PROCYMIDONE

First draft prepared by
I. Dewhurst[1] & A. Boobis[2]

[1]Pesticides Safety Directorate, Department for Environment, Food and Rural Affairs, Kings Pool, York, England
[2]Experimental Medicine and Toxicology, Division of Medicine, Faculty of Medicine, Imperial College London, London, England

Explanation 350
Evaluation for acceptable daily intake 350
1. Biochemical aspects 350
1.1 Absorption, distribution and excretion 350
(a) Oral route 350
(b) Dermal route 355
1.2 Biotransformation 356
2. Toxicological studies 360
2.1 Acute toxicity 360
(a) Lethal doses 360
(b) Dermal and ocular irritation and dermal sensitization 362
2.2 Short-term studies of toxicity 362
(a) Oral route 362
(b) Dermal route 367
2.3 Long-term studies of toxicity and carcinogenicity 369
2.4 Genotoxicity 371
2.5 Reproductive toxicity 371
(a) Multigeneration studies 371
(b) Developmental toxicity 376
2.6 Special studies 380
(a) Neurotoxicity 380
(b) Mechanism of action 380
3. Studies on metabolites 386
(a) 3,5-Dichloroaniline (DCA) 386
(b) Carboxyprocymidone (PCM-NH-COOH) 386
(c) 1,2-Dimethylcyclopropane-dicarboxylic acid (DMCPA) . 386
(d) Hydroxyprocymidone (PCM-CH_2OH) 386
(e) Interconversion of metabolites 387
4. Observations in humans 387
Comments 388
Toxicological evaluation 393
References 396

Explanation

Procymidone is the International Organization for Standardization (ISO) approved name for *N*-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide (International Union of Pure and Applied Chemistry, IUPAC), Chemical Abstracts Service, CAS No. 32809-16-8. It is a dicarboximide fungicide that is used on a range of vegetables, fruits, soya bean, sunflowers, tobacco and oil seed rape, as well as on ornamental plants and flower bulbs. The mechanism of pesticidal action involves the inhibition of triglyceride synthesis in fungi.

Procymidone was previously evaluated by JMPR in 1981, 1982 and 1989 (Annex 5, references *37*, *39*, *58*). No acceptable daily intakes (ADIs) were established when procymidone was evaluated by the JMPR in 1981 and 1982. In 1989, an ADI of 0–0.1 mg/kg bw was established based on the NOAEL of 12.5 mg/kg bw per day identified in studies of reproductive toxicity in rats. Procymidone was re-evaluated by the present Meeting as part of the periodic review programme of the Codex Committee on Pesticide Residues (CCPR). A range of new studies was submitted to the present Meeting; these studies addressed kinetics, developmental toxicity and hormonal effects in different species.

Many of the conventional studies of toxicity with procymidone were relatively old, were performed before the widespread use of good laboratory practice (GLP) and some contained relatively limited information. Overall, the Meeting considered that the database was adequate for the risk assessment.

Procymidone used in the main studies of toxicity was considered to be representative of current production material, which is typically of > 99% purity. The Food and Agriculture Organization (FAO) specification for procymidone specifies a minimum content of 98.5% w/w[1]. Procymidone has also been known under the development code S7131 or 'Sumilex'; 'Sumisclex' is a 50% formulation.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

(a) Oral route

Mice

Groups of five male ICR mice were given [phenyl-^{14}C]procymidone (radiochemical purity, > 99%; specific activity, 22.8 mCi/mmol (843.6 MBq/mmol) as a single oral dose at 100 mg/kg bw in corn oil. Urine and faeces were collected for 7 days from one group of mice. Other groups of mice were killed 2, 4, 6, 8, 12, 24 and 72 h after dosing and a range of tissues removed for radiochemical analysis. Rapid elimination was observed, with 92% of the administered dose excreted in the first 24 h, mainly in the urine (73.5%). Over a 7-day period, faeces and urine accounted for 22% and 82%, respectively, of the administered dose. There was no evidence of tissue accumulation in this study; the highest concentration of radioactivity (429 µg equivalent/g) was found in fat 2 h after dosing, but the concentration had declined to low concentrations (2 µg equivalent/g) by the end of the experimental period. Radioactivity in adrenals, blood, brain, kidneys, liver, prostate, epididymis and testes reached a maximum 2–8 h after dosing (133, 17, 27, 58, 67, 88, 48 and 17 µg equivalent/g respectively) and subsequently decreased with a half-life of 4–14 h. This study did not claim to be compliant with GLP (Kimura et al., 1988).

[1] http://www.fao.org/ag/AGP/AGPP/Pesticid/Specs/docs/Pdf/new/procymid.pdf

Rats

Groups of five male and female Wistar rats were given single oral doses of [phenyl-^{3}H]procymidone (radiochemical purity, > 99%; specific activity, 15.8 mCi/mmol (584.6 MBq/mmol) or [carbonyl-^{14}C]procymidone (radiochemical purity, > 99%; specific activity, 4.67 mCi/mmol (172.79 MBq/mmol) at 25 mg/kg bw, formulated in 10% Tween 80. A similar group received seven doses of [carbonyl-^{14}C]procymidone at 25 mg/kg bw per day. Samples of urine, faeces and carbon dioxide were taken for 7 days after the last dose. A limited number of tissue samples were taken from rats killed at 3, 6, 12, 24, 48 and 168 h after dosing and analysed for ^{14}C. Radioactivity was rapidly absorbed and eliminated. Almost identical patterns of excretion were found in both sexes and with both labelled forms, primarily in the urine (85–90%). Peak mean concentrations of radioactivity in tissues were reached at 6–12 h after dosing, the highest concentrations being in the kidneys (28 µg equivalent/g) and liver (19 µg equivalent/g) followed by muscle, stomach, lungs, and heart (15, 15, 12 and 12 µg equivalent/g respectively). Fat samples were not taken before 48 h. The tissue concentrations of radioactivity declined rapidly and except for fat (0.3 µg equivalent/g) were < 0.1 µg equivalent/g at 168 h after the last dose. Repeated dosing did not alter the pattern of excretion, but tissue residues were 3–10-fold those seen with a single dose at 168 h after the last dose. This study did not claim to be compliant with GLP (Mikami & Yamamoto, 1976).

Excretion and tissue distribution were investigated in groups of five male Sprague-Dawley rats given a single oral dose of [phenyl-^{14}C]procymidone (radiochemical purity, > 99%; specific activity, 22.8 mCi/mmol (843.6 MBq/mmol) at a dose of 100 mg/kg bw in corn oil. Urine and faeces were collected from five rats for 7 days after dosing. Tissues were removed from other groups at intervals up to 72 h after dosing. Radioactivity was rapidly and almost completely eliminated in the urine. Fifty-nine per cent of the dose was eliminated on the first day and 96% overall (urine, 84%; and faeces, 13%). The highest concentration of radioactivity was found in fat at 8 h (555 µg equivalent/g), declining to 5 µg equivalent/g at 72 h after dosing. Radioactivity in adrenals, blood, brain, kidneys, liver, prostate, epididymis and testes reached a maximum 8–12 h after dosing (77, 15, 26, 49, 67, 65, 28 and 11 µg equivalent/g, respectively) and subsequently decreased with an overall half-life of 7–12 h. This study did not claim to be compliant with GLP (Kimura et al., 1988).

Three groups of five male and five female Crl:CD®(SD)BR rats were given a single oral dose of [phenyl-^{14}C]procymidone (radiochemical purity, > 98%; specific activity, 242 µCi/mg (8.95 MBq/mg) at a dose of 1 or 250 mg/kg bw in corn oil. An additional group of rats received 14 consecutive daily doses of unlabelled procymidone at 1 mg/kg bw before being given a single dose of [phenyl-^{14}C]procymidone at 1 mg/kg bw. Urine and faeces were collected for 7 days after dosing; the rats were then killed and tissues removed for analysis. After oral administration of procymidone at a dose of 1 mg/kg bw, absorption was rapid and extensive, with approximately 80% of the administered dose being excreted in the urine in 24 h (Table 4). At the higher oral dose of 250 mg/kg bw, the proportion of radioactivity in the urine declined to 63–67% of the administered dose, with a concomitant increase in faecal radioactivity to 24–33% (Table 4). Radioactivity exhaled in the expired air accounted for < 0.03% of the administered dose. There was an indication of more rapid urinary excretion, but no substantial differences in the pattern of excretion for single or multiple lower doses. At either dose, radioactivity retained in the carcass and in the tissues 168 h after dosing accounted for < 0.3% of the dose and ≤ 0.01% of the administered dose respectively, demonstrating almost complete excretion. At 168 h, concentrations of radioactivity in all tissues of rats at 1 mg/kg bw were close to or less than 0.001 µg equivalent/g, except for fat (0.002–0.006 µg equivalent/g) and kidneys (0.001–0.002 µg equivalent/g). Fat also contained the highest concentration of radioactivity (5.0 µg equivalent/g) in rats at 250 mg/kg bw. This study claimed compliance with GLP and US EPA guidelines for studies of metabolism with pesticides (Struble, 1992a).

Comparative kinetics between species

To further investigate the species differences in findings in studies of developmental and reproductive toxicity, the kinetics and metabolism of procymidone were investigated in female rats, rabbits and monkeys, after single and repeated doses given to non-pregnant animals and to pregnant animals.

Single doses

Groups of four female Sprague-Dawley Crj:CD(SD)IGS rats were given a single oral dose of [phenyl-^{14}C]procymidone (specific activity, 13.7 MBq/mg; purity, > 97.8%) at 37.5, 62.5, 125, 250 or 500 mg/kg bw. Groups of three, female New Zealand White rabbits and female cynomolgus monkeys were given [phenyl-^{14}C]procymidone at a dose of 62.5, 125, 250 or 500 mg/kg bw. Corn oil was the vehicle in the studies in rats, while in studesi in rabbits and monkeys the vehicle was 0.5% methylcellulose. It is uncertain what effect the use of different vehicles would have had on the results. Each dose was given to two groups, blood was collected from one group at 1, 2, 4, 6, 8, 10, 12, 24, 48 and 72 h after dosing and excreta were collected from the other group at 6 (urine only), 24, 48, 72 and 120 h after dosing. The concentration of radioactivity was determined in excreta and plasma. Urine, faeces and plasma were analysed by thin-layer chromatography (TLC) either directly or after solvent extraction and metabolites were identified by co-chromatography with reference compounds.

The results (Table 1) showed marked species differences. Absorption in the rabbit was much more rapid than in rats and monkeys. Monkeys had much lower maximum concentration (C_{max}) values were much lower in monkeys than in rabbits and rats, but area under the curve of concentration–time (AUC) values were relatively low in rabbits given doses of up to 125 mg/kg bw owing to rapid elimination. In monkeys, the predominant compound in plasma was procymidone; in rabbits, acid metabolites and glucuronides of alcohol metabolites were the major components in plasma; in rats, free alcohol metabolites predominated. These results led to a hypothesis that the species differences in developmental effects observed were due to the high concentrations of free alcohol metabolites in rats, which lead to hypospadias (Sugimoto, 2005a, 2005b; Mogi, 2005a).

Repeated dosing

Groups of four female Sprague-Dawley Crj:CD(SD)IGS rats or groups of three female cynomolgus monkeys received 14 daily doses of [phenyl-^{14}C]procymidone (specific activity, 13.7 MBq/mg; purity, > 98.1%) at 37.5 (rats only), 62.5, 125, 250 or 500 (monkeys only) mg/kg bw per day. Corn oil was the vehicle in the studies in rats, for monkeys the vehicle was 0.5% methylcellulose. Blood was collected 2, 4, 8 and 24 h after the 1st, 3rd, 7th, 10th and 14th dose and additionally 48 and 72 h after the 14th dose. Urine and faeces were collected over a 24-h period after the 1st and 14th dose, at 2-day intervals at other times during the dosing period and at 24–72 h and 72–120 h after the final dose. Plasma, urine and faeces were analysed for radioactivity and for metabolites. The results are summarized in Table 2.

These results suggest that a steady state was reached after administration of repeated doses for approximately 3 days in rats, but only after 14 days or longer in monkeys. Although C_{max} and AUC were significantly higher in rats after a single dose, after 14 doses values were similar for rats and monkeys. These findings show that the doses used in the studies of developmental toxicity in cynomolgus monkeys would have produced plasma concentrations of total radioactivity that were similar to those produced in rats at doses resulting in hypospadias, but with different metabolite profiles. In monkeys, most of the radiolabel was unextractable and was not identified. On the data available, the metabolite patterns were similar to those seen with single doses, with unchanged procymidone beng the major component in plasma in monkeys, and alcohol metabolites predominating in rats (Sugimoto, 2005c; Mogi, 2005b).

Table 1. Pharmacokinetic parameters of radioactivity in plasma of female rats, rabbits and monkeys given a single oral dose of [phenyl-^{14}C]procymidone

Parameter	Dose (mg/kg bw per day)				
	37.5	62.5	125	250	500
Rats					
C_{max} (µg equivalent/ml)	11.48	16.42	30.06	35.67	50.33
T_{max} (h)	8	12	24	24	24
$T_{1/2}$: from C_{max} to 120 h (h)	18.3	15.6	16.3	15.9	14.6
$AUC_{0–120\ h}$ (µg equivalent/h per ml)	450	574	1108	1389	2317
$AUC_{0–\infty}$ (µg equivalent/h per ml)	454	578	1117	1401	2331
Radioactivity in urine (%)	83	84	81	67	47
Radioactivity in faeces (%)	12	11	15	29	49
Peak PCM (µg equivalent/ml)	3.3	6.1	11.4	10.6	19.4
Peak PCM-NH-COOH[a](µg equivalent/ml)	0.8	0.9	1.6	1.9	1.7
Peak PCM-CH_2OH (µg equivalent/ml)	5.4	7.1	14.4	8.2	19.4
Peak PA-CH_2OH (µg equivalent/ml)	2.9	7.3	8.3	16.7	8.4
Peak PA-COOH (µg equivalent/ml)	0.7	0.7	1.0	1.7	—
Peak glucuronides (µg equivalent/ml)[b]	0.5	0.3	0.4	0.7	—
Rabbits					
C_{max} (µg equivalent/ml)	—	19.36	29.96	37.22	50.38
T_{max} (h)	—	1	1	1	4
$T_{1/2}$: from C_{max} to 48 h (h)	—	7.0	6.9	7.6	17.6
$T_{1/2}$: from 48 to 120 h (h)	—	64.1	47.9	53.4	—
$AUC_{0–120\ h}$ (µg equivalent/h per ml)	—	180	251	457	1244
$AUC_{0–\infty}$ (µg equivalent/h per ml)	—	189	258	474	1260
Radioactivity in urine (%)	—	93	81	75	64
Radioactivity in faeces (%)	—	4	16	22	35
Peak PCM (µg equivalent/ml)	—	0	0.2	0.7	1.0
Peak PCM-COOH (µg equivalent/ml)	—	5.1	4.7	7.3	8.0
Peak PCM-CH_2OH (µg equivalent/ml)	—	0	0	0	0
Peak PA-CH_2OH (µg equivalent/ml)	—	0.3	0.8	1.8	2.5
Peak PA-COOH (µg equivalent/ml)	—	4.2	8.8	12.7	16.5
Peak glucuronides (µg equivalent/ml) [b]	-	7.0	10.7	11.9	17.8
Monkeys	—				
C_{max} (µg equivalent/ml)	—	5.92	4.43	8.76	8.69
T_{max} (h)	—	10	6	10	4
$T_{1/2}$: from C_{max} to 120 h (h)	—	81.7	78.9	58.5	84.5
$AUC_{0–120\ h}$ (µg equivalent/h per ml)	—	295	379	563	522
$AUC_{0–\infty}$ (µg equivalent/h per ml)	—	427	600	754	836

Radioactivity in urine (%)	—	57	36	29	12
Radioactivity in faeces (%)	—	42	66	72	87
Peak PCM (µg equivalent/ml)	—	2.8	2.3	3.4	3.6
Peak PCM-NH-COOH (µg equivalent/ml)	—	0.08	0.14	0.24	0.25
Peak PCM-CH_2OH [a] (µg equivalent/ml)	—	1.1	0.65	1.2	0.91
Peak PA-CH_2OH (µg equivalent/ml)	—	0.24	0.13	0.32	0.22
Peak PA-COOH (µg equivalent/ml)	—	0.26	0.16	0.40	0.71
Peak glucuronides (µg equivalent/ml) [b]	—	0.06	0.08	0.15	0.96

From Sugimoto (2005a, 2005b) and Mogi (2005a)
PA, procymidone acid; PA-CH_2OH, hydroxyprocymidone acid; PA-COOH, carboxyprocymidone acid; PCM, procymidone; PCM-CH_2OH, hydroxyprocymidone; PCM-NH-COOH, carboxyprocymidone.
[a] Included PCM-COOH.
[b] Glucuronides were of PA-CH_2OH and PCM-CH_2OH.

Table 2. Pharmacokinetic parameters of radioactivity in plasma of female rats and monkeys given 14 oral doses of [phenyl-^{14}C]procymidone

No. of doses	Parameter	Dose (mg/kg bw per day)				
		37.5	62.5	125	250	500
Rats						
Single dose	C_{max} (µg equivalent/ml)	11.7	15.7	29.6	42.1	—
	T_{max} (h)	8	8	24	24	—
	$AUC_{2-24\ h}$ (µg equivalent/h per ml)	189	325	520	599	—
Seven doses	C_{max} (µg equivalent/ml)	19.0	32.9	47.2	72.9	—
	T_{max} (h)	8	8	4	8	—
	$AUC_{2-24\ h}$ (µg equivalent/h per ml)	301	485	693	1187	—
Fourteen doses	C_{max} (µg equivalent/ml)	17.7	24.8	47.0	68.3	—
	T_{max} (h)	8	8	8	8	—
	$AUC_{2-24\ h}$ (µg equivalent/h per ml)	269	369	689	1038	—
	$AUC_{0-\infty}$ (µg equivalent/h per ml)	498	599	1111	1588	—
	Radioactivity in urine (%)	81	77	79	61	—
	Radioactivity in faeces (%)	15	18	16	35	—
	Peak PCM (µg equivalent/ml)	2.6	2.6	6.4	5.1	—
	Peak PCM-NH-COOH [a] (µg equivalent/ml)	—	—	—	—	—
	Peak PCM-CH_2OH (µg equivalent/ml)	9.9	9.8	32	51	—
	Peak PA-CH_2OH (µg equivalent/ml)	3.3	7.8	4.0	1.9	—
	Peak PA-COOH(µg equivalent/ml)	0.3	0.5	0.6	1.4	—
	Peak glucuronides (µg equivalent/ml)	0.6	0.9	0.7	2.9	—
Monkeys						
Single dose	C_{max} (µg equivalent/ml)	—	2.5	5.2	5.2	9.8
	T_{max} (h)	—	4	4	8	24
	$AUC_{2-24\ h}$ (µg equivalent/h per ml)	—	53.3	115	115	194

Seven doses	C_{max} (µg equivalent/ml)	—	10.1	17.3	23.8	31.6
	T_{max} (h)	—	2	8	4	4
	$AUC_{2-24\ h}$(µg equivalent/h per ml)	—	195	380	465	667
Fourteen doses	C_{max} (µg equivalent/ml)	—	15.1	26.9	30.0	46.9
	T_{max} (h)	—	2	2	2	2
	$AUC_{2-24\ h}$(µg equivalent/h per ml)	—	300	531	624	945
	$AUC_{0-\infty}$ (µg equivalent/h per ml)	—	1287	2195	2714	4167
	Radioactivity in urine (%)	—	39	25	19	18
	Radioactivity in faeces (%)	—	55	70	72	78
	Peak PCM (µg equivalent/ml)	—	3.6	6.6	6.8	11.5
	Peak PCM-NH-COOH (µg equivalent/ml)	—	0.1	0.2	0.1	0.2
	Peak PCM-CH_2OH [a] (µg equivalent/ml)	—	1.3	3.1	2.1	3.6
	Peak PA-CH_2OH (µg equivalent/ml)	—	0.3	0.8	0.5	0.5
	Peak PA-COOH(µg equivalent/ml)	—	0.1	0.3	0.1	1.1
	Peak glucuronides (µg equivalent/ml)	—	0.2	1.2	0.6	3.3

From Sugimoto (2005c) and Mogi (2005b)
AUC, area under the curve of concentration–time; PA, procymidone acid; PA-CH_2OH, hydroxyprocymidone acid; PA-COOH, carboxyprocymidone acid; PCM, procymidone; PCM-CH_2OH, hydroxyprocymidone; PCM-NH-COOH, carboxyprocymidone.
[a] Included PCM-COOH.

