4 ^{c)}
Carboxylated-PCM	0.4	61.9	0.2	62.5	0.5	49.3	0.1	49.9	0.1 ^{d)}	4.1 ^{d)}	1.4 ^{d)}	5.5 ^{d)}	0.2 ^{d)}	1.5 ^{d)}	0.0 ^{d)}	1.8 ^{d)}
Hydroxylated-PCM-glucuronide	11.6	3.7	—	15.3	18.8	4.0	—	22.8	1.1	20.0	0.3	21.4	5.7	13.8	0.0	19.5

* Data were obtained from each pooled sample. **: Data were obtained from one animal.

a) PCM-COOH and PCM-CH₂OH could not be separated. b) PA-CH₂OH (1) and PA-COOH (1) could not be separated. c) Data were produced by the following equation (as the worst scenario): a+b+PA-CH₂OH (2). d) Data were produced by the following equation (as the worst scenario): a+b + PA-COOH (2). —: Not determined.

As shown in Table 7, in monkeys administered with ^{14}C -procymidone at 125 mg/kg, the amounts absorbed (mg/kg) were similar to those in rats administered with ^{14}C -procymidone at 37.5 mg/kg.

3. Biliary excretion of procymidone in rats, rabbits, and monkeys

As shown in Table 8, in rats at doses of 62.5 mg/kg, the ^{14}C excretion in bile accounted for 19.3% of the dose within 48 hr after administration. Hydroxylated-PCM-glucuronide was mainly detected in the bile, accounting for 18.8% of the administered dose (more than 97% of the bile sample) at 62.5 mg/kg. Hydroxylat-

ed-PCM-glucuronide in urine was 4.0% of the dose at 62.5 mg/kg (Table 9). The excretion profiles at a dose of 3.5 mg/kg were similar to those at 62.5 mg/kg. The ^{14}C excretion in rabbit and monkey bile was 1.2% and 6.0% of the dose within 48 hr after administration, respectively (Table 8). Hydroxylated-PCM-glucuronide was detected in rabbit and monkey bile, accounting for 1.1% and 5.7% of the administered dose (more than 97% and 95% of the bile sample), respectively, at 125 mg/kg. The major metabolite in rabbit and monkey urine was Hydroxylated-PCM-glucuronide (20.0% and 13.8% of the dose, respectively) (Table 9). PCM was the major component of metabolites in feces and

Fig. 5. Metabolites in urine after a single oral administration of ^{14}C -procymidone to female rats, rabbits, and monkeys. Data were obtained from 125 mg/kg dose groups.

Fig. 6. Metabolites in urine and plasma after a single oral administration of ^{14}C -procymidone to female rats. Data were obtained from 125 mg/kg dose groups.

Fig. 7. Species differences in biliary excretion of the glucuronide of Hydroxylated-PCM.

Table 10. Excretion ratio of Hydroxylated-PCM-glucuronide to bile

	Rats		Rabbit	Monkey
	3.5 mg/kg	62.5 mg/kg	125 mg/kg	125 mg/kg
Biliary excretion ratio*	3.14	4.70	0.06	0.41

* Data were obtained from the following equation: Biliary excretion ratio = biliary excretion rate (% of dose)/urinary excretion rate (% of dose).

intestinal contents.

Discussion

In teratogenicity studies, developmental toxicity (abnormalities of external genitalia in male rats) was observed in rats but not in rabbits or monkeys. The lack of apparent toxic observations in rabbit and monkey studies was thought to be explained by their lower internal exposure to procymidone as compared to rats. If higher internal exposure could be achieved, developmental toxicity might possibly be observed even in rabbits and monkeys. In response to these concerns, we have carried out a series of studies to determine whether there are differences in internal doses among rats, rabbits, and monkeys. Furthermore, we have conducted a series of mechanistic studies to determine whether there are species differences in metabolism, excretion, placental transfer, and protein binding among rats, rabbits, monkeys, and humans.

In the present investigation, the pharmacokinetics and excretion of procymidone in female rats, rabbits, and monkeys were clarified. In the single-administration pharmacokinetic and excretion studies, the $C_{\max}$ of ^{14}C in rabbits administered with ^{14}C -procymidone and the amounts absorbed from the gastrointestinal tracts at each dose were similar to those in rats. In rabbits, however, the AUC_{0-120} and $AUC_{0-\infty}$ of ^{14}C in plasma were much lower than those in rats, indicating that the metabolism and excretion of ^{14}C -procymidone in rabbits are much faster than in rats. PCM concentrations in rats were higher than in rabbits and monkeys following a single oral administration. The $C_{\max}$ of PCM in monkeys at a non-teratogenic dose level of 125 mg/kg/day was lower than that in rats at teratogenic dose levels. However, the AUC_{0-120} and $AUC_{0-\infty}$ of PCM in monkey plasma at 125 mg/kg were similar to those in rats at 62.5 mg/kg. Therefore, it was demonstrated that the AUC of PCM in monkeys at a non-teratogenic dose level was larger than that in rats at the lowest teratogenic dose level (37.5 mg/kg).