(b) Dermal route

The dermal absorption of procymidone from a formulated product has been measured in rats in vivo and in rat and human skin membranes in vitro.

Groups of four male Sprague-Dawley rats were given [phenyl-^{14}C]procymidone (radiochemical purity, 99.4%; specific activity, 449 µCi/mg (16.61 MBq/mg) formulated as Sumisclex SC at a dose of 0.002, 0.02 or 0.2 mg/cm^2 given as an application to the shaved back. Rats were exposed for 0.5, 1, 2, 4, 10 and 24 h, the application site was then washed and the rats were killed and radioactivity measured by liquid scintillation counting (LSC). An additional group of rats at each dose was washed 10 h after application and excreta were collected until 168 h after dosing. Recoveries of radioactivity were > 90%. Radioactivity was readily removed from the skin by washing and most of the excreted radioactivity was eliminated in the urine. In the groups exposed for 10 h and then killed 168 h after dosing, 1.2–2.5% of the administered dose remained in skin; this was considered to be bound radioactivity that was unavailable for absorption, as excretion was essentially complete at 120 h. Dermal absorption after a 10-h exposure was calculated to be 13%, 9% and 4% for dermal exposures of 0.002 mg/cm^2, 0.02 mg/cm^2 and 0.2 mg/cm^2 respectively. This study claimed compliance with GLP and complied with OECD test guideline 427 (Savides, 2002).

In a study of dermal penetration in vitro, [phenyl-^{14}C]procymidone (radiochemical purity, 99.4%; specific activity 449 µCi/mg), formulated as Sumisclex SC, was applied at a dose of 0.295, 1.48 or 500 g/l, corresponding to application concentrations of 0.003, 0.015 and 5 mg/cm^2, to rat and human epidermal membranes. Membranes were checked for integrity on the basis of electrical resistance. Samples of receptor fluid (ethanol/water; 1 : 1) were taken at intervals up to 24 h after application of the formulation and the membranes were then washed. Human, but not rat membranes were stripped with tape to remove the stratum corneum. Absorption was calculated as percentage of

the applied dose in receptor fluid plus unstripped epidermal membrane. Twenty-four hours after the application of procymidone at 0.295, 1.48 or 500 g/l, absorption through rat epidermis was 79%, 45% and 0.8% of the applied radioactivity respectively. Absorption through human membranes was lower than rat membranes: 5.3%, 4.7% and 0.022% of applied doses of 0.295, 1.48 and 500 g/l. The study claimed GLP compliance and complied with OECD test guideline 428 (Owen, 2002).

1.2 Biotransformation

See also section on special studies.

Mice

Groups of five male ICR mice were given a single oral dose of [phenyl-^{14}C]procymidone (radiochemical purity, > 99%; specific activity 22.8 mCi/mmol (843.6 MBq/mmol) at a dose of 100 mg/kg bw in corn oil. Urine and faeces were collected over 2 days and analysed for metabolites by TLC and comparison with standards. A range of tissues was extracted and analysed for metabolites by TLC. The predominant metabolites in the urine were the acid derivatives of procymidone (Table 3). Procymidone was the main component in faeces. In tissues and blood, the major component was procymidone, with alcohol derivatives being present at higher concentrations than the acids. (Kimura et al., 1988)

Rats

Male Sprague-Dawley rats received a single oral dose of [phenyl-^{14}C]procymidone (purity, > 99%; specific activity, 22.8 mCi/mmol (843.6 MBq/mmol) at 100 mg/kg bw in corn oil. Urine and faeces were collected for 2 days after dosing. Tissues were removed from other groups of rats at intervals up to 72 h after dosing. Samples of excreta, blood and tissues were prepared and analysed for metabolites by TLC and comparison with standards. The predominant metabolites in the urine were the acid derivatives of procymidone (Table 3). Procymidone was the main component in the faeces. In tissues and blood, the major component was procymidone, with alcohol derivatives being present at higher concentrations than the acids. The metabolic profile in rats was similar to that in mice (Table 3) (Kimura et al., 1988). A similar pattern of acid metabolites in excreta and alcohol derivatives in tissues was reported after the administration of procymidone at a dose of 25 mg/kg bw (Mikami & Yamamoto, 1976).

Table 3. Metabolites in excreta from rats and mice given [phenyl-^{14}C]procymidone a single dose at 100 mg/kg bw

Metabolite[a]	Recovery (% of administered dose)			
	Mice		Rats	
	Urine	Faeces	Urine	Faeces
PCM	2.6	7.0	0.2	5.2
PCM-CH_2OH	7.1	2.1	3.2	1.1
PA-CH_2OH	1.4	0.5	1.5	0.3
PCM-COOH	20	1.4	22	0.4
PA-COOH	39	3	47	0.6
DCA	< 1	< 1	< 1	< 1

From Kimura et al. (1988)
DCA, 3,5-dichloroaniline; PA, procymidone acid; PA-CH_2OH, hydroxyprocymidone acid; PA-COOH, carboxyprocymidone acid; PCM, procymidone; PCM-CH_2OH, hydroxyprocymidone; PCM-NH-COOH, carboxyprocymidone.
[a] See Figure 1 for key.

Three groups of five male and five female Crl:CD®(SD)BR rats were given a single oral dose of [phenyl-^{14}C]procymidone (radiochemical purity, > 98%; specific activity, 242 µCi/mg (8.95 MBq/mg) at 1 or 250 mg/kg bw in corn oil. An additional group of rats received 14 consecutive daily doses of unlabelled procymidone at 1 mg/kg bw before receiving [phenyl-^{14}C]procymidone at a dose of 1 mg/kg bw. Urine and faeces were collected for 7 days after dosing, after which the rats were killed and tissues removed for analysis. Samples were processed, including incubation with glucuronidase. Metabolites in excreta collected for up to 48 h (for rats at 1 mg/kg bw) and to 72 h (for rats at 250 mg/kg bw) after dosing were isolated by TLC and identified by co-chromatography with standards by mass spectral analysis. Overall, up to 42 urinary and nine faecal metabolites were found. Unchanged procymidone in the faeces accounted for < 5% of the administered dose in the group at 1 mg/kg bw, but was a major faecal component (18–27% of the administered dose) at 250 mg/kg bw. There were no significant differences in metabolism between the sexes, or after repeated dosing (Table 4). The findings were consistent with the results of earlier studies, with acid metabolites predominating in the urine (Struble, 1992b).

The proposed metabolic pathway is shown in Figure 1. The major metabolic reactions for procymidone were oxidation of the methyl groups to hydroxymethyl or carboxylic acid derivatives; cleavage of the imide; and glucuronide conjugation of the resultant metabolites.

Human-derived tissues

In an initial study, [phenyl-^{14}C]procymidone (purity, > 98%; specific activity, 15.6 MBq/mg) at a concentration of approximately 1 µmol/l was incubated with human hepatocytes at 37 °C for 46 h . The reaction was terminated by the addition of acetonitrile. Metabolites were identified by

Table 4. Metabolites in excreta from rats given [phenyl-^{14}C]procymidone at a dose at 1 or 250 mg/kg bw

Metabolite[a]	Recovery (% of administered dose)					
	Dose					
	1 mg/kg bw				250 mg/kg bw	
	Single dose		Fourteen doses			
	Male	Female	Male	Female	Male	Female
Urine	71	70	67	75	58	61
PCM	0	0	0	0	0	0
PCM-CH_2OH	< 1	< 1	< 1	< 1	< 1	1
PCM-CH_2OH-glucuronide	2	1	2	3	—	—
PA-CH_2OH	< 1	1.8	< 1	< 1	< 1	< 1
PCM-COOH	2	0	3	1	2	1
PA-COOH	50	48	45	56	44	44
DCA	0.3	0.5	0.1	1	0.8	0.5
Unknown/unextracted	10	11	11	19	11	11
Faeces	15	10	11	6	32	23
PCM	3	1	< 1	< 1	26	18
PCM-CH_2OH	2	1	1	< 1	2	1
Unknown/unextracted	10	7	8	6	7	6

From Struble (1992b)

DCA, 3,5-dichloroaniline; PA, procymidone acid; PA-CH_2OH, hydroxyprocymidone acid; PA-COOH, carboxyprocymidone acid; PCM, procymidone; PCM-CH_2OH, hydroxyprocymidone; PCM-NH-COOH, carboxyprocymidone.

qualitative autoradiography of TLC plates run with two solvent systems. The polar components were reported to be glucuronides of hydroxyprocymidone (PCM-CH_2OH) and hydroxyprocymidone acid (PA-CH_2OH). No radioactive compounds other than procymidone were detected in samples from the controls. The hepatocytes came from four different donors and some variations were evident in the autoradiograph patterns from different preparations (Tarui, 2005b).

Groups of four male, chimeric mice with humanized livers (i.e. liver repopulated with human hepatocytes) (from PhoenixBio, Hiroshima, Japan) and four male mice (uPA$^{-/-}$SCID) serving as controls were given a single oral dose of [phenyl-^{14}C]procymidone (purity, > 95%; specific activity, 15.8 MBq/mg) at 37.5 mg/kg bw in corn oil. Urine and faeces were collected at 24-h intervals for 72 h after dosing and analysed for radioactivity and for metabolites using high-performance liquid chromatography (HPLC)/LSC and TLC/autoradiography. A glucuronidase/sulfatase preparation (± glucuronidase inhibitor) was used to release conjugates, but only qualitative data were presented. Radioactivity was excreted at similar rates in the urine and faeces of control and chimeric mice (Table 5). The main difference in metabolism was the higher concentration of glucuronides (of PCM-CH_2OH and PA-CH_2OH) and lower concentration of acid metabolites excreted in the chimeric mice (Table 5) (Ohzone, 2005a).

Comparison of metabolism between species

Procymidone [phenyl-^{14}C]procymidone (specific activity, 15.8 MBq/mg) and PCM-CH_2OH (specific activity, 3.3 MBq/mg) were incubated for 60 min at 37 ^{0}C with S9 fractions prepared from pooled livers of women (two Caucasians and one African-American), rats (Sprague-Dawley), rabbits (New Zealand White) and monkeys (cynomolgus). A single concentration of 50µmol/l was used. Metabolites were identified by co-chromatography with reference standards. Only limited information was given in the study report. The rates of hydroxylation of procymidone (to alcohol or acid derivatives) or oxidation of PCM-CH_2OH (to acid derivatives) were relatively constant over the assay period. S9 from rabbit liver had the highest activity for hydroxylation of procymidone, with activity in rat liver being lower than that in other species (Table 6). S9 from monkey liver had the highest activity for the oxidation of PCM-CH_2OH, with the activity of S9 from rats being much lower than that from other species tested (Table 6) (Matsui, 2005b).

Table 5. Metabolite excretion profiles in chimeric (humanized) mice (n = 4) and control mice (n = 4) given [phenyl-^{14}C]procymidone as a single dose at 37.5 mg/kg bw

Metabolite	Recovery (% of administered dose)			
	Chimeric mice[a]		Control mice	
	Urine	Faeces	Urine	Faeces
Excretion in 24 h	59	22	65	17
Excretion in 72 h	73	24	76	20
PCM (72 h)	< 1	1.8	1.4	< 1
PCM-COOH (72 h)	21	4.6	34	6
PA-CH_2OH (72 h)	2	< 1	2.5	1
PA-COOH (72 h)	12	2.6	26	3
Glucuronides (72 h)	35	7.5	7	2
Others/unextracted (72 h)	3	7	5	6

From Ohzone (2005a)

[a] Chimeric mice possessed humanized livers (i.e. liver repopulated with human hepatocytes).

PA-CH_2OH, hydroxyprocymidone acid; PA-COOH, carboxyprocymidone acid; PCM, procymidone; PCM-CH_2OH, hydroxyprocymidone; PCM-NH-COOH, carboxyprocymidone.

The excretion of radioactivity in bile, urine and faeces, and the metabolites present, were investigated in groups of bile-duct cannulated females given a single oral dose of [carbonyl-^{14}C]procymidone (purity, > 98.5%; specific activity, 8 MBq/mg). Groups of four Sprague-Dawley rats received [carbonyl-^{14}C]procymidone at a dose of 3.5 or 62.5 mg/kg bw in corn oil; a rabbit (New Zealand White) and a cynomolgus monkey received [carbonyl-^{14}C]procymidone at a dose of 125 mg/kg bw in 0.5% methylcellulose. Bile, urine and faeces were collected for 48 h after dosing; samples were analysed for radioactivity and for metabolites. At termination, the gastrointestinal tract and contents were removed and analysed for radioactivity. Metabolites were separated by TLC and identified by co-chromatography with reference standards either before or after enzyme hydrolysis. The results are summarized in Table 7.

Interpretation of the data from the rabbit and monkey were complicated by the high residues in the gastrointestinal contents and carcass. Excretion of glucuronides in the urine was lower in rats than the other two species, but with higher concentrations of acid derivatives. The indications were that the glucuronides in rat bile are subsequently deconjugated and reabsorbed (Sugimoto, 2005d; Mogi, 2005c, 2005d).

Table 6. Specific enzyme activities related to the metabolism of procymidone in S9 from liver of four species

Metabolic conversion	Enzyme activity (pmol/min per mg protein)			
	Human	Rat	Monkey	Rabbit
Procymidone hydroxylation	36	10	30	61
PCM-CH_2OH oxidation	13	1	33	26

From Matsui (2005b)
PCM-CH_2OH, hydroxyprocymidone.
S9, 9000 × *g* supernatant.

Table 7. Metabolite excretion profiles in bile-duct canulated females given a single dose of [carbonyl-^{14}C]procymidone

Excretion parameter	Recovery (% of administered dose)					
	Rat (62.5 mg/kg bw)		Rabbit (125 mg/kg bw)		Monkey (125 mg/kg bw)	
Faeces 0–48 h	7		3.5		<1	
Carcass	7		22		25	
Gastrointestinal tract/contents	4		50		53	
	Urine	Bile	Urine	Bile	Urine	Bile
Excretion in 48 h	59	19	24	1	15	6
PCM (48 h)	0	0	< 0.1	< 0.1	< 0.1	< 0.1
PCM-COOH/CH_2OH (72 h)	28	0	2	< 0.1	1	< 0.1
PA-CH_2OH (72 h)	2	0	< 0.1	< 0.1	< 0.1	< 0.1
PA-COOH (72 h)	21	0.5	1.4	< 0.1	0.4	< 0.1
Glucuronides (72 h)	4	19	20	1	14	6
Others/unextracted (72 h)	2	0	< 0.1	< 0.1	0	< 0.1

From Sugimoto (2005d) and Mogi (2005c, 2005d)
PA, procymidone acid; PA-CH_2OH, hydroxyprocymidone acid; PA-COOH, carboxyprocymidone acid; PCM, procymidone; PCM-CH_2OH, hydroxyprocymidone; PCM-NH-COOH, carboxyprocymidone.

Binding to plasma proteins

The plasma-protein binding of [phenyl-^{14}C]procymidone (specific activity, 15.8 MBq/mg) and its metabolite [phenyl-^{14}C]PCM-CH_2OH (specific activity, 3.3 MBq/mg) was determined in humans, Sprague-Dawley rats, cynomolgus monkeys and New Zealand White rabbits; plasma from females was used fo each species. Plasma-protein binding at pH 7.0 was investigated in vitro at concentrations of 1, 3, 10 and 30 µg/ml using an ultrafiltration method. Both compounds exhibited a high level of protein binding in plasma from all species (Table 8). Plasma-protein binding of procymidone was similar in all species tested and ranged from 92% to 98% over the range of concentrations tested. The plasma-protein binding of PCM-CH_2OH was slightly lower than that of procymidone; the value for human plasma (90–91%) was slightly greater than that for rats (82–90%), monkeys (77–85%) and rabbits (83–86%). Since rats make much more PCM-CH_2OH (tenfold), these results indicated that there are likely to be much higher levels of free PCM-CH_2OH in rats than in other species (Matsui, 2005a).

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of studies of acute toxicity with procymidone administered by the oral, dermal, subcutaneous, intraperitoneal (i.p) and inhalation routes, are presented in Table 9. Procymidone was of low acute toxicity by all routes. All these studies were performed before GLP was instituted and are limited in detail; however, there are no reasons to doubt the findings.

Clinical signs of toxicity were typically increased respiration and reduced motor activity. These were evident at doses of ≥ 250 mg/kg bw orally and subcutaneously and ≥ 100 mg/kg bw i.p., but showed no clear trend with dose administered. There were no adverse findings at postmortem examination.

Table 8. Plasma-protein binding in vitro and total and calculated plasma concentrations of free procymidone and PCM-CH_2OH (µg equivalent/ml) in female mice, rats, monkeys and humans

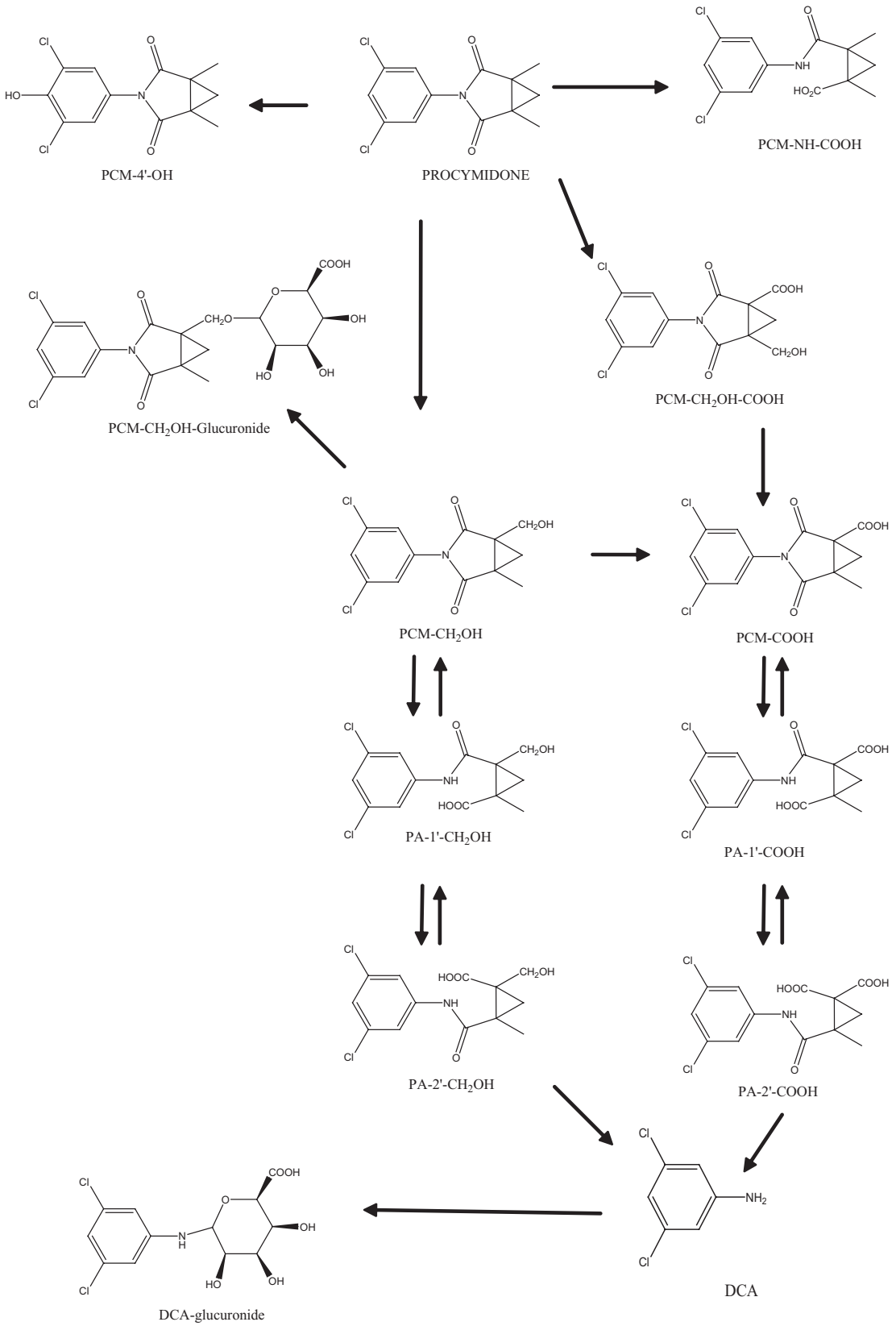
Concentration (µg/ml) / dose (mg/kg bw per day)	Human		Rat		Monkey		Rabbit	
	PPB (%)	PPB (%)	Total (µg equivalent/ml)	Free[a] (µg equivalent/ml)	PPB (%)	Total (µg equivalent/ml)	Free[a] (µg equivalent/ml)	PPB (%)
Procymidone								
1 / 37	95.5	97.9	2.6	0.06	95.4	ND	ND	97.6
3 / 67	95.5	97.5	2.6	0.06	94.6	3.6	0.19	97.3
10 / 125	95.5	96.9	6.4	0.19	93.9	6.6	0.4	97.4
30 / 250	95.4	96.3	5.1	0.15	92.4	6.8	0.4	97.1
PCM-CH_2OH								
1 / 37	90.9	90.4	9.9	1.3	85.3	ND	ND	85.6
3 / 67	90.9	98.9	9.8	1.3	83.9	1.3	0.2	85.6
10 / 125	90.5	87.1	32	5.7	80.2	3.1	0.5	84.3
30 / 250	90.4	82.1	51	9.2	76.9	2.1	0.3	82.7

From Matsui (2005a)

ND, no data; PCM-CH_2OH, hydroxyprocymidone; PPB, plasma-protein binding.

[a] Concentration of free substance was calculated from binding percentage and total concentration.

Figure 1. Proposed metabolic pathways of procymidone



DCA, 3,5-dichloroaniline; PA, procymidone acid; PA-CH_2OH, hydroxyprocymidone acid; PA-COOH, carboxyprocymidone acid; PCM, procymidone; PCM-CH_2OH, hydroxyprocymidone; PCM-NH-COOH, carboxyprocymidone.

Table 9. Results of studies of acute toxicity with procymidone

Species	Strain	Sex	Route	Vehicle	LD_{50} (mg/kg bw)	LC_{50} (mg/l air)	Purity (%)	Reference
Rat	SD	M & F	Oral	Corn oil	> 5 000	—	92.3	Kadota et al. (1976a)
							96.5	Segawa (1977)
Mouse	dd	M & F	Oral	Corn oil	> 5 000	—	92.3	Kadota et al. (1976a)
							96.5	Segawa (1977)
Rat	SD	M & F	Dermal	Corn oil	> 2 500	—	92.3	Kadota et al. (1976a)
							96.5	Segawa (1977)
Mouse	dd	M & F	Dermal	Corn oil	> 2 500	—	92.3	Kadota et al. (1976a)
							96.5	Segawa (1977)
Rat	SD	M & F	SC	Corn oil	> 10 000	—	92.3	Kadota et al. (1976a)
							96.5	Segawa (1977)
Mouse	dd	M & F	SC	Corn oil	> 10 000	—	92.3	Kadota et al. (1976a)
							96.5	Segawa (1977)
Rat	SD	M & F	IP	Corn oil	850 (M) 730 (F)	—	92.3	Kadota et al. (1976a)
Rat	SD	M & F	IP	Corn oil	1 440 (M) 1 450 (F)	—	96.5	Segawa (1977)
Mouse	dd	M & F	IP	Corn oil	1 560 (M) 1 900 (F)	—	92.3	Kadota et al. (1976a)
Mouse	dd	M & F	IP.	Corn oil	2 030 (M) 2 050 (F)	—	96.5	Kadota et al. (1976a); Segawa (1977)
Rat	SD	M & F	Inhalation	Fine dust	—	> 1.5 mg/l MMAD < 2.4 µm	99.5	Suzuki & Kato (1988); Tsuji (1994)

IP, intraperitoneal; F, female; M, male; MMAD, mass median aerodynamic diameter; SC; subcutaneous; SD, Sprague-Dawley.

(b) Dermal and ocular irritation and dermal sensitization

Procymidone was a slight transient irritant to rabbit skin (all signs had disappeared by 24 h; Kadota & Miyamoto, 1976), but produced no irritation to the rabbit eye (Kadota & Miyamoto, 1976). Procymidone was not a skin sensitizer in guinea-pigs in a repeated injection (10 + 1 doses) protocol using 1% or 5% w/v preparations in corn oil (Okuno, 1975), nor in a GLP-compliant Magnusson & Kligman maximization study using concentrations of 1% w/v for intradermal induction, 25% w/v for topical induction and 25% w/v for challenge application (Nakanishi et al., 1991).

2.2 Short-term studies of toxicity

(a) Oral route

Mice

Groups of 15 male and 15 female ICR mice received diets containing procymidone (purity, 96.9%) at a concentration of 0, 50, 150 or 500 ppm for 13 weeks. The mice were observed twice per day for clinical signs of toxicity and mortality and body weights were determined weekly. Food and

water consumption were determined on three consecutive days each week. At the end of the treatment period, an ophthalmological examination was conducted and blood was taken for measurement of biochemical and haematological parameters. At necropsy, organs were weighed and tissues were examined microscopically.

Achieved intakes are given in Table 10. Behaviour, appearance and survival were not affected and no treatment-related effects were apparent in body weight, food and water intake, clinical pathology or macroscopic findings. An increase in serum glucose concentrations in females at the highest dose was not considered adverse as the absolute value was similar to typical mean values for the controls (114 vs 113 mg/dl) Absolute liver weights were increased for all males receiving procymidone and for females at 500 ppm, but relative liver weights were unaffected at ≤ 150 ppm (Table 10). Histopathology results showed a treatment-related centrilobular hypertrophy of hepatocytes in males at 500 ppm and hepatocyte granuloma in females (Table 10). Relative kidney weights were decreased in females at 150 and 500 ppm, but this was not considered to be treatment-related because there was no effect on absolute kidney weights or on histopathology.

The no-observed-adverse-effect level (NOAEL) was 150 ppm, equal to 22 mg/kg bw per day, on the basis of histopathological findings in the liver of males at 500 ppm. This study did not claim GLP compliance (Arai et al., 1980a).

Groups of 12 male and 12 female B6C3F$_1$ mice received diets containing procymidone (purity, 99.8%) at a concentration of 0, 100, 500, 2500 or 10 000 ppm for 13 weeks. All mice were observed daily for mortality and overt signs of toxicity, detailed clinical examinations were conducted weekly and body weight and food consumption were recorded at the same intervals. Blood and urine were collected for haematology, clinical chemistry and urine analysis at the start and end of the treatment period, respectively. Necropsies were performed at the end of the treatment period and organ weights were recorded. Tissues from the control group and from the group at the highest dose, and livers from the other groups, were processed for histopathology.

Actual intakes of procymidone were not calculated. A slightly depressed overall body weight (< 10%) was recorded for males at 10 000 ppm, although food intakes were not influenced by treatment. Behaviour, appearance and survival of the treated mice were not affected. Clinical pathology revealed higher activities of serum alanine aminotransferase (ALT) in males at 10 000 ppm and higher cholesterol concentrations in females at the highest dose. Blood urea nitrogen (BUN) was reduced in all groups of males, but the values were within the range for historical controls. Creatinine concentrations were also statistically lower than those of the controls in all groups (Table 11). At termination, organ-weight analysis revealed significantly higher liver-to-body-weight ratios and/or liver weights

Table 10. Liver weights and pathology findings in mice (n = 15) fed diets containing procymidone for 13 weeks

Finding	Dietary concentration (ppm)							
	0		50		150		500	
	Male	Female	Male	Female	Male	Female	Male	Female
Intake (mg/kg bw per day)	0	0	7	11	22	26	71	84
Liver weight (g)	1.4	1.3	1.5*	1.2	1.6*	1.2	1.6*	1.4*
Relative liver weight (%)	3.6	4.1	3.8	3.9	3.8	3.8	4.0	4.4
Hepatocyte hypertrophy	0	0	0	0	1	0	3	1
Hepatocytic granuloma	0	2	0	2	1	2	0	4

From Arai et al. (1980a)
* $p < 0.05$.

for males and females at 2500 or 10 000 ppm. Histopathology revealed changes in the liver of mice at 10 000 ppm and 2500 ppm. The changes consisted of multifocal coagulative necrosis of hepatic parenchyma, together with centrilobular cytoplasmic swelling, nuclear enlargement, coarsely dispersed chromatin and multinucleate hepatocytes (Table 11). Decreases in kidney and spleen weights were also noted at dietary concentrations of 2500 ppm and greater (Table 11).

The NOAEL was 100 ppm (reportedly equal to 19.6 mg/kg bw per day), on the basis of liver pathology (coagulative necrosis) in males at dietary concentrations of 500 ppm and greater. This study did not claim GLP compliance (Weir et al., 1984).

Groups of 20 male and 20 female ICR mice weer given diets containing procymidone (purity, 96.9%) at a concentration of 0, 50, 150 or 500 ppm for 26 weeks. The mice were observed twice per day for clinical signs of toxicity and mortality and body weights were determined weekly. Food and water consumption were determined on three consecutive days each week. At the end of the treatment period, an ophthalmological examination was conducted and blood was taken for measurement of biochemical and haematological parameters. All surviving mice were necropsied immediately after blood was taken, organs were weighed and tissues were examined microscopically.

Achieved intakes are presented in Table 12. There were 11 deaths during the study; these occurred in all groups and there was no indication of a relationship to treatment. No treatment-related effects were detected on behaviour, appearance, survival, body weights, food and water intakes, ophthalmological examination, or macroscopic pathology. There was a statistically significant decrease in the leukocyte count in males at 150 and 500 ppm (Table 12), the deficit being primarily in lymphocytes. Since no comparable change was found in other studies in mice, these effects were considered to be unrelated to treatment. There was also a statistically significant increase in creatinine concentration and cholinesterase activity in male mice at 500 ppm and a reduction in cholinesterase activity in female mice of that group; these are considered to be sporadic findings unrelated to treatment. Organ weights were similar across all groups; an increase in pituitary weight in all groups of males was without a dose–response relationship, not reproduced in females and was considered to be a chance

Table 11. Findings in mice (n = 12) fed diets containing procymidone for 13 weeks

Finding[a]	Dietary concentration (ppm)									
	0		100		500		2500		10000	
	M	F	M	F	M	F	M	F	M	F
Creatinine (mg/dl)	1.2	0.9	1.0	0.7	0.7*	0.6	0.6*	0.6	0.5*	0.8
Cholesterol (mg/dl)	139	116	139	139	158	141	158	128	154	164*
BUN (mg/dl)	41	29	31	25	24*	26	29*	27	23*	22
ALT (U/l)	268	229	353	260	256	125	531	216	795*	327
Liver weight (g)	1.7	1.5	1.7	1.5	1.7	1.7	2.0*	2.0*	2.4*	2.3*
Relative liver weight (%)	6.6	6.4	6.3	6.6	6.8	7.2*	8.2*	8.7*	10.1*	10.5*
Relative kidney weight (%)	2.4	2.1	2.3	2.0	2.3	2.0	2.2	1.9	1.9*	1.7*
Relative spleen weight (%)	0.33	0.45	0.35	0.43	0.32	0.47	0.35	0.40	0.31	0.39*
Centrilobular hepatocyte swelling	0	0	1	0	6	0	11	7	12	12
Liver, coagulative necrosis	0	0	0	0	1	1	5	0	10	7

From Weir et al. (1984)

ALT, alanine aminotransferase; BUN, blood urea nitrogen; F, females; M, males.

[a] Values are means for each group of 12 mice.

* $p < 0.05$.

finding. Histopathology revealed that the incidence of a testicular atrophy was higher than that among the controls, achieving statistical significance at 500 ppm (Table 12). Liver weights and histological findings were unaffected by treatment.

The NOAEL was 150 ppm, equal to 20 mg/kg bw per day, on the basis of the statistically significant increase in testicular atrophy at 500 ppm. This study did not claim GLP compliance (Arai et al., 1980b).

An additional 6-month study was conducted to further investigate the testicular atrophy reported by Arai et al. (1980b). Groups of 20 male Alpk/AP albino mice received diets containing procymidone (purity, 99.8%) at a concentration of 0, 10, 30, 100 or 300 ppm. Mice were checked daily and a detailed clinical examination was given weekly. Body weight was monitored weekly and food consumption was recorded weekly for the first 12 weeks and then for 7 days every 4 weeks for the remainder of the study. At termination, mice were given a full postmortem, the testes and epididymides from mice in all groups were weighed and examined by histopathology. Mean achieved intakes were 0, 1.1, 3.6, 11 and 37 mg/kg bw per day.

One mouse at the lowest dose died. No treatment-related clinical signs or deaths were reported. Body-weight gain, food consumption and food use were not influenced by treatment. After 26 weeks, organ-weight analysis and histopathology of testes and epididymides revealed no evidence of a treatment-related effect (Table 13). The NOAEL was 300 ppm, equal to 37 mg/kg bw per day, on the basis of the absence of dose-related effects. The study was checked by a quality assurance unit (Kinsey et al., 1985).

Rats

Groups of 12 male and 12 female Sprague-Dawley rats were fed diets containing procymidone (purity, 98.7%) at a concentration of 0, 150, 500 or 1500 ppm for 6 months. Additional groups of 15 males and 15 females were fed diets containing procymidone at 0 or 1500 ppm for 9 months, or 0 or 1500 ppm for 6 months and then placed on a control diet for another 3 months. Rats were observed

Table 12. Leukocyte and testicular findings in mice (n = 20) fed diets containing procymidone for 6 months

Finding	Dietary concentration (ppm)							
	0		50		150		500	
	Males	Females	Males	Females	Males	Females	Males	Females
Intake (mg/kg bw per day)	0	0	6.5	7.3	20	24	72	83
Leukocytes (10^2/mm^3)	48	41	47	46	39*	41	34*	37
Testicular atrophy (all grades;	2	—	5	—	6	—	10*	—
			(p = 0.2)		(p = 0.1)		(p = 0.01)	

From Arai et al. (1980b)
* $p < 0.05$.

Table 13. Testes and epididymides weights in mice fed diets containing procymidone for 6 months

Organ weight	Dietary concentration (ppm)				
	0	10	30	100	300
Testes (mg)	213	240	225	257*	233
Epididymis (mg)	104	107	105	115	103

From Kinsey et al. (1985) * p<0.05

daily for signs of toxicity; body weight was measured two or three times during the first 2 weeks of treatment and then weekly; food consumption was also measured weekly. Urine analysis was conducted after 3, 6 and 9 months of treatment; haematological and blood chemistry examinations were made at 6 and 9 months. A complete necropsy was conducted on each rat at termination, organ weights were recorded and tissues were examined by histopathology.

Achieved intakes of procymidone were not calculated. No compound-related effects were observed on mortality, clinical signs, food and water consumption, urine analysis and clinical chemistry. Depression of body-weight gain was observed in males and females at 1500 ppm. There were slight decreases (< 10%) in packed cell volume (PCV; haematocrit) and haemoglobin values in males and females at 1500 ppm for 6 months, but not in those fed the same dose for 9 months. Serum ALT activity was increased at ≥ 500 ppm after 6 months but not after 9 months. Liver-weight to body-weight ratio was increased after 6 months at 1500 ppm in males and at 500 (< 10%) and 1500 ppm in females (Table 14), but this was reversible and had returned to normal in males after the 3-month recovery period. The spleen to body-weight ratio was also increased in male rats at 500 and 1500 ppm at 6 months; however, this increase was not apparent after treatment for 9 months and was therefore considered not related to treatment (Table 14). Relative adrenal weight was significantly increased at 1500 ppm in males and 500 and 1500 ppm in females, and relative pituitary weight at all doses in females after 6 months, but with no dose–response relationship; there were no histopathological changes in these organs and therefore alterations in weight were considered to be of no toxicological significance. After 9 months, absolute and relative testes weights were increased. The only histopathological lesion that could be attributed to treatment with procymidone was swelling and vacuolar degeneration of the liver cells in males fed 1500 ppm for 6 and 9 months. All the findings showed clear evidence of returning to normal after the recovery period.