In teratogenicity studies, maternal rats are generally dosed repeatedly. Treatment starts during early organogenesis and throughout the potential period of development of abnormal external genitalia. Therefore, repeated-administration pharmacokinetic studies are more relevant to procymidone teratology because the dosing regime is the same. In the repeated-administration studies, the differences between rats and monkeys were reduced because the internal exposure levels increased with the number of administrations in monkeys; thus, monkeys were comparable to rats in this regard. Also, it is possible that induction of metabolic enzymes might occur with repeated oral administration of procymidone in rats (hypermetabo-

lism). The AUC_{2-24} of ^{14}C and PCM in monkeys administered with ^{14}C -procymidone at 125 mg/kg were nearly equal to those in rats administered with ^{14}C -procymidone at 62.5 mg/kg, and the amounts absorbed from the gastrointestinal tracts in monkeys administered with ^{14}C -procymidone at 125 mg/kg were nearly equal to those in rats administered with ^{14}C -procymidone at 37.5 mg/kg. Therefore, PCM concentrations in monkeys at a non-teratogenic dose level of 125 mg/kg/day were much higher than those in rats at teratogenic dose levels, following repeated administration.

From these results, it was concluded that procymidone itself (PCM) and/or total (^{14}C) of procymidone and its metabolites were not direct causes of the developmental toxicity. Because it was thought that the cause of developmental toxicity is related to anti-androgenic action, we previously conducted a study of anti-androgen assay. In the previous study,⁹⁾ we demonstrated that there were no species differences for binding to androgen receptors, and only PCM and Hydroxylated-PCM, being approximately half of the PCM level, had anti-androgenic activities, and other metabolites (Carboxylated-PCM and Hydroxylated-PCM-glucuronide) had no anti-androgenic activity. Based on the above findings, it was speculated that the apparent toxic observations in rats were distinguished from the absence of toxicity in rabbits and monkeys by higher internal exposure of its metabolite with anti-androgenic characteristics, Hydroxylated-PCM, not procymidone (PCM). In the present studies, remarkable species differences in plasma concentrations of Hydroxylated-PCM were observed. All data showed that the concentration and the AUC in rat plasma were much higher than those in rabbit and monkey plasma. Furthermore, the Hydroxylated-PCM concentration was much higher than the PCM concentration in rat plasma. Therefore, it was considered that Hydroxylated-PCM could be a key factor in the observed species differences in developmental toxicity between rats and monkeys.

In an analysis of metabolites in urine obtained from single-administration pharmacokinetic and excretion studies, it was observed that Hydroxylated-PCM-glucuronide is a major metabolite in the urine of monkeys and rabbits but only a minor metabolite in rat urine (Fig. 5). The difference in excretion of Hydroxylated-PCM-glucuronide into urine was thought to be related to the higher Hydroxylated-PCM plasma concentration in rats. Furthermore, although the major metabolite in rat plasma was Hydroxylated-PCM, it was not significant in rat urine (Fig. 6). Therefore, Hydroxylated-PCM is considered to be reabsorbed in the kidney due to its lipophilicity, and biotransformation from Hydroxylated-PCM to Carboxylated-PCM is very slow. The difference in urinary excretion of Hydroxylated-PCM-

Fig. 8. Flow diagrams of procymidone and its metabolites in rabbits and monkeys. (Simple excretion: rapid urinary excretion.) Thick arrows show main pathways.

Fig. 9. Flow diagrams of procymidone and its metabolites in rats. (Complex excretion: biliary excretion and enterohepatic circulation.) Thick arrows show main pathways.

glucuronide appears to be due to a species difference in its biliary excretion, as reported extensively in the literature.^{10–12)} The difference might be derived from species differences in transporters in the liver.^{13–16)} The glucuronic acid conjugate, Hydroxylated-PCM-glucuronide, has a molecular weight (MW) of 476, which means that it should pass more readily into the bloodstream in rabbits, monkeys, and humans than in rats (Fig. 7).¹⁷⁾ It follows that the biliary excretion rate is higher in rats and that urinary excretion predominates in the other species. Based on

this hypothesis, biliary excretion experiments have been conducted in the present study. As described above, the biliary excretion rate of Hydroxylated-PCM-glucuronide was much higher in rats than in rabbits and monkeys. As shown in Table 10, the biliary excretion ratio [biliary excretion rate (% of the dose)/urinary excretion rate (% of the dose)] of Hydroxylated-PCM-glucuronide in rats was 78 times higher than that in rabbits and 12 times higher than that in monkeys.

In an extensive series of studies, the following conclusions

were obtained regarding species differences in the developmental toxicity of procymidone: in rabbits and monkeys, procymidone is absorbed from the gastrointestinal tract into the liver *via* the portal vein, where it is hydroxylated (Hydroxylated-PCM) and transformed into a glucuronic acid conjugate (Hydroxylated-PCM-glucuronide), which in turn is transferred to the blood circulation and rapidly excreted in urine (Fig. 8). On the other hand, in rats, after glucuronidation of Hydroxylated-PCM, the glucuronide (Hydroxylated-PCM-glucuronide) is excreted into the bile, deconjugated in the gastrointestinal tract, and reabsorbed, resulting in enterohepatic circulation (Fig. 9). This can be considered important because it results in sustained high plasma concentrations of Hydroxylated-PCM. Therefore, the species differences in the developmental toxicity of procymidone are mainly caused by species differences of Hydroxylated-PCM concentrations in plasma (retention of Hydroxylated-PCM in the animal body), and these differences can be attributed to variation in the biliary excretion rate of the glucuronic acid conjugate (Hydroxylated-PCM-glucuronide). As mentioned above, as the threshold of biliary excretion in humans is similar to that in rabbits and monkeys, the possibility of teratogenicity of procymidone in humans is considered to be very low. To confirm this hypothesis, further studies are needed.

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