Table 14. Findings in rats fed diets containing procymidone for 13 weeks

Finding	Dietary concentration (ppm)							
	0		150		500		1500	
	M	F	M	F	M	F	M	F
Body weight (g) at 6 months)	669	349	687	337	672	327	632	306*
ALT activity (U/l):								
6 months	15	18	17	19	21*	23	21	23
9 months	33	42	—	—	—	—	26	23
Relative liver weight (%):								
6 months	2.8	3.0	2.9	2.8	2.9	3.2*	3.2*	3.3*
9 months	2.9	2.8	—	—	—	—	3.3*	3.4*
Recovery	2.9	2.8	—	—	—	—	2.8	3.0*
Relative testes weight (%):								
6 months	0.55	—	0.56	—	0.54	—	0.59	—
9 months	0.42	—	—	—	—	—	0.58*	—
Recovery	0.42	—	—	—	—	—	0.49	—
Relative spleen weight (%)								
6 months	0.12	0.14	0.13	0.14	0.13	0.16*	0.13	0.18*
9 months	0.12	0.16	—	—	—	—	0.13	0.16

From Fujita et al. (1976) and Hosokawa (1984)
ALT, alanine aminotransferase; F, females; M, males.
* $p < 0.05$.

The NOAEL was 500 ppm, equivalent to 25 mg/kg bw per day, on the basis of reduced body-weight gain, a range of altered organ weights and other effects at 1500 ppm. The study did not claim GLP compliance (Fujita et al., 1976; Hosokawa, 1984).

(b) Dermal route

Rats

Groups of 10 male and 10 female Crj:CD(SD) rats were given procymidone (purity, 99.6%) at a dose of 0, 180, 450 or 1000 mg/kg bw per day as ground powder that was moistened with water and applied daily to the shaved backs for 28 days. Clinical observations were carried out daily during the treatment period and any skin reactions were noted. Body weight and food consumption were recorded weekly and an ophthalmological examination was conducted during the last week of treatment. Haematological and clinical parameters were measured on blood taken at termination and urine collected during week 4 was also analysed. A gross necropsy was performed at termination, organs were weighed and tissues were examined microscopically.

There was no evidence of local effects at the application site. Increases in adrenal and heart weights, and decreases in prostate and spleen weights were without a dose–response relationship and/or within typical values for controls. There were no effects on the liver or testes. Histopathology and clinical chemistry findings showed no treatment-related effects. The NOAEL was 1000 mg/kg bw per day, the highest dose tested. The study contained GLP compliance statements and complied with OECD test guideline 410 (Ogata, 2002).

Dogs

Groups of six male and six female beagle dogs were given gelatin capsules containing procymidone (purity, > 95%) at an oral dose of 0 (empty capsule), 20, 100 or 500 mg/kg bw per day for 26 weeks. Clinical signs and food consumption were recorded daily and body weight was measured weekly. Water consumption was measured over a 3-day period after 7, 16 and 25 weeks of treatment and urine analysis was performed at the same intervals. Ophthalmology, haematology, and clinical chemistry examinations were made after 4, 8, 12, 16, 21 and 25 weeks. At the end of the treatment period, the dogs were dissected, organ weights were recorded and microscopic investigations were made on stained tissues and organs.

All dogs survived the study. Vomiting and diarrhoea were seen in all groups, including controls, but was more prevalent in dogs at 500 mg/kg bw per day, the highest dose. The incidences of diarrhoea in males at the highest dose and emesis in both sexes was statistically significantly increased relative to controls ($p < 0.02$, Mann-Whitney U test subsequent to a Kruskal-Wallis ANOVA). Body weights, food and water intakes, ophthalmoscopy and urine analysis parameters were not affected by treatment. There were statistically significant changes in clinical chemistry. Serum alkaline phosphatase (ALP) activity was increased in male and female dogs at 500 mg/kg bw per day. At the same dose, BUN, glucose and calcium concentrations were increased in males (Table 15). BUN and calcium concentrations were also increased in dogs at 100 and 20 mg/kg bw per day (Table 15); the changes at 20 and 100 mg/kg bw per day were rather sporadic, possibly linked to pre-test values and were considered to be not related to administration of procymidone. Results of urine analysis and kidney pathology were unremarkable. Absolute and relative heart weights were significantly decreased in females at 20 or 500 mg/kg bw per day, but without any dose–response relationship or histopathological correlate.

The NOAEL was 100 mg/kg bw per day on the basis of the compound-related emesis, diarrhoea and serum chemistry changes at 500 mg/kg bw per day. The study was inspected by a quality assurance unit (Nakashima et al., 1984).

Groups of four male and four female beagle dogs received capsules containing procymidone technical (purity, 98.5%) once daily at a dose of 0 (empty capsule), 20, 100 or 500 mg/kg bw per day for 1 year. All dogs were observed twice per day for mortality, moribundity and clinical signs of toxicity and were given a detailed physical examination weekly. Body weight and food consumption were recorded weekly. Ophthalmoscopic assessments were made during weeks 26 and 52. Clinical pathology and haematology parameters were measured in blood sampled before dosing began and during weeks 13, 26, 39 and 52. All dogs were given a gross postmortem examination, selected organs were weighed and tissues were examined microscopically.

One male in the group at 100 mg/kg bw per day was killed following an irreparable intussusception of the large intestine in week 19. Emesis and soft faeces were observed in dogs in all groups, including the controls, but appeared to be more prevalent at 500 mg/kg bw per day; the incidence of diarrhoea/soft faeces in males at this, the highest dose, was statistically significantly increased relative to values for the controls ($p = 0.02$, Mann-Whitney U test subsequent to a Kruskal-Wallis ANOVA). Dogs in the groups receiving procymidone generally consumed more food and put on more weight than did the controls. There was an increase in serum globulin concentration in weeks 39 and 52 amongst male dogs at 500 mg/kg bw per day, resulting in a reduced albumin : globulin ratio; a less marked effect was present at 100 mg/kg bw per day. Serum ALP activity was increased in males at the highest dose (Table 16). Serum concentrations of creatinine, glucose, BUN and calcium were similar between groups. No treatment-related changes were detected on organ weights or by gross or microscopic pathology examination.

Table 15. Findings in dogs (n = 6) fed capsules containing procymidone for 26 weeks

Finding	Dose (mg/kg bw per day)							
	0		20		100		500	
	M	F	M	F	M	F	M	F
ALP activity (U/l)	149	143	164	133	160	142	196	242
BUN (mg/dl)	18	21	20*	21	20	20	21*	23
Glucose (mg/dl)	92	94	93	95	94	94	102*	89
Calcium (mg/dl) (before dosing)	11.1 (11.1)	11.3 (11.2)	11.3 (11.0)	11.5 (11.2)	11.4* (11.2)	11.4 (11.3)	11.5* (11.2)	11.3 (11.4)
Heart weight (g)	103	93	99	83	100	97	102	85
Relative heart weight (%)	0.83	0.88	0.79	0.74*	0.78	0.86	0.83	0.76*

From Nakashima et al. (1984)
ALP, alkaline phosphatase; BUN, blood urea nitrogen; F, females; M, males.
* $p < 0.05$.

Table 16. Findings in dogs (n = 4) fed capsules containing procymidone for 52 weeks

Finding	Dose (mg/kg bw per day)							
	0		20		100		500	
	Males	Females	Males	Females	Males	Females	Males	Females
Alkaline phosphastase activity (U/l)	27	40	31	46	29	34	53*	44
Total protein (g/dl)	5.8	5.8	5.8	5.6	6.6*	6.1	6.6*	6.1
Globulin (g/dl)	2.4	2.3	2.3	2.1	2.9	2.5	3.2*	2.3
Albumin : globulin ratio	1.5	1.6	1.5	1.6	1.3	1.5	1.1	1.6

From Dalgard & Machotka (1992)
* $p < 0.05$.

The NOAEL was 100 mg/kg bw per day on the basis of increased emesis and diarrhoea/soft faeces, and clinical chemistry changes at 500 mg/kg bw per day. The study claimed GLP compliance and complied with OECD test guideline 409 (Dalgard & Machotka, 1992).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 50 male and 50 female B6C3F$_1$ mice received diets containing procymidone (purity, 99.8%) at a concentration of 0, 30, 100, 300 or 1000 ppm for 104 weeks. In addition, satellite groups of 10 males and 10 females were used for interim investigations at 26, 52 and 78 weeks. All mice were observed twice per day for mortality and signs of toxicity and a detailed examination, that included palpation, was conducted weekly. Body weights and food consumption were recorded weekly for the first 14 weeks and every 2 weeks thereafter. An ophthalmological examination was performed before the start of treatment and at weeks 26, 52, 78 and 105, and blood and urine samples were collected at the same intervals. A complete gross necropsy was performed on all mice, organs were weighed and samples of tissues and bone-marrow smears were taken for microscopic examination.

Mean intakes of procymidone over the study were presented in an addendum (Moore, 1993; Table 17). Treatment had no influence on survival. Survival for male mice at 0, 30, 100, 300 or 1000 ppm was 82%, 88%, 80%, 82% and 80%, respectively, and survival for females was 82%, 74%, 64%, 78% and 72%, respectively. There were no treatment-related effects on behaviour or appearance, body weights, food and water intakes or clinical pathology investigations. Organ-weight analysis revealed higher liver weights and liver-to-body-weight ratios for both sexes at 300 and 1000 ppm. Absolute and relative liver weights were statistically significantly increased at weeks 26 in mice at the highest dose, progressing with duration of dosing, such that absolute liver weights at week 105 were significantly higher than those of mice in the control group for males and females at 300 ppm and in males at 100 ppm. Histopathology revealed treatment-related changes only in the liver. After 52 weeks, there was an increase in a very mild hepatocellular change recorded as centrilobular cytologic alteration. After 105 weeks, high incidences of centrilobular cytomegaly were detected in males at 1000 ppm and to a lesser extent in males at 300 ppm and females at 1000 ppm. Focal or multifocal hepatocellular hyperplasia, eosinophilic foci and fatty change were also recorded at 1000 ppm. Neoplastic changes were an increased incidence of hepatocellular adenomas and carcinomas combined in males at 30, 100 and 1000 ppm, but without a dose–response relationship, and females at 300 and 1000 ppm. The incidence of hepatoblastomas was increased in males at 1000 ppm. Although the incidences were reported to be within the published range for control mice of this strain, clear increases are evident in the number of females at the highest dose with hepatocellular adenomas and the number of males at the highest dose with hepatoblastomas when compared with concurrent controls and groups at lower doses (Table 17). A subsequent statistical analysis (Thakur, 1988) concluded that there was a statistically significant trend in females for adenomas and adenomas plus carcinomas combined and in males for hepatoblastomas.

The NOAEL for toxicity was 100 ppm, equal to 15 mg/kg bw per day, on the basis of the overall range of liver pathologies at 300 ppm. The increased liver weight in males at 100 ppm was not considered to be adverse as there were no associated clinical chemistry or histopathological findings. Procymidone was carcinogenic to B6C3F$_1$ mice, inducing hepatocellular adenomas in females and hepatoblastomas in males at 1000 ppm; the NOAEL for neoplastic toxicity was 300 ppm, equal to 46 mg/kg bw per day. The study claimed GLP compliance and met the essential elements of OECD test guideline 453 (Filler & Parker, 1988).

Table 17. Hepatic findings in a long-term study of carcinogenicity in mice fed diets containing procymidone

Finding	Incidence (No. of mice per group of 50)									
	Dietary concentration (ppm)									
	Male					Female				
	0	30	100	300	1000	0	30	100	300	1000
Mean compound intake (mg/kg bw per day)	0	4.6	15	46	153	0	6.4	23	65	206
Liver weight (g)	1.6	1.8	2.1*	1.9*	2.4*	1.4	1.5	1.6	1.7*	2.2*
Centrilobular cytomegaly	0	0	1	6*	43*	0	0	0	0	5*
Multifocal hyperplasia	2	0	1	5	8	0	0	0	2	2
Eosinic foci	1	0	1	5	3	0	1	0	2	10*
Multifocal fatty change	2	1	3	5	11*	0	0	0	4	3
Hepatocellular adenomas	7	11	12	9	10	1	1	0	3	7*
Hepatocellular carcinomas	5	6	9	5	10	1	1	2	4	2
Hepatoblastomas	1	0	0	2	5	0	0	0	0	0
Malignant hepatocellular neoplasms	5	6	9	7	11	1	1	2	4	2

From Filler & Parker (1988) and Moore (1993)
* $p < 0.05$, Fisher exact test (one-sided).

Rats

Groups of 50 male and 50 female Osborne-Mendel rats were given diets containing procymidone (purity, 99.8%) at a concentration of 0, 100, 300, 1000 or 2000 ppm for 104 weeks. Satellite groups of 50 males and 50 females received the same treatment and were used for blood analyses and interim termination at 26, 52 or 78 weeks. Groups of 50 males and 50 females, and 60 males and 60 females, respectively, served as controls for the main and satellite groups. All rats were observed daily for mortality and overt signs of toxicity and were given a detailed clinical examination weekly. Body weights, food consumption and water consumption were recorded weekly for the first 13 weeks and at 2-week intervals thereafter. An ophthalmology assessment was performed on 10 males and 10 females per group at weeks 26, 52, 78 and 104, blood and urine were collected for clinical pathology from 10 males and 10 females per group at the same intervals. Testosterone analyses were performed on blood samples from males in the control group (n = 2–8) and males at the highest dose (n = 3–5) at 18 and 24 months. At termination, a necropsy was performed, organ weights were recorded and tissues were examined microscopically.

Survival at termination in the groups investigated for carcinogenicity was 22–34% for males and 50–70% for females; in males, 50% survival was maintained until week 90. There were no biologically significant or consistent compound-related effects on mortality, clinical signs, food and water consumption, ophthalmology, urine analysis, and haematology or blood chemistry. Reduced body weight (> 10%) was noted in males and females at 1000 and 2000 ppm (Table 18); body weight was also lower at 300 ppm during the early part of the study and, although occasionally statistically significant, varied by less than 5%. The incidence of palapable masses was higher in males at the highest dose than in other groups from week 26 onwards. Increased relative weights of liver in both sexes and testes and ovaries were recorded at 1000 and 2000 ppm. In females, the kidney to body-weight and the brain to body-weight ratios were also increased at 2000 ppm, but there was no pathological evidence of a treatment-related abnormality in either organ. Seminal vesicle weights were decreased

at 2000 ppm. Histopathology revealed an increased incidence of hepatic centrilobular cytomegaly in males and females at 1000 and 2000 ppm and a statistically significant increased incidence of ovarian stromal hyperplasia in females at 2000 ppm. There was no increase in the total number of rats with benign or malignant tumours. An increased incidence of testicular interstitial-cell tumours and interstitial-cell hyperplasia was seen in males at 1000 and 2000 ppm (Table 18). Testosterone concentrations were increased in males at 2000 ppm (the only dose sampled) at 18 and 24 months.

The NOAEL for toxicity was 300 ppm, equal to 14.0 mg/kg bw per day, on the basis of reduced body weight and liver pathology at 1000 ppm and greater. The NOAEL for tumour incidence was 300 ppm, equal to 14 mg/kg bw per day, on the basis of the increase in interstitial-cell tumours of the testes at 1000 ppm and greater. The study claimed GLP compliance and met the requirements of OECD test guideline 453 (Keller & Cardy, 1986).

2.4 Genotoxicity

Procymidone was not genotoxic in a range of studies in vitro and in vivo (Table 19). Many of the studies were relatively old and contained moderate levels of detail, but the information they contained did not indicate that procymidone has any genotoxic potential. Three more modern studies in vitro, which complied with GLP and with the essential elements of contemporary test guidelines, and provided negative results.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Groups of 30 male and 30 female Alpk:APfSD (Wistar-derived) rats (F_0 generation) were given diets containing procymidone (purity, > 99%) at a concentration of 0, 50, 250 and 750 ppm. The doses

Table 18. Findings in a long-term study of carcinogenicity in rats fed diets containing procymidone

Finding	Dietary concentration (ppm)									
	Males					Females				
	0	100	300	1000	2000	0	100	300	1000	2000
Mean intake (mg/kg bw per day)	0	4.6	14	48	97	0	6	18	60	125
Body weight (g), week 81	582	572	563	535*	513*	313	297	289	278*	278*
Relative testes/ovary weight (%):										
12 months	0.84	0.86	0.84	0.87	0.92	0.046	0.048	0.050	0.053	0.058
18 months	0.78	0.81	0.85	0.90	0.90	0.041	0.056	0.054	0.046	0.049
24 months	0.71	0.80	0.84	1.06*	1.04*	0.042	0.043	0.042	0.048*	0.055*
Relative liver weight (%) at 24 months	4.1	3.7	4.4	4.6	4.9*	3.2	3.4	3.6	4.1*	4.4*
Liver centrilobular cytomegaly	0	0	0	11*	17*	0	0	2	25*	36*
Testes, interstitial-cell tumour	1	1	0	10*	20*	—	—	—	—	—
Interstitial-cell hyperplasia	2	0	1	7	12*	—	—	—	—	—
Ovaries, stromal hyperplasia	—	—	—	—	—	0	0	0	2	8*

From Keller & Cardy (1986)
* $p < 0.05$.

Table 19. Results of studies of genotoxicity with procymidone

End-point	Test object	Concentration	Purity (% w/w)	Result	Reference
In vitro					
Reverse mutation [a]	*S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* (WP2 *hcr-)*	10–1 000 µg/plate	96.9	Negative +S9[d] Negative – S9	Moriya & Kato (1977)
Reverse mutation[b]	*S. typhimurium* strains TA98, TA100, TA1535, TA1537	10–10 000 µg/plate	96.3	Negative +S9[d] Negative –S9	Suzuki & Miyamoto (1976)
Reverse mutation[b,c,f]	*S. typhimurium* strains TA98, TA100, TA1535, TA1537, TA1538 and *E. coli* (WP2 *uvrA)*	5–5 000 µg/plate	99.4	Negative +S9[d] Negative –S9	Kogiso (1991)
Gene mutation (*Hprt* locus)	Chinese hamster lung cells (V79)	0.7–6.0 mmol/l	Sumisclex 50% granule	Negative +S9[d] Negative –S9	Principe et al. (1980) Nunziata (1982)
Chromosomal aberration [g]	Chinese hamster ovary cells (CHO-K1)	75–300 µg/ml	99.4	Negative +S9[d] Negative –S9	Hara (1991b)
DNA damage	*Bacillus subtilis* (H17 and M45*rec-*)	20–2 000 µg/disk	96.9	Negative	Moriya & Kato (1977)
DNA damage[b]	*Bacillus subtilis* (H17 and M45*rec-*)	10–10 000 µg/disk	96.3	Negative	Suzuki & Miyamoto (1976)
Sister chromatid exchange[h]	Primary cultures from ICR mouse embryos	1–100 µmol/l	96.9	Negative +S9[d] Negative –S9	Suzuki (1980)
Unscheduled DNA synthesis[i]	Heteroploid human epithelial cells	6 µmol/l–6.0 mmol/l	Sumisclex 50% granule	Negative +S9[d] Negative –S9	Principe et al. (1980) Nunziata (1982) (supplement)
Unscheduled DNA synthesis[c, f, j]	Primary rat hepatocytes	3–300 µg/ml	99.4	Negative	Hara (1991a); Hara (1992)
In vivo					
Chromosomal aberration[k]	Male ddY mice	400, 800 or 1 600 mg/kg bw intraperitoneally in corn oil	96.9	Negative	Hara & Suzuki (1980)
Reverse mutation, host-mediated[b]	Male ICR mice, *S. typhimurium* (G46)	1 000 or 2 000 mg/kg bw by gavage in DMSO	96.3	Negative	Suzuki & Miyamoto (1976)
Reverse mutation host-mediated[b, l]	Male ICR mice, *S. typhimurium* (G46)	2 × 200 and 2 × 500 mg/kg bw by gavage in 5% gum arabic	96.9	Negative	Moriya & Kato (1977)

Positive controls were included in all tests for genotoxicity and were performed adequately.
DMSO, dimethyl sulfoxide; S9, 9000 × *g* supernatant from rat livers.
[a] Each test was conducted in duplicate.

[b] Each test was conducted in triplicate.
[c] Two separate experiments were conducted.
[d] S9 was prepared from livers of Sprague Dawley rats pre-treated with polychlorinatedbiphenyl mixture..
[e] S9 was prepared from livers of Sprague Dawley rats pre-treated with phenobarbital and -naphthoflavone.
[f] The study was performed in compliance with good laboratory practice and according to United States Environmental Protection Agency (EPA) study guideline 84-2.
[g] One hundred cells from duplicate cultures were analysed at 10 and 18 h after treatment.
[h] Fifty metaphases were analysed at each concentration.
[i] Three replicates of 50 cells were analysed in each experiment.
[j] Hepatocytes were prepared from Sprague-Dawley rats; at each concentration, six replicates of 50 cells were analysed.
[k] Six mice were treated for 6, 24 and 48 h after intraperitoneal administration; 50 metaphases in bone-marrow cells were analysed for each mouse.
[l] Six mice were treated at each concentration.

were based on a preliminary study that showed decreased litter size and feminization of male pups at ≥ 1000 ppm. After 12 weeks, the rats were mated and allowed to rear the F_{1a} litters to weaning and then paired again to produce the F_{1b} litters. F_1 parents selected from the F_{1a} litters were mated to produce two further litters (F_{2a} and F_{2b}). Body weight, food intake and clinical condition were recorded weekly. Reproductive parameters (fertility, length of gestation, precoital interval, litter size, viability and number of live and dead pups) were recorded for each generation and litter. The parental rats were given a postmortem, the liver and reproductive organs were weighed and tissues were examined histopathologically. Clinical observations, body weights, organ weights and pathology findings were also recorded for the offspring. Additional pathological examinations were performed subsequently and were reported in addenda.

Achieved intakes of procymidone throughout the study were not tabulated; data for premating periods only were presented in Wickramaratne & Milburn (1990), but actual concentrations, stability and homogeneity in diet were confirmed as acceptable. All parental rats at the highest dose showed reductions in body-weight gain from the start of compound administration; statistically significant for F_o rats of both sexes and F_1 and F_2 females. There were no consistent effects on body weight or body-weight gain at 250 ppm. Precoital interval was generally reduced in pairings at 750 ppm. In F_1 males at the highest dose (but not F_o), there was a significant reduction in fertility (Table 20), associated with hypospadias (Table 20). Relative liver and relative testes weights were increased at 250 and 750 ppm (Table 20). The duration of gestation was similar in all groups. Litter size was reduced in the F_{1b} mating at 750 ppm, but not in any other matings. Penile abnormalities were noted at both gross and microscopic examinations in rats at 750 ppm. No effects were observed on sperm parameters. Sexing pups at birth was made difficult by reductions in anogenital distance (qualitative assessment) in male offspring at 750 ppm. Liver weight and testes-weight findings in parents were repeated in offspring; the increase in testes weights at 50 ppm in the F_{1b} pups was < 10%, and not statistically significant in the F_{2b} pups and was not considered to be adverse. Reductions in weights of the prostate and epididymis were seen at 250 ppm (F_{1b}) and 750 ppm (Table 20). There were no adverse effects on the sex organs of female pups.

The NOAEL for pup development was 50 ppm, equivalent to 3 mg/kg bw per day, on the basis of alterations in testes, prostate and epididymis weights at 250 ppm. The NOAEL for reproductive effects was 250 ppm, equivalent to 17 mg/kg bw per day, on the basis of reduced male fertility at 750 ppm. The NOAEL for parental toxicity was 250 ppm on the basis of reduced body weights at 750 ppm. The study was inspected by a quality assurance unit and complied with OECD test guideline 416 (1983) (Wickramaratne et al., 1988a; Milburn & Moreland, 1991).

A modified study of reproduction in rats was performed to provide information on the development of reproductive organs of F_1 males aged 5 weeks and at sexual maturity (aged 10 weeks). Groups of 26 male and 26 female Alpk:APfSD (Wistar-derived) rats received diets containing procymidone (purity, 98.7–99.1%) at a constant dose equal to 0, 2.5, 12.5 or 37 mg/kg bw per day throughout the

Table 20. Findings in offspring and parental rats fed diets containing procymidone for two generations

Finding	Dietary concentration (ppm)							
	0		50		250		750	
	Males	Females	Males	Females	Males	Females	Males	Females
Fertile rats (%):								
F_{1b}	93	100	85	97	85	93	97	97
F_{1b}	76	97	88	86	81	90	89	100
F_{2a}	79	97	96	93	93	100	42*	86
F_{2b}	92	93	78	93	81	96	46*	97
Precoital time (days):								
F_{1a}	2.5		3.8		2.0		1.9	
F_{1b}	2.0		2.0		2.1		1.9	
F_{2a}	2.4		2.6		2.1		2.9	
F_{2b}	3.2		2.0		2.0		2.0	
Adjusted liver weight (g):								
F_{o}	20.6	12.2	20.3	12.1	21.7*	12.4	22.9*	12.9*
F_{1}	21.5	14.8	21.6	14.7	22.4*	15.5	24.9*	16.7*
F_{2}	19.8	10.2	19.6	10.3	20.5	10.9*	21.5*	11.2*
Adjusted testes weight (g):								
F_{o}	3.8	—	3.8	—	3.9*	—	4.1*	—
F_{1}	3.6	—	3.7	—	3.9*	—	4.0	—
F_{2}	3.7	—	3.6	—	3.8	—	3.9*	—
Penile abnormalities:								
F_{0} [a]	0/30	—	0/30	—	0/30	—	0/30	—
F_{1} [a]	0/30	—	0/30	—	0/30	—	12/30	—
F_{2} [a]	0/30	—	0/30	—	0/30	—	8/30	—
F_{2b} [b]	—	—	—	—	—	—	21/40	—
Adjusted prostate weight (g):								
F_{1b}	0.22	—	0.22	—	0.19*	—	0.17*	—
F_{2b}	0.22	—	0.20	—	0.19	—	0.17*	—
Adjusted testes weight (g):								
F_{1b}	1.22	—	1.29*	—	1.31*	—	1.39*	—
F_{2b}	1.22	—	1.29	—	1.34*	—	1.36*	—
Adjusted epididymis weight (g)								
F_{1b}	0.16	—	0.16	—	0.14*	—	0.13*	—
F_{2b}	0.17	—	0.15	—	0.15	—	0.12*	—

From Wickramaratne et al. (1988a) and Milburn & Moreland (1991)

[a] Parents.

[b] Pups, week 7.

* $p < 0.05$.

experimental period. Rats were observed daily for changes in clinical condition and behaviour and were given a detailed examination weekly. Body weight and food consumption were also recorded weekly. Rats were mated 12 weeks after the start of treatment and allowed to litter and wean the pups. Reproductive parameters were assessed. The clinical condition of pups was recorded daily and body weights were recorded at intervals after birth. F_1 males were separated from their mothers on postnatal day 22 and killed at either 5 or 10 weeks after birth. F_1 females were killed at postnatal day 22. F_1 males were given a postmortem examination, organs were weighed and reproductive tissues were examined by histopathology.

Treatment at 37.5 mg/kg bw per day slightly impaired the body-weight gain of F_0 males during the period before mating. Among females, reduced body-weight gain and food consumption was recorded during the same period at 37 and 12.5 mg/kg bw per day (Table 21); however, the decreases at 12.5 mg/kg bw per day were less than 5% and were not considered to be adverse. There were no significant effects on body weight during pregnancy and lactation. Doses of up to 37 mg/kg bw per day had no effect on fertility or mating success; however, at 37 mg/kg bw per day, litter size was lower than in the control group and the resulting superior pup weight at birth was maintained through to weaning. Total litter weights were unaffected. Body-weight gain in F_1 males was reduced in rats at 37 mg/kg bw per day. At age 5 and 10 weeks, F_1 males at 37 mg/kg bw per day showed an increase in absolute and relative testes weight and a decrease in the weight of the prostate with seminal vesicles at 10 weeks. At 37 mg/kg bw per day, hypospadias was observed in 1 out of 46 and 2 out of 47 F_1 males at age 5 and 10 weeks, respectively (typical incidence in the control group was zero). Histopathology did not reveal any significant differences between groups.

The NOAEL for parental toxicity was 12.5 mg/kg bw per day on the basis of the reductions in body weight and food consumption in females, before mating, at 37 mg/kg bw per day. The NOAEL for effects on reproduction was 12.5 mg/kg bw per day on the basis of the reduced litter size at 37 mg/kg bw per day. The NOAEL for offspring toxicity was 12.5 mg/kg bw per day on the basis of the incidence of hypospadias, increased testes weight and decreased weights of the accessory sex organ at 37 mg/kg bw per day. The study claimed GLP compliance (Hodge et al., 1991).

Table 21. Findings in rats fed diets containing procymidone in a study of reproduction/ developmental toxicity

Finding	Dose (mg/kg bw per day)							
	0		2.5		12.5		37	
	Males	Females	Males	Females	Males	Females	Males	Females
Food consumption before mating (g/day), weeks 1–12	28.8	20.9	28.6	20.4	28.8	19.9*	28.3	19.6*
Body weight (g), week 12	383	186	381	186	381	175*	370	169*
Litter size, day 1	11.3		11.8		10.9		9.6*	
F_1 body-weight adjusted testes weight (g):								
Week 5	1.03	—	1.06	—	1.03	—	1.12*	—
Week 10	3.11	—	3.12	—	3.08	—	3.30*	—
F_1 body-weight adjusted prostate weight (g):								
Week 5	0.182	—	0.192	—	0.179	—	0.175	—
Week 10	1.47	—	1.50	—	1.44	—	1.31*	—

From Hodge et al. (1991)
* $p < 0.05$.

(b) Developmental toxicity

Rats

Groups of 25 (presumed) pregnant female Sprague-Dawley rats received procymidone technical (purity, 99.6%) at a dose of 0, 30, 100 or 300 mg/kg bw per day in corn oil by oral gavage on days 6–15 of gestation. Doses were based on the results of a range-finding study (Pence et al., 1980a). Rats were observed twice per day and weighed at intervals during the study. Dams were terminated on day 20 of gestation, fetuses were delivered by caesarean section and a uterine examination was performed. The number, sex and weights of fetuses were recorded; visceral examinations (Wilson technique) were performed on 33% of the fetuses and skeletal examinations (alizarin staining) conducted on the remainder.

All dams survived the study, pregnancy rates were acceptable and there were no treatment-related clinical signs. Body-weight gains were reduced (60%) during days 6–11 of gestation at 300 mg/kg bw per day, although parity with the controls was regained by the end of the dosing period; food consumption was not measured. The proportion of males was reduced relative to controls (57.5%) at the intermediate (50%) and highest (46%) doses, but the values were within the normal ranges for studies of developmental toxicity in rats. There was no effect of treatment on litter values or the incidence of fetal abnormalities at any dose. The NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of reduced body-weight gain on days 6–11 of gestation. The NOAEL for developmental toxicity was 300 mg/kg bw per day, the highest dose tested, on the basis of the absence of a treatment-related effect on embryo-fetal development. The study claimed compliance with GLP and complies with the essential elements of OECD test guideline 414 of 1981 (Pence et al., 1980b).

To more fully evaluate the incidence and significance of the genital abnormalities observed in the multigeneration study of reproduction (Wickramaratne et al., 1988a), an additional study of developmental toxicity was conducted. For dose-range finding, groups of eight (presumed) pregnant female Sprague-Dawley rats received procymidone (purity, 99.4%) at a dose of 0, 12.5, 300, 500, 750 or 1000 mg/kg bw per day in corn oil by oral gavage on days 6–19 of gestation. The NOAEL for maternal toxicity was 12.5 mg/kg bw per day on the basis of clinical signs at 300 mg/kg bw per day, and the NOAEL for developmental toxicity was 12.5 mg/kg bw per day on the basis of hypospadias (71% vs 0% in controls), and reduced anogenital distance at 300 mg/kg bw per day (Hobermann, 1992a).

In the main study, groups of 45 (presumed) pregnant female Sprague-Dawley rats received procymidone (purity, 99.4%) at a dose of 0, 3.5, 12.5, 125 or 500 mg/kg bw per day in corn oil by oral gavage on days 6–19 of gestation. Approximately half the females in each group were terminated on day 20 of gestation. Uterine examinations were performed and litter parameters were evaluated for fetuses on day 20 of gestation. The fetuses were weighed and examined for visceral and skeletal abnormalities. The remaining females were allowed to deliver and rear their pups to weaning. Male offspring were retained until postnatal day 45 and females until postnatal days 51 to 53. Rats were observed daily for clinical signs of toxicity, body weights and food consumption were recorded daily in the periods during and after dosing. Pups delivered normally were weighed and anogenital distance measured on postnatal days 1, 21 and 45. At necropsy, these pups were given a detailed examination and reproductive organs were weighed and examined histopathologically.

There were no deaths during the study. Maternal toxicity was apparent at 125 and 500 mg/kg bw per day as clinical signs (ungroomed coat, urine-stained fur), initial body-weight loss and reduced food intake. Developmental toxicity was apparent at 125 and 500 mg/kg bw per day as lower fetal weights, reduced anogenital distance (Table 22), higher incidences of male offspring with undescended testes,

hypospadias, testicular atrophy, distended preputial grand, inflammatory changes in the accessory sex organs (seminal vesicles, prostate and coagulating glands), and lower organ weights of testes and prostate. At 500 mg/kg bw per day, there was a significant increase in the incidence of bifid thoracic vertebral centra in fetuses and prostate lesions in male offspring. Male offspring at 12.5 and 3.5 mg/kg bw per day were not affected by treatment. No treatment-related influence on sexual differentiation and development of female offspring was observed at any dose.

The NOAEL for maternal toxicity was 12.5 mg/kg bw per day, on the basis of reduced weight gain and food intakes at 125 mg/kg bw per day and above. The NOAEL for developmental toxicity was 12.5 mg/kg bw per day, on the basis of the reduction of anogenital distance at 125 mg/kg bw per day and above in males. These studies contained GLP compliance statements (Hoberman, 1992a, 1992b, 1992c).

A study was performed to determine the minimum toxic dose of procymidone required to produce the external effects on male genitalia of rats. Groups of 20 pregnant Crj:Sprague-Dawley rats were given procymidone (purity, 99.6%) at a dose of 37.5 or 62.5 mg/kg bw per day during the critical period of development of fetal external genitalia (between days 6 and 19 of gestation). Dams were allowed to deliver and, together with female pups, were euthanized on postnatal day 21. Male pups were weaned and euthanized on postnatal day 56. Maternal rats and offspring were observed daily for clinical signs of toxicity and body weights were recorded at intervals. Maternal rats and offspring were given a gross necropsy at euthanasia. Observations on the offspring included macroscopic examination of the external genitalia for all males, organ weights were also measured. In maternal rats, samples of blood were taken for analysis of procymidone concentrations. Blood samples were collected from additional groups of rats on days 6 and 19 of gestation, at 2, 4, 8 and 24 h after dosing and analysed for procymidone.

There were no treatment-related effects on clinical or gross necropsy observations in the maternal rats. Maternal body-weight gains were suppressed in both groups. The blood concentration of procymidone was at a maximum (C_{max}) between 2 h and 4 h after dosing and higher C_{max} values were found at the end of the dosing period (day 19 of gestation) than at the beginning (day 6 of gestation), but the rate of elimination of procymidone from plasma appeared to be more rapid on day 19 of gestation than on day 6 of gestation. The kinetic data showed only slightly higher systemic exposures at the higher dose (Table 23). There were no effects on pregnancy outcome or pup viability. One pup in the group at 62.5 mg/kg bw per day died, but there were no effects on clinical signs or body weight in the surviving offspring. Gross necropsy observations on the offspring included a dose-related increase in misshapen penis (hypospadias, unseparated prepuce) and undescended and abnormal testes size. There were no statistically significant effects on organ weights in the male pups, although prostate and seminal vesicle weights were approximately 10% lower in the group at the highest dose. No abnormalities were detected in female offspring.

Table 22. Anogenital distances in male rats exposed to procymidone in utero

Time-point	Anogenital distance (mm)				
	Dose (mg/kg bw per day)				
	0	3.5	12.5	125	500
Postnatal day 1	2.6 ± 0.2	2.6 ± 0.2	2.6 ± 0.4	1.8 ± 0.2*	1.3 ± 0.1*
Postnatal day 21	12.3 ± 1.1	11.9 ± 0.7	11.8 ± 1.3	9.7 ± 1.4*	8.3 ± 1.1*
Termination (postnatal day 45)	33.0 ± 1.1	32.8 ± 1.6	32.3 ± 1.7	27.2 ± 2.2*	23.1 ± 2.2*

From Hoberman (1992a, 1992b, 1992c)
* $p < 0.05$.

The study did not identify a NOAEL as the lowest dose (37.5 mg/kg bw per day) produced effects on male external genitalia of rats (Inawaka, 2005).

Rabbits

Groups of 18 inseminated New Zealand White rabbits received procymidone (purity, 99.6%) at a dose of 0, 30, 150, 750 or 1000 mg/kg bw per day in corn oil by oral gavage on days 7–19 of gestation. Clinical observations, food consumption and body weight were monitored and dams were terminated on day 30 of gestation. Uteri were examined for live fetuses and intrauterine deaths. Fetuses were weighed and examined for external, visceral (dissection) and skeletal abnormalities (alizarin staining). Specific examinations of anogenital distance and external genitalia were not performed.

There was no compound-related maternal toxicity; pregnancy outcome was unaffected and no evidence of teratogenic potential of procymidone was recorded in any of the groups. The degree of ossification of the fifth and sixth sternebrae was reduced at 1000 mg/kg bw per day. The NOAEL for maternal toxicity and teratogenicity was 1000 mg/kg bw per day, the highest dose tested. The NOAEL for developmental toxicity was 750 mg/kg bw per day on the basis of reduced sternebrae ossification. The study and procedures were inspected by a quality assurance unit and complied with the essential elements of OECD test guideline 414 of 1981 (Wickramaratne, 1988; Wickramaratne et al., 1988b)

Groups of 26 pregnant New Zealand White rabbits were given procymidone (purity, 99.2%) at a dose of 0 or 125 mg/kg bw per day in a vehicle of 0.5% methylcellulose by gavage on days 6 to 28 of gestation. Clinical signs of toxicity, body weight and food consumption were recorded. The dams were terminated on day 29 of gestation and received an extensive uterine examination. Live fetuses were weighed, sexed and examined for abnormalities. A detailed examination of the external genitalia was performed, the anogenital distance and phallus boundary-genital distance were measured using a micrometer and the diameter and the ventral gap of the preputial lamella were measured using a microscope.

Signs of maternal toxicity (reduced food consumption, anorexia, abortion) were evident in the group receiving procymidone. There were no treatment-related effects on fetal parameters, although the litter size was lower in the procymidone group than controls (7.4 ± 2.7 vs 9.1 ± 2.8) this was within the normal range for rabbits. Anogenital distance, the phallus boundary-genital distance and the diameter of the preputial lamina in live fetuses were similar in both groups (Table 24). The ventral gap of the preputial lamina was unaffected in male fetuses, but was increased in female fetuses in the treated group. As a result, the ratio of the ventral gap to the diameter of preputial lamella was also increased. The relative ventral gap of the preputial lamina to body-weight ratio was not statistically significantly altered, indicating that this effect might be secondary to pup body weight, although pup weight was only slightly greater in the group receiving procymidone (Table 24). Only

Table 23. Blood plasma kinetic values in pregnant rats given procymidone by gavage

Parameter	Dose (mg/kg bw per day)			
	37.5	62.5	37.5	62.5
Gestation day	6	6	19	19
C_{max} (µg/ml)	3.11	3.91	4.01	4.43
T_{max} (h)	2	2	4	4
AUC (µg.h/ml)	33	46	38	36

From Inawaka (2005)
AUC, area under the curve of concentration–time.

a single dose, which produced overt maternal toxicity, was used in the study, and the fetal findings were considered to be of uncertain toxicological relevance. The NOAEL for developmental toxicity was 125 mg/kg bw per day, the highest dose tested. A NOAEL for maternal toxicity was not identified (Inawaka, 2003).

Primates

Groups of four pregnant cynomolgus monkeys (*Macaca fascicularis*) were given procymidone (purity, 99.0%) at a dose of 62.5 or 125 mg/kg bw per day by gavage in 0.5% methylcellulose during the critical period of differentiation of the fetal external genitalia (days 20–99 of gestation). Monkeys were observed twice per day during the dosing period and once per day thereafter. Body weight and food consumption were measured at intervals up to day 100 of gestation. Blood was taken for clinical chemistry and haematology on days 19 and 100 and 2, 4, 8 and 24 h after the first and last doses on days 20 and 99 for analysis of procymidone concentration. Fetal heartbeat and size were monitored by ultrasound at intervals during pregnancy. The fetuses and placentae were removed under anaesthesia by caesarean section on day 100 or 102 of gestation. The dams were then terminated and selected organs were removed, fetuses and placenta were weighed; the fetuses were then given an external examination followed by an internal examination of visceral organs and internal genitalia.

None of the monkeys died in either group and there were no signs of toxicity or treatment-related effects on body weight, food consumption, clinical chemistry or haematology. One dam in each group aborted. The major organs were unaffected. Blood concentrations of procymidone reached a maximum 2–4 h after dosing and C_{max} and AUC increased in a linear manner with dose on day 20 but were equal at day 99 (Table 25). There were two male and one female fetuses in the group at the lower dose and three males in the group at the higher dose. There were no effects on the weight of the fetus or placenta and no increase in adverse external or visceral findings including external genitalia of all five male fetuses treated with procymidone (Fukunishi, 2003a).

A more extensive examination of the fetuses was made in a second study in which groups of 16 pregnant cynomolgus monkeys (*Macaca fascicularis*) were given procymidone (purity, 99.0%) at a dose of 0 or 125 mg/kg bw per day by gavage in 0.5% methylcellulose during the critical period of differentiation of the fetal external genitalia (days 20–99 of gestation). Monkeys were observed twice per day for clinical signs of toxicity during the dosing period and once per day during the non-dosing period; body weight and food consumption were measured at intervals. Fetal heartbeat and size were monitored by ultrasound during the treatment period to confirm pregnancy status and fetal

Table 24. Findings in fetuses of female rabbits given procymidone by gavage

Finding[a]	Dose (mg/kg bw per day)			
	0		125	
	Males	Females	Males	Females
Pup body weight (g)	41.5 ± 6.5	39.8 ± 6.1	40.5 ± 8.2	40.8 ± 8.3
Anogenital distance (mm)	1.48 ± 0.18	1.34 ± 0.23	1.41 ± 0.16	1.35 ± 0.10
Phallus boundary-genital distance (μm)	434 ± 94	286 ± 91	368 ± 113	259 ± 76
Preputial lamella diameter (μm)	940 ± 58	836 ± 59	934 ± 60	833 ± 70
Ventral gap of preputial lamella (μm)	46 ± 40	421 ± 114	38 ± 19	498* ± 71
Ventral gap : preputial lamella ratio	0.05	0.50	0.04	0.60*
Relative ventral gap : body weight (μm/g)	1.1 ± 0.8	11 ± 3.4	1.0 ± 0.6	13 ± 3.5

From Inawaka (2003)

[a] Mean ± standard deviation.

* $p < 0.05$ relative to females in the control group.

Table 25. Blood plasma kinetic values in pregnant monkeys given procymidoneby gavage

Parameter	Dose (mg/kg bw per day)			
	62.5	125	62.5	125
Gestation day	20	20	99	99
C_{max} (µg/ml)	0.45	0.96	1.2	1.2
T_{max} (h)	3.3	2.7	3.3	3.3
AUC (µg.h/ml)	7.1	12.8	19.9	20.8

From Fukunishi (2003a)
AUC, area under the curve of concentration–time.

presence. Fetuses were delivered by caesarean section on days 100–102 of gestation, placentae were also removed. Each fetus was examined for viability and sexed. The placenta and fetus were weighed and measurements or observations were made of the crown–rump length, body form, symmetry of the head, facial form, mandibular formation, eyes and eyelids, hair on the head, nipple formation, anus, fingers, toes, finger and toe nails, ears, tail, upper and lower extremities, external genitalia, vertebral column, umbilical cord and palate. After the external examination each fetus was necropsied, a macroscopic assessment was made of the visceral organs and sex was determined from the internal genitalia.

No dams died or aborted in either group and there was no effect of treatment on clinical signs of toxicity, body weight, food consumption and necropsy. There were six male and ten female fetuses in the control groups, and eight male and eight female fetuses in the groups receiving procymidone. No test article-related effects were noted in external genitalia or in any of the other measurements or observations. The study claimed GLP compliance (Fukunishi, 2003b).

2.6 Special studies

(a) Neurotoxicity

No specific investigations of neurotoxic potential with procymidone had been performed. In studies of acute toxicity, there were some signs that might have been linked to a neurotoxic action at all doses (> 250 mg/kg bw per day), e.g. reduced motor activity, but these could also be related to general malaise after the administration of a bolus of corn oil. There was no indication from repeat-dose studies that procymidone has any neuropathic potential.

(b) Mechanism of action

(i) Binding to androgen receptors

Binding to androgen receptors in × 105 000 *g* prostate cytosols from castrated rats (Sprague-Dawley, age 10 weeks) and mice (ICR, age 14 weeks) was examined in a competitive assay using [^{3}H]dihydroxytestosterone (DHT; 135 Ci/mmol (4995 GBq/mmol). The affinity constants (Kd) for DHT were 0.23 nmol/l and 0.1 nmol/l in rats and mice respectively. The relative binding affinity—the ratio of the DHT concentration required to inhibit activity by 50% (IC_{50}) to the IC_{50} of the other compound—for procymidone was relatively low at 0.065% in rats and 0.07% in mice (DHT = 100%). Procymidone-3-Cl, a plant metabolite, was similar to parent compound with a relative affinity of 0.05%, while 2-(3,5-dichlorophenyl carbamoyl)-1,2 dimethylcyclopropane-1-carboxylic acid (carboxyprocymidone, PCM-NH-COOH) and cyclopropane dicarboxylate metabolites produced no inhibitory effects (Murakami et al., 1988b).

The anti-androgenic activities and androgen-receptor (AR) binding activities of procymidone (purity, 99.6%) and its metabolites PCM-CH_2OH (purity, 99.9%), PCM-COOH (purity, 96.4%), PA-CH_2OH (purity, 98.8%), carboxyprocymidone acid, PA-COOH (purity, 96.9%), PCM-NH-COOH (purity, 98.6%) and PCM-CH_2OH glucuronide were evaluated using androgen-receptor-mediated assays in vitro. Anti-androgenic activity was determined in HeLa cells transfected with an ARE×3/luciferase reporter gene and full length human and rat AR expression vectors (AR reporter assay). AR-binding activity was also determined by a competitive ligand-binding assay on the basis of the fluorescence polarization method using a rat AR kit. Concentrations used were up to 30 µmol/l for the AR reporter assay and up to 150 µmol/l for the assay for competitive binding. Acceptable performance of the three assay systems was demonstrated by conducting reporter gene assays with the known androgen antagonists, flutamide, hydroxyflutamide and the binding assay with DHT.

In all the assays, procymidone exhibited almost identical binding to flutamide, with metabolites of procymidone having varying but lower levels of activity (Table 26). It is possible that the non-enzymic interconversion of the metabolites might have influenced the results of this assay (Suzuki, 2005).

(ii) Serum hormone concentrations

Groups of 30 male Sprague-Dawley rats received diets containing procymidone (purity, 99.1%) at concentrations of 100 to 6000 ppm for durations ranging from 14 days to 6 months, with and without recovery periods. The schedule of doses and termination times are shown below.

Ten male rats per group were used and the anti-androgen cadmium chloride (at a subcutaneous dose of 5.5 mg/kg bw) was used to treat a positive-control group. Body weights were determined at the start of treatment and at termination. Serum testosterone, luteinizing hormone (LH) and, in the first experiment, estradiol concentrations were measured at each termination. Reproductive organs were weighed and examined by microscopic pathology. In the first experiment, body weight was depressed and testes weights were slightly increased in rats at 2000 or 6000 ppm. Epididymis weights were reduced at 2000 and 6000 ppm after 14 days and 1 month, but there was no statistically significant effect after 3 months. Testosterone concentrations were increased in a dose-related

Table 26. IC_{50} values (µmol/l) for binding of procymidone and its metabolites to androgen receptors

Compound	IC_{50} (µmol/l)		
	Androgen-receptor reporter assay		Competitive-binding assay
	Rat	Human	
Flutamide	0.34	0.23	11
Procymidone	0.31	0.29	12
PCM-CH_2OH	3.4	2.4	26
PA-CH_2OH	4.1	3.5	227
PCM-NH-COOH	1.4	1.2	100
PCM-COOH	No activity	No activity	No activity
PA-COOH	No activity	No activity	No activity
PCM-CH_2OH-glucuronide	No activity	No activity	No activity

From Suzuki (2005)
IC50, concentration required to inhibit activity by 50%; PA, procymidone acid; PA-CH_2OH, hydroxyprocymidone acid; PA-COOH, carboxyprocymidone acid; PCM, procymidone; PCM-CH_2OH, hydroxyprocymidone; PCM-NH-COOH, carboxyprocymidone.

Table 27. Dosing schedule and termination times in a study of serum hormone concentrations in rats given diets containing procymidone

Parameter	Experiment 1	Experiment 2	Experiment 3
Dietary concentrations (ppm)	700, 2000, 6000	100, 300, 700 and 2000	6000
Duration	Up to 3 months	Up to 6 months	One month
Termination times	Fourteen days, 1 month and 3 months after the start of treatment	One, 3 and 6 months after the start of treatment	One month after the start of treatment, then 2 weeks, 1, 3 and 6 months after treatment

From Murakami et al (1986)

and significant manner at 700 ppm and greater; LH concentrations were significantly increased at 6000 ppm. In the second experiment, serum testosterone concentrations were increased at 700, and 2000 ppm. Serum concentrations of testosterone and LH returned to normal after 1 month of the recovery period. Serum estradiol concentrations were close to the limit of detection in all groups. In the positive-control group, administration of cadmium chloride produced a reduction in testes, seminal vesicle, epididymes and prostate weights, an increase in LH concentration and a decrease in testosterone indicating a different mechanism of action from procymidone. The NOAEL for hormonal effects was 300 ppm, equivalent to 30 mg/kg bw per day. The report was checked by a quality assurance unit (Murakami et al, 1986).

Tests for testicular function

Rodents

Groups of 30 male ICR mice received diets containing procymidone (purity, > 99.3%) at a concentration of 0, 1000, 5000 or 10 000 ppm for up to 13 weeks. Groups of 12 male Sprague-Dawley rats received diets containing procymidone at a concentration of 0, 700, 2000 or 6000 ppm for 13 weeks. Routine examinations were performed during the study. Rodents were terminated after 2, 4 and 13 weeks of treatment. Body weights and food consumption were recorded weekly. At termination, reproductive organs were removed and weighed. Concentrations of testosterone were determined in serum and testes and LH was determined in serum and pituitary. The testicular response to stimulation by human chorionic gonadotrophin (hCG) and the binding of hCG to the LH/hCG receptor in testes were determined in vitro.

In mice, mean achieved intakes over 13 weeks were 133, 638 and 1338 mg/kg bw per day. There was no consistent effect on body weight, food consumption or organ weights. Testosterone concentrations were elevated in serum (165%) and testes (248%) after 2 weeks of treatment at 10 000 ppm and levels returned to normal at 4 and 13 weeks. LH concentrations in serum and pituitary were elevated for the first 4 weeks at 1000 ppm and greater; returning to normal thereafter. There was a slight increase in hCG-stimulated testosterone production at 10 000 ppm after 2 weeks but this returned to control levels after 3 months. The binding affinity of hCG decreased in mice at 5000 and/or 10 000 ppm during the 3-month treatment period.

In rats, mean achieved intakes over 13 weeks were 47, 132 and 388 mg/kg bw per day. There was no consistent effect on body weight or food consumption. Seminal vesicle weight was decreased and testis weight increased at 6000 ppm after 2 weeks. An increase in testis weight was also apparent at all doses (approximately 10%, but no dose–response relationship) after 4 weeks and at 6000 ppm after 13 weeks (10%). In rats at 6000 ppm, testosterone concentrations in serum were increased (155%) throughout the experimental period and increases were noted in testes after 4 weeks (220%) and 13 weeks (160%). LH concentrations were only elevated in serum for the first 4 weeks at 6000 ppm (300%). In the pituitary, LH concentrations were elevated at all doses after 4 weeks (138–180%)

but only at 6000 ppm after 13 weeks (131%). The assay in vitro showed that basal production of testosterone was increased at all termination times at 6000 ppm. The hCG-stimulated production of testosterone was elevated at all times in rats at 2000 and 6000 ppm and after 13 weeks in rats at 700 ppm. There were no treatment-related changes in the number of binding sites or the binding affinity of hCG to the LH/hCG receptor (Murakami et al., 1988a).

Primates

In a dose range-finding study, no effects on ejaculate mass, sperm counts, testosterone and LH concentrations or organ weights were seen in groups of two male cynomolgus monkeys (*Macaca fascicularis*) that received procymidone (purity, 98.7%) at doses up to 1000 mg/kg bw per day by gavage for seven consecutive days (Zühlke, 1991).

In the main study, groups of five male cynomolgus monkeys (*Macaca fascicularis*) received procymidone (purity, 98.7%) at a dose of 0, 30 100 or 300 mg/kg bw per day by gavage as a suspension in 1% sodium carboxymethyl cellulose for 13 weeks. Clinical signs and food consumption were recorded daily and body weights weekly. Sperm was taken for analysis and testicular dimensions recorded six times before dosing and in weeks 4, 8 and 13. Serum testosterone and LH concentrations were measured in samples taken before dosing and weekly during the treatment phase. The monkeys were necropsied at the end of the treatment period and the epididymides, prostate gland, seminal vesicles and testes were weighed and examined microscopically.

All monkeys survived the study with no signs of reaction to treatment. Body-weight variations were similar across the groups. There was no clear evidence of a treatment-related effect on food intakes, testicular size or LH concentrations. Histopathology of the reproductive organs did not detect any treatment-related change. There were indications of effects on ejaculate mass, sperm counts (Table 28), and serum testosterone concentrations (Figure 2) particularly during the first 4 weeks, and on testes weights (Table 28). There was no statistically significant change when compared with values for concurrent controls, other than relative testes weights, which did not exhibit any dose–response relationship. When values after dosing were compared with values before dosing for each individual, using a repeated measures ANOVA with post-hoc comparisons using the least significant differences (LSD) test, ejaculate mass was found to be statistically significantly reduced ($p < 0.05$) at 100 and 300 mg/kg bw per day. The small group size, absence of a clear dose–response relationship and large degree of inter- and intra-group variation before and after dosing made it difficult to reach firm conclusions on the findings of this study.

The NOAEL was 30 mg/kg bw per day on the basis of statistically significant effects on testes weights and ejaculate mass at 100 mg/kg bw per day. The study contained statements of compliance with GLP (Bee, 1992).

(iii) Fetal distribution

Groups of three pregnant rats (Crj:CD(SD)IGS), New Zealand White rabbits or cynomolgus monkeys were given [phenyl-^{14}C]procymidone (purity, > 97.5%; specific activity, 15.8 MBq/mg) at a dose of 125 mg/kg bw on day 17, 21 or 54 of gestation, respectively. Groups of rats also received doses on: (i) day 16; (ii) days 16 and 17; or (iii) days 16, 17 and 18 of gestation. Corn oil was the vehicle in the studies in rats, for rabbits and monkeys the vehicle was 0.5% methylcellulose. The rats and monkeys were killed 6 and 24 h after dosing, rabbits after 2 and 24 h. Samples of maternal blood, amniotic fluid and fetal blood were taken. The maternal liver and kidneys, the placenta, fetus, fetal brain, fetal heart, fetal lung, fetal liver and fetal kidneys were removed at necropsy. These samples were analysed for radioactivity and the amounts of procymidone and its metabolites were also determined.

Table 28. Ejaculate mass and testes weights in monkeys (n = 5) given procymidone by gavage for up to 13 weeks

Parameter[a]	Dose (mg/kg bw per day)			
	0	30	100	300
Ejaculate mass (g):				
Week –6	0.28 ± 0.27	0.39 ± 0.27	0.65 ± 0.60	0.59 ± 0.47
Week –1	0.69 ± 0.56	0.55 ± 0.22	0.46 ± 0.34	0.63 ± 0.23
Week +4	0.52 ± 0.43	0.41 ± 0.21	0.16 ± 0.19*	0.18 ± 0.18*
Week +8	0.70 ± 0.49	0.26 ± 0.21	0.27 ± 0.22	0.31 ± 0.17
Minimum before dosing	0.28 ± 0.27	0.24 ± 0.14	0.46 ± 0.34	0.41 ± 0.16
Sperm count (10^6):				
Week –6	167 ± 223	118 ± 139	372 ± 535	133 ± 58
Week –1	174 ± 207	202 ± 151	201 ± 251	349 ± 195
Week +4	133 ± 143	187 ± 83	80 ± 87	75 ± 81
Week +8	121 ± 118	158 ± 149	141 ± 119	180 ± 138
Minimum before dosing	66 ± 54	73 ± 54	76 ± 52	133 ± 58
Relative testes weights (%), week 13	0.60 ± 0.11	0.87 ± 0.11**	0.79 ± 0.21	0.85 ± 0.04**

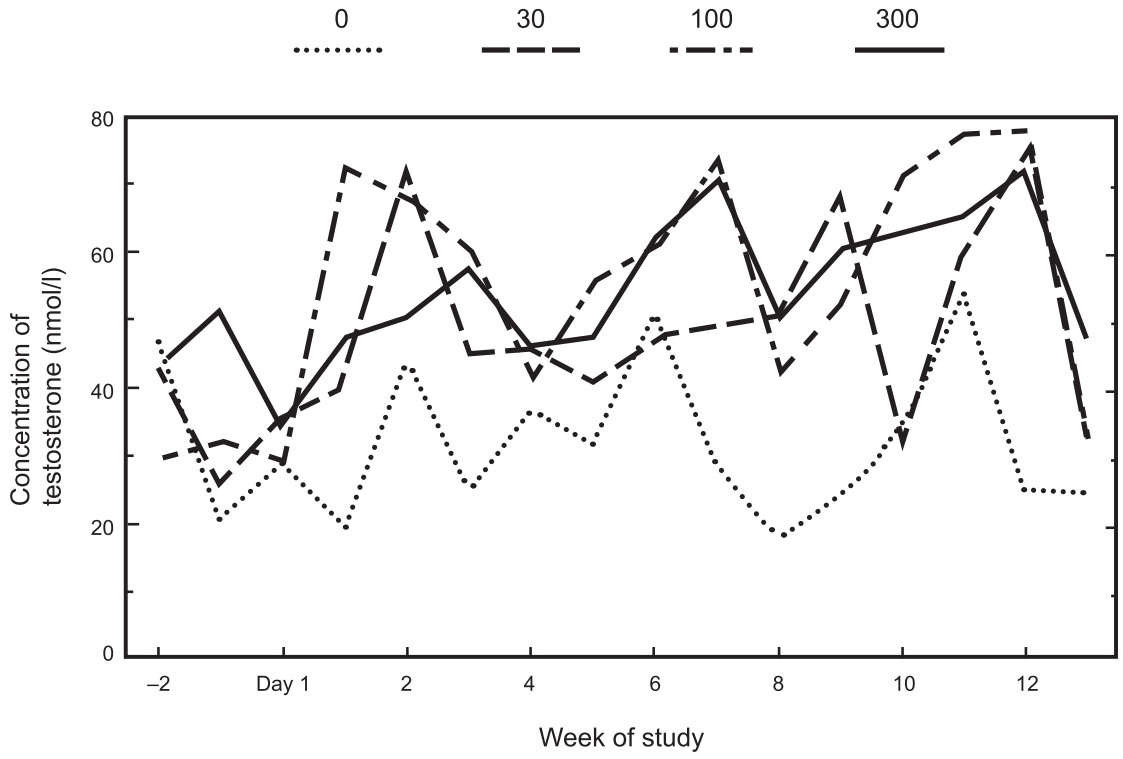
From Bee (1992)

[a] Mean ± standard deviation.

* $p < 0.05$ vs pre-test (ANOVA).

** $p < 0.01$ vs values for controls.

Figure 2. Serum testosterone concentrations in monkeys given procymidone by gavage for up to 13 weeks



From Bee (1992)

The results are summarized in Table 29. The metabolic profile in fetal-rat tissues is the same as that in the plasma of non-pregnant and pregnant rats (Sugimoto, 2005e, 2005f). In rats, procymidone was the major component in maternal plasma 6 h after the first dose, followed by PCM-NH-COOH (and/or PCM-COOH) and PA-CH_2OH but at all other sampling times, the main plasma metabolites were PCM-NH-COOH (and/or PCM-COOH), PA-CH_2OH and PA-COOH. Unchanged procymidone was also the main component in the placenta and fetus 6 h after the first dose followed by PCM-CH_2OH. At other sampling times, however, PCM-CH_2OH was the major component. PA-CH_2OH was also the main metabolite in amniotic fluid and its concentration increased with time. The transfer ratios of metabolites showed that PCM-CH_2OH accumulated more readily in the fetus than did procymidone (Tomigahara, 2005d).

In rabbits, the main metabolites present in the fetus were procymidone and PCM-CH_2OH. PCM-COOH and PA-COOH, plus glucuronides of PCM-CH_2OH and PA-CH_2OH, were hardly transferred to the fetus, although PA-COOH was the major component of maternal plasma (Sugimoto, 2005g).

In monkeys, procymidone was also the major component in the placenta and fetus, PCM-CH_2OH and/or PCM-COOH and PCM-NH-COOH were lesser metabolites. Other metabolites were only detected at trace levels in fetuses (Mogi, 2005e).

These results show that the transfer of procymidone and metabolites to the fetus is significantly greater (approximately 10-fold) in rats than in rabbits or monkeys. In rats, there was also a transfer of PCM-CH_2OH, the postulated developmental toxin, from the dam to the fetus.

Table 29. Pharmacokinetic parameters in plasma and tissues of pregnant rats, rabbits and monkeys given oral doses of [phenyl-^{14}C]procymidone at 125 mg/kg bw

Parameter	Rats				Rabbits		Monkeys	
	Single dose		Three doses		Single dose		Single dose	
	6 h	24 h	6 h	24 h	2 h	24 h	6 h	24 h
Dams								
Concentration of ^{14}C (µg equivalent/g):								
Plasma	18	28	35	30	40	2.3	5.5	3.7
Liver	53	56	—	—	43	3.8	33	28
Kidney	32	59	—	—	143	8.7	30	21
Whole fetus								
Concentration of ^{14}C (µg equivalent/g):								
Single dose	10	16	7.4	10	2.5	0.36	1.2	1.2
Three doses	—	—	21	15	—	—	—	—
Ratio of concentrations of ^{14}C in whole fetus and in maternal plasma								
PCM	2.7	1.1	0.9	0.6	NM	NM	0.5	0.8
PCM-CH_2OH	16	10	48	22	2.1	NM	0.2	0.2
PCM-NH-COOH	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	0.4	0.2	0.2
PA-CH_2OH	0.8	0.8	0.2	0.5	< 0.1	NF	< 0.1	<0.1
PA-COOH	—	—	0.2	0.1	NF	< 0.1	< 0.1	<0.1
Glucuronides	—	—	0.4	0.5	< 0.1	< 0.1	<0.1	<0.1

From Tomigahara (2005d), Sugimoto (2005g) and Mogi (2005e)

NF, not in fetus; NM, not in maternal plasma but present in fetus; PA, procymidone acid; PA-CH_2OH, hydroxyprocymidone acid; PA-COOH, carboxyprocymidone acid; PCM, procymidone; PCM-CH_2OH, hydroxyprocymidone; PCM-NH-COOH, carboxyprocymidone.

3. Studies on metabolites

(a) 3,5-Dichloroaniline (DCA)

3,5-Dichloroaniline (DCA) in corn oil vehicle was of moderate acute toxicity to *dd* mice, having an oral median lethal dose (LD_{50}) of approximately 850 mg/kg bw and a subcutaneous LD_{50} of approximately 1270 mg/kg bw (Kohda et al., 1980).

(b) Carboxyprocymidone (PCM-NH-COOH)

PCM-NH-COOH (2-(3,5-dichlorophenyl carbamoyl)-1,2 dimethylcyclopropane-1-carboxylic acid) in corn oil, was of moderate acute toxicity to *dd* mice, having an oral LD_{50} of approximately 1450 mg/kg bw (Kohda et al., 1980).

(c) 1,2-Dimethylcyclopropane-dicarboxylic acid (DMCPA)

1,2-Dimethylcyclopropane-dicarboxylic acid (DMCPA), in Tween 80, was of low acute toxicity to *dd* mice, having an oral LD_{50} of approximately 4400 mg/kg bw and a subcutaneous LD_{50} of approximately 2400 mg/kg bw (Kohda et al., 1980). DMCPA (purity, 98.7%) gave negative results in an assay for gene mutation in *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98, TA100) and *E. coli* (WP2*uvr*A), in the presence and absence of metabolic activation, at concentrations of up to 5000 µg/plate (Kogiso et al., 1992). DMCPA (purity, 98.7%) gave negative results in an assay for micronucleus formation in ICR mice given doses of up to 320 mg/kg bw intraperitoneally. The study included three harvest times and used five male and five female mice per group (Murli,1992a, 1992b).

The absorption, distribution and metabolism of DMCPA was investigated in groups of five male and five female Sprague-Dawley rats. [Carboxy-^{14}C]DMCPA was administered orally at a dose of 0.56 mg/kg bw in corn oil to groups of five male and five female rats. Rats were killed 7 days after dosing. Excretion was rapid, with 87% (males) and 84% (females) of the administered dose being excreted within the first 24 h, and equally distributed between the urine and faeces. Retention of radioactivity in tissues was low, with concentrations of less than 2 ng equivalent/g at 7 days; the only tissue with quantifiable levels was bone. The only component identified in excreta corresponded to non-metabolized DMCPA accounting for 77% (male) and 74% (female) of the administered dose at 24 h (Saito, 1992).

(d) Hydroxyprocymidone (PCM-CH_2OH)

Two groups of 12 female Sprague-Dawley rats were given PCM-CH_2OH (purity, 100%) orally at dose of 62.5 or 125 mg/kg bw per day in corn oil by gavage on days 6–19 of gestation. The rats were allowed to deliver and wean the pups. Clinical signs were observed twice per day for dams during the treatment period and once daily for the remainder of the study. Offspring were observed once daily. Body weights of dams and offspring were recorded regularly. Live newborns were weighed, sexed and examined for external abnormalities and the anogenital distance was measured. At the time of weaning, on postnatal day 21, all dams and all female offspring were necropsied. All male offspring were necropsied on day 56 after birth.

Decreases in locomotor activity, and increases in bradypnoea and/or prone position were observed in the dams in both groups receiving PCM-CH_2OH from the first day of dosing. Body weights of dams and offspring were similar in all groups. No abnormal findings were seen in dams or female offspring. In the male offspring, a dose-related shortening of the anogenital distance was seen (3.35, 2.67 and 2.35 mm). Increases in the incidence of male offspring with abnormities of the external genitalia were observed in both groups receiving PCM-CH_2OH. Hypospadias were observed in 19 out of 86 males (8 out of 12 litters) in the group at 62.5 mg/kg bw per day and 75 out of 84

males (all litters) in the group at 125 mg/kg bw per day. In addition, small testes and epididymis and undescended testes and epididymis were observed occasionally in both groups. These results were similar to those seen with procymidone (Inawaka, 2005). A benchmark-dose (BMD) assessment of the incidence of hypospadia (Table 30) was performed by the Meeting, using a nested logistic model on incidences in individual litters. The 95% lower limit for a 10% response ($BMDL_{10}$) for hypospadias was 43.8 mg/kg bw per day for PCM-CH_2OH and 23.7 mg/kg bw per day for procymidone (Izumi, 2005).

(e) Interconversion of metabolites

The interconversion of metabolites of procymidone was investigated in female rats in vivo and and in vitro. After the subcutaneous administration of [phenyl-^{14}C]PA-CH_2OH at a dose of 62.5 mg/kg bw to a group of three Sprague-Dawley rats, imide-bond cleaved and uncleaved metabolites were found in tissues and plasma (Table 31) (Tomigahara, 2005a), indicating reversal of the cleavage reaction that produced PA-CH_2OH from procymidone (see Figure 1). In an identical study but with [phenyl-^{14}C]PCM-CH_2OH, both cleaved and uncleaved metabolites were seen in plasma and tissues (Table 31) (Tomigahara, 2005b). A comparative study with oral and subcutaneous administration showed that a subcutaneous dose of 125 mg/kg bw was equivalent, in terms of metabolite profile, C_{max} and AUC, to an oral dose of 62.5 mg/kg bw (Tomigahara, 2005c). The variations in the relative levels of metabolites between tissues (pH approximately 6.8) and plasma (pH approximately 7.4) were investigated in vitro. [phenyl-^{14}C]Procymidone and [phenyl-^{14}C]PCM-CH_2OH were incubated at pH 2.0, 4.0, 6.8, 7.4, 8.0 or 11.0 at room temperature for 16 h. The reaction mixtures were analysed by TLC and the ratio of procymidone to PCM-NH-COOH and of PCM-CH_2OH to PA-CH_2OH were calculated. Under acid conditions (pH < 6.8), procymidone and PCM-CH_2OH were stable, but under alkaline conditions (pH > 7.4) the imide bond was cleaved to give PCM-NH-COOH and PA-CH_2OH (Tarui, 2005a). These studies indicated that procymidone metabolites have the potential to interconvert non-enzymatically when transferred from plasma to tissues and vice versa.

4. Observations in humans

Annual medical records for workers involved with the packaging of procymidone were examined for 1979–1983. A total of 20 workers was included in the survey. Of these, 16 had been engaged in packing procymidone for more than 9 h per year for an average of 4 years. None of the workers had any symptoms that could be associated with exposure to procymidone (Harada, 1983).

A further review was completed in 2001. The medical records and operational details of 15 workers who had been engaged in the manufacture and packing of technical-grade procymidone into drums were examined. Operational details included number of hours spent packing per year, number of years involved in packing, age and use of personal protective equipment. Annual medical

Table 30. Incidence of hypospadia in pups from studies of developmental toxicity with procymidone and PCM-CH_2OH

Dose (mg/kg bw per day)	Day 22 (observation)		Day 56 (gross examination)	
	Procymidone	PCM-CH_2OH	Procymidone	PCM-CH_2OH
0	0/119	0/67	0/119	0/67
37.5	2/122	Not dosed	19/122	Not dosed
62.5	13/135	3/86	59/134	16/86
125	Not dosed	26/86	Not dosed	71/84

From Izumi (2005) and Inawaka (2005)
PCM-CH_2OH, hydroxyprocymidone.

Table 31. Metabolite concentrations (µg equivalent/g) in plasma and tissues from female rats (n = 3) receiving procymidone metabolites at 62.5 mg/kg bw

Metabolite	Concentration of metabolite (µg equivalent/g)			
	Plasma	Liver	Kidney	Ovary
Six h after dosing with PCM-CH_2OH				
PCM-CH_2OH	2.9	33.8	20.9	16.8
PCM-COOH	7.3	3.0	15.1	1.8
PA-CH_2OH	12.9	6.0	6.2	3.1
PA-COOH	2.3	1.4	2.8	0.5
Others	3.1	1.4	2.6	0.6
Four h after dosing with PCM-CH_2OH				
PCM-CH_2OH	0.6	16.4	12.7	10.6
PCM-COOH	2.7	5.1	10.4	2.0
PA-CH_2OH	12.8	2.0	5.4	0.5
PA-COOH	3.0	2.9	3.8	0.7
Others	4.2	0.6	1.3	0.3

From Tarui (2005a)
PA, procymidone acid; PA-CH_2OH, hydroxyprocymidone acid; PA-COOH, carboxyprocymidone acid; PCM, procymidone; PCM-CH_2OH, hydroxyprocymidone; PCM-NH-COOH, carboxyprocymidone.

examination records were checked for 1991–2000 for adverse effects on body weight, vital capacity, hearing acuity, chest X-ray, blood pressure, urine analysis, haematology and clinical biochemistry. An annual questionnaire that was completed at the time of the medical examination was also examined. This recorded headaches, dizziness, irritability, restlessness, fatigue, loss of appetite, full stomach, diarrhoea, cough, dyspnoea, hearing problems, tinnitus, abnormal taste, gastroenteric problems or any other abnormalities.

The number of hours spent packing ranged from 10 to 83 per year. Four of the workers had been engaged in packing for 10 years or more, four for between 5 and 9 years and seven for less than 4 years. Five of the workers were aged at least 50 years, two were aged between 40 and 49, five were aged between 30 and 39 and three were aged less than 29 years. All workers wore protective equipment consisting of long-sleeved cotton clothes, plastic helmets, masks, glasses and gloves. No abnormalities were apparent from the medical records that could be related to the manufacture or handling of technical-grade procymidone (Kurihara, 2001). A similar study in 2007 confirmed that there were no adverse health effects in the monitored workforce (Kumagai, 2007).

The sponsor reported there were no documented cases of intoxication with procymidone and no information on effects of exposure of the general population to procymidone.

Comments

Biochemical aspects

Studies with ^{14}C-labelled procymidone showed that radiolabel was rapidly absorbed (C_{max}, 2–8 h) and rapidly eliminated (> 80% in 24 h) in mice and rats. Absorption was extensive, as shown by the high level of urinary excretion (> 80% of the administered dose), with similar results obtained in mice and rats receiving doses of up to 100 mg/kg bw. At higher doses, the proportion of the administered dose excreted in the faeces increased: at 250 mg/kg bw, 24–33% was eliminated by that route after 168 h; the increase in faecal radioactivity was mainly attributable to an increase in unabsorbed

procymidone (18–27% of the administered dose). Only low concentrations (< 0.3%) of radioactivity were retained 168 h after oral administration; the highest concentrations were found in fat. The major metabolic pathway for procymidone in mice and rats involved the oxidation of the methyl groups to hydroxymethyl or carboxylic acid derivatives, cleavage of the imide, and glucuronide formation of the resultant metabolites. In cynomolgus monkeys, rabbits and chimeric mice with humanized livers (i.e. liver repopulated with human hepatocytes), there was more extensive urinary excretion of glucuronide conjugates than in rats or normal mice. In rats, the glucuronides were formed, but were present in the bile and appear to be deconjugated and the aglycone was reabsorbed giving a prolonged elimination phase and relatively high AUC. Studies in vitro with liver preparations from humans, rabbits, cynomolgus monkeys and rats showed that the rate of metabolism of procymidone and PCM-CH_2OH was significantly lower in rats than in the other species studied.

In female cynomolgus monkeys, there was an increase in C_{max} and AUC values after repeated doses. In other species, the routes and rates of tissue distribution, biotransformation and excretion of procymidone were similar in males and females and after single or repeated administration.

Procymidone has a high binding affinity in vitro (92–98% bound) for plasma proteins when incubated with plasma from female rats, cynomolgus monkeys, rabbits and humans. The alcohol metabolite PCM-CH_2OH had a slightly lower binding affinity (77–91% in all species) than procymidone, with the highest affinity being for human plasma proteins.

Kinetic studies in rats, rabbits and cynomolgus monkeys (dams and fetuses of each species) have been performed as part of investigations of the effects on male rat reproductive tissues. After a single dose, cynomolgus monkeys had much lower C_{max} values for total radioactivity than did rabbits and rats, but at doses of up to 125 mg/kg bw rabbits had relatively low AUC values owing to rapid elimination. In cynomolgus monkeys, the predominant compound in plasma was procymidone; in rabbits, acid metabolites and glucuronides of alcohol metabolites were the major components in plasma; in rats, free alcohol metabolites predominated. After 14 doses at 37.5–500 mg/kg bw per day, C_{max} and AUC values for total radioactivity were similar for rats and cynomolgus monkeys. These findings show that the doses used in the studies of developmental toxicity in cynomolgus monkeys would have produced similar plasma concentrations of total radioactivity, but with different metabolite profiles, to those in rats at doses resulting in hypospadias. The investigations of the transfer of procymidone and metabolites to fetuses again showed species differences. After dosing at 125 mg/kg bw per day, the concentrations of procymidone and metabolites in the fetus were significantly greater (10-fold or greater) in rats than in rabbits or cynomolgus monkeys. In rats, the relative concentrations of PCM-CH_2OH in the fetus versus the dam were much higher than in rabbits or cynomolgus monkeys.

Toxicological data

Procymidone was of low acute toxicity by the oral (LD_{50} > 5000 mg/kg bw) and dermal (LD_{50} > 2500 mg/kg bw) routes, and after a 4-h exposure by inhalation (LC_{50} > 1.5 mg/l). Procymidone is not an eye irritant, but is a slight, transient skin irritant. Procymidone did not produce delayed contact hypersensitivity in guinea-pigs in either the maximization or repeat-injection tests.

The primary target organs in rats and mice exposed to procymidone in repeat-dose studies were the liver and testes. The major effect of short-term dietary administration of procymidone in ICR mice was on the liver. Centrilobular hepatocyte hypertrophy was noted in male mice and hepatocyte granuloma in females that received procymidone at 500 ppm, equal to 71 mg/kg bw per day, for 13 weeks; the NOAEL was 150 ppm, equal to 22 mg/kg bw per day. In a subsequent study with $B6C3F_1$ mice, multifocal coagulative necrosis of hepatic parenchyma, centrilobular cytoplasmic swelling, nuclear enlargement, coarsely-dispersed chromatin and multinucleate hepatocytes were noted in mice receiving procymidone at 10 000 ppm, equivalent to 1430 mg/kg bw per day. This was accompanied by increased liver weight and serum ALT activity in males and higher cholesterol concentrations in

females. The histopathological effects were apparent to a lesser extent in animals treated with procymidone at 500 or 2500 ppm, equivalent to 71 and 355 mg/kg bw per day respectively. The NOAEL was 100 ppm, equal to 19.6 mg/kg bw per day. An increase in the incidence of testicular atrophy was noted in mice receiving procymidone at 500 ppm for 26 weeks, the NOAEL was 150 ppm, equal to 20 mg/kg bw per day. In an additional 6-month study, the NOAEL for effects on the testes was 300 ppm, equal to 37 mg/kg bw per day. The Meeting considered that the overall NOAEL in three short-term studies in mice was 300 ppm, equal to 37 mg/kg bw per day.

The only short-term study in rats included a 6-month exposure period with or without a 3-month recovery phase, and a 9-month exposure period. In Sprague-Dawley rats, there was a significant reduction in body-weight gain among females receiving procymidone at 1500 ppm, equivalent to 75 mg/kg bw per day, for 6 months (although this was not significant after an exposure of 9 months) and an increase in liver-to-body-weight ratio in both sexes. The liver-to-body-weight ratio was also increased in female rats at 500 ppm, equivalent to 25 mg/kg bw per day, and there was an increase in spleen-to-body-weight ratio at 500 and 1500 ppm. Absolute and relative weights of the testes were increased after 9 months at 1500 ppm. The only treatment-related histopathological effect was swelling of the liver cells in male rats at 1500 ppm. The findings showed clear evidence of reversal over the 3-month recovery phase. The NOAEL was 500 ppm, equivalent to 25 mg/kg bw per day.

In a 28-day study of dermal exposure, there were no treatment-related local or systemic changes when doses of up to 1000 mg/kg bw per day were applied to the shaved backs of rats.

Emesis and diarrhoea were the principal signs of toxicity in dogs given procymidone at 500 mg/kg bw per day for 26 weeks. At that dose, there was also an increase in serum ALP activity, BUN and calcium concentrations. The NOAEL was 100 mg/kg bw per day. In a 52-week study in dogs, there was an increase in emesis and soft faeces and increases in serum globulin and ALP activity at 500 mg/kg bw per day. The NOAEL was 100 mg/kg bw per day.

Procymidone gave negative results in an adequate range of assays for genotoxicity in vitro and in vivo. The Meeting concluded that procymidone was unlikely to be genotoxic.

The toxicity and carcinogenicity of procymidone had been investigated in long-term studies in mice and rats. In the study in mice, treatment-related effects were limited to the liver. Higher liver weights and liver-to-body-weight ratios were apparent in males at 100 ppm, equal to 15 mg/kg bw per day, and in both sexes at 300 and 1000 ppm, equal to 46 and 153 mg/kg bw per day respectively, and there were histopathological changes in the liver, comprising increased incidences of centrilobular cytomegaly in males at 1000 and 300 ppm and in females at 1000 ppm. In addition, focal or multifocal hepatocellular hyperplasia, and eosinophilic foci were noted in females at 1000 ppm. There was an increased incidence of hepatocellular adenomas in females at the highest dose. There was also an increase in the incidence of hepatoblastomas in males receiving procymidone at 1000 ppm. The NOAEL for toxicity was 100 ppm, equal to 15 mg/kg bw per day, on the basis of a range of liver effects at 300 ppm. The NOAEL for carcinogenicity was 300 ppm, equal to 46 mg/kg bw per day, on the basis of increases in liver tumours in males and females at 1000 ppm.

In the long-term study in rats, the liver was a target organ and reproductive organs were also affected. There was a reduction in body-weight gain among rats given procymidone at 1000 and 2000 ppm , equal to 48 and 97 mg/kg bw per day respectively. Effects on organ weights were noted at both these doses and consisted of increased relative and absolute weight of the liver in both sexes and increased weights of the testes and ovaries at dietary concentrations of 1000 ppm and greater. Histopathology revealed an increased incidence of centrilobular cytomegaly in the liver of both sexes at dietary concentrations of 1000 ppm and greater. At 1000 and 2000 ppm, there were increases in the incidence of interstitial cell tumours and interstitial cell hyperplasia in the testes; the incidence of ovarian stromal hyperplasia was statistically significantly increased at 2000 ppm. The NOAEL for general toxicity and for carcinogenicity was 300 ppm, equal to 14 mg/kg bw per day.

Procymidone induced liver tumours in mice and testicular tumours in rats. No specific mechanistic studies have been performed to investigate the liver tumours; however, hepatocellular hypertrophy was present in shorter-term studies, and mice are sensitive to the production of liver tumours in response to such effects produced by high concentrations of xenobiotics. Procymidone gave negative results in assays for genotoxicity in vitro and in vivo. A clear threshold for induction of liver tumours was identified in the study of carcinogenicity in mice. Investigative work on the endocrine effects of procymidone indicated that it binds to the androgen receptor with a similar potency to that of the prostate-cancer drug flutamide, and that in rats the mechanism of hormonal action appeared to be via binding to the androgen receptor, disrupting the feedback controls on LH and testosterone concentrations. Overall, the data suggest that procymidone may induce testicular interstitial cell tumours via an endocrine-mediated mechanism. Although this mode of action is relevant to humans, there is good evidence to suggest that humans are less sensitive to chemically-induced interstitial cell tumours of the testis than rats, owing to differences in sensitivity to LH on the basis of Leydig-cell receptor number and control of LH-receptor expression (e.g. via prolactin in rodents but not in humans).

The Meeting concluded that procymidone was unlikely to present a carcinogenic risk to humans at typical levels of dietary exposure.

The reproductive toxicity of procymidone had been investigated in two studies in rats. The developmental toxicity of procymidone had been studied in conventional studies in rats and rabbits and in special investigative studies in rats, rabbits and cynomolgus monkeys.

In the first generation of the two-generation study in rats, there was no effect on mating and reproduction, but at 750 ppm, equivalent to 50 mg/kg bw per day, there was an increase in the number of male pups with hypospadias and reduced anogenital distance. In the second generation, male fertility was reduced and the incidence of hypospadias was increased at 750 ppm. In parents and pups, there were increases in testes weights and decreases in body-weight gain, prostate and epididymis weights at 750 ppm, with the organ-weight changes also present in pups at 250 ppm. There were no adverse effects on female reproductive performance or on female offspring. The NOAELs for effects on parents and on reproduction were 250 ppm, equivalent to 17 mg/kg bw per day. The NOAEL for pup development was 50 ppm, equivalent to 3 mg/kg bw per day, on the basis of the alterations in weights of the testes, prostate and epididymis at 250 ppm. In a subsequent one-generation study, designed to investigate the effects on male pups in the previous study, there was a reduction in body-weight gain and reduced litter size at 37 mg/kg bw per day. Increases in the incidence of hypospadias and in testes weights, and decreases in weights of the prostate and seminal vesicles were seen in pups at 37 mg/kg bw per day. The NOAELs for parental toxicity, pup development and reproduction were 12.5 mg/kg bw per day.

Studies of developmental toxicity with procymidone have been performed in rats, rabbits and cynomolgus monkeys. In a conventional study of developmental toxicity in rats, there were significant (60%) reductions in maternal body-weight gain during the first 6 days of dosing at 300 mg/kg bw per day, but no adverse findings in pups. This study did not include specific investigations of anogenital distance or the external genitalia. The NOAEL for fetotoxicity and teratogenicity was 300 mg/kg bw per day, the highest dose tested. The NOAEL for maternal effects was 100 mg/kg bw per day. In a modified study of developmental toxicity, pregnant rats were dosed with procymidone on days 6–19 of gestation; on day 20, half the dams had caesarian sections and the other half were allowed to deliver normally. Examinations focused on the male reproductive tract. Maternal toxicity (body-weight loss and poor appearance) was evident at doses of 125 mg/kg bw per day and greater. A range of effects was seen on male fetuses and offspring from dams receiving doses of 125 mg/kg bw per day and greater: reduced anogenital distance, undescended testes, hypospadias, testicular atrophy, distended preputial gland, inflammatory changes in the accessory sex organs (seminal vesicles, prostate and coagulating glands), and lower organ weights of testes and prostate. At 500 mg/kg bw per day, there

was a significant increase in the incidence of bifid thoracic vertebral centra in fetuses and prostate lesions in male offspring. The NOAEL for maternal and developmental toxicity was 12.5 mg/kg bw per day. A further investigative study of developmental toxicity, in which dams were allowed to deliver normally, confirmed the production of hypospadias and undescended testes at 37 mg/kg bw per day, the lowest dose tested.

In rabbits, an initial study of developmental toxicity found no evidence of maternal toxicity or teratogenicity, but there was a reduction in sternebrae ossification at 1000 mg/kg bw per day, the highest dose tested. The NOAEL for developmental toxicity was 750 mg/kg bw per day. In a subsequent study that concentrated on the external genitalia of fetuses, maternal toxicity (anorexia and abortions) was seen at 125 mg/kg bw per day, the only dose used. There were equivocal effects on measurements of the external genitalia in female offspring. Only a single dose, which produced overt maternal toxicity, was used in the study, and the findings are considered to be of uncertain toxicological relevance.

In an initial study focusing on effects on male fetuses, groups of four pregnant cynomolgus monkeys received doses of 62.5 or 125 mg/kg bw per day. There were no indications of maternal toxicity or fetotoxicity. In a more extensive investigation in groups of 16 pregnant cynomolgus monkeys, there was no maternal toxicity or effects on the external genitalia of offspring (six and eight male offspring in controls and in the test group, respectively) at 125 mg/kg bw per day, the only dose tested.

The characteristics of procymidone were similar in assays for binding to androgen receptors in rats and humans. The concentration required to inhibit activity by 50% (IC_{50}) values for procymidone (approximately 0.3 µmol/l) were similar to those of the anti-androgen prostate-cancer drug flutamide. The IC_{50} values for the procymidone metabolites PCM-CH_2OH, PA-CH_2OH and PCM-NH-COOH were approximately 5–10-fold that of procymidone; PCM-CH_2OH-glucuronide, PCM-COOH and PA-COOH showed no activity. However, these results might have been influenced by the non-enzymatic interconversion of procymidone metabolites. Under acid conditions (pH < 6.4), procymidone and PCM-CH_2OH are stable, but under alkaline conditions (pH ≥ 7.8) the imide bond was shown to be cleaved to give PCM-NH-COOH and PA-CH_2OH.

The mechanism of the effect of procymidone on the reproductive organs has been investigated by measuring hormone concentrations in mice, rats and cynomolgus monkeys. Procymidone had no adverse effects on testes, sperm and serum or tissue levels of LH in cynomolgus monkeys receiving procymidone at doses of up to 1000 mg/kg bw per day for 7 days or up to 100 mg/kg bw per day for 91 days. There was evidence of a reduction in ejaculate mass in cynomolgus monkeys at a dose of 100 mg/kg bw per day or greater, but there was no statistically significant effect on ejaculate sperm counts. The small group size and extent of variation between animals and in values recorded before dosing makes it difficult to reach firm conclusions about the toxicological significance of these results. The NOAEL in this study was considered to be 30 mg/kg bw per day. Two studies in rats provided qualitatively similar results, but although the protocols were similar and the same strain was used, there were differences in the doses that produced effects. There were increases in hCG-stimulated production of testosterone and circulating testosterone concentrations in rats receiving diets containing procymidone at 700 ppm, equal to 47 mg/kg bw per day, for 13 weeks. LH concentrations were increased at 6000 ppm after 2 weeks. Epididymis weights were reduced after exposure to procymidone at a dietary concentration of 2000 ppm or greater for 2 weeks, but not after 3 months. Testes weights were increased at dietary concentrations of 700 ppm and greater. All effects showed signs of reversal during a recovery phase. Findings in mice were similar to those in rats, but the stimulation of testosterone production by hCG had returned to control levels after 3 months owing to a decrease in the binding affinity of hCG to the LH/hCG receptor.

The Meeting concluded that the existing database on procymidone was adequate to characterize the potential hazards to fetuses, infants and children

Procymidone has not been studied specifically for neurotoxicity, but there were no indications from the results of conventional studies that it has significant neurotoxic potential.

A number of studies have been performed with procymidone metabolites. DCA was of moderate acute toxicity in *dd* mice, having an oral LD_{50} of approximately 850 mg/kg bw. PCM-NH-COOH was also of moderate acute toxicity in *dd* mice, with an oral LD_{50} of approximately 1450 mg/kg bw. DMCPA was of low acute toxicity in *dd* mice, having an oral LD_{50} of approximately 4400 mg/kg bw and a subcutaneous LD_{50} of approximately 2400 mg/kg bw. DMCPA gave negative results in an Ames test and an assay for micronucleus formation in vivo. DMCPA was rapidly absorbed and excreted with minimal metabolism. Retention of radioactivity in tissues was low.

PCM-CH_2OH was investigated in a modified study of developmental toxicity in which rats were allowed to deliver normally. No abnormal findings were seen in dams or female offspring. In the male offspring, a dose-related shortening of the anogenital distance was seen and there were increases in the incidence of male offspring and litter with abnormities of the external genitalia. The incidence of hypospadias was increased in pups from dams receiving procymidone at 62.5 mg/kg bw per day, the lowest dose tested. In addition, small testis and epididymis and undescended testis and epididymis were observed occasionally in groups treated with PCM-CH_2OH. These results are similar to those seen with procymidone at similar doses. The $BMDL_{10}$ for hypospadias was 43.8 mg/kg bw per day for PCM-CH_2OH and 23.7 mg/kg bw per day for procymidone. The Meeting considered the data on the relative androgen receptor and plasma-protein binding affinities of hyroxy-procymidone and procymidone, and the marked species differences in formation and elimination of procymidone and its metabolites. The Meeting concluded that while PCM-CH_2OH might be a significant contributor to the effects seen in rats, due to the much higher systemic and fetal exposure, the contribution of procymidone could not be discounted in other species, particularly due to limitations in the studies in cynomolgus monkeys.

Surveys of the medical records of production-plant workers had not identified any symptoms or diseases related to the manufacture of procymidone. There were no documented cases of procymidone intoxication nor any significant effects associated with its use.

Toxicological evaluation

The Meeting established an ADI of 0–0.1 mg/kg bw based on a NOAEL of 12.5 mg/kg bw per day in a two-generation study of reproductive toxicity and a study of developmental toxicity in rats, on the basis of hypospadias and alterations in testes, prostate and epididymis weights, and a safety factor of 100. The ADI was supported by NOAELs of 14 mg/kg bw per day in the 2-year study in rats and 15 mg/kg bw per day in the 2-year study in mice.

The Meeting established an ARfD of 0.1 mg/kg bw based on a NOAEL of 12.5 mg/kg bw on the basis of hypospadias, which might have been a consequence of a single exposure, in a study of developmental toxicity in rats, and using a safety factor of 100. The Meeting concluded that the effects on organ weights observed in the multigeneration study were largely a consequence of postnatal exposure over a period of time and therefore not appropriate for the establishment of the ARfD. The Meeting considered that, on the basis of the observed differences between species in terms of kinetics, metabolism and toxicological sensitivity to procymidone, this ARfD might be conservative. However, uncertainties regarding potential responses in species other than rats were such that it was not possible to modify the default safety factor.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	Two-year studies of toxicity and carcinogenicity[a]	Toxicity	100 ppm, equal to 15 mg/kg bw per day	300 ppm, equal to 46 mg/kg bw per day
		Carcinogenicity	300 ppm, equal to 46 mg/kg bw per day	1000 ppm, equal to 153 mg/kg bw per day
Rat	Two-year studies of toxicity and carcinogenicity [a]	Toxicity	300 ppm, equal to 14 mg/kg bw per day	1000 ppm, equal to 48 mg/kg bw per day
		Carcinogenicity	300 ppm, equal to 14 mg/kg bw per day	1000 ppm, equal to 48 mg/kg bw per day
	Multigeneration study of reproductive toxicity[ae]	Parental toxicity	250 ppm, equivalent to 17 mg/kg bw per day	750 ppm, equivalent to 50 mg/kg bw per day
		Offspring toxicity	12.5 mg/kg bw per day	250 ppm, equivalent to 17 mg/kg bw per day
	Developmental toxicity[b,e]	Maternal toxicity	100 mg/kg bw per day	125 mg/kg bw per day
		Embryo/fetotoxicity	12.5 mg/kg bw per day	37.5 mg/kg bw per day
Rabbit	Developmental toxicity[b,e]	Maternal toxicity	—	125 mg/kg bw per day[f]
		Embryo/fetotoxicity	750 mg/kg bw per day	1000 mg/kg bw per day
Monkey	Developmental toxicity[b,e]	Maternal toxicity	125 mg/kg bw per day[c]	—
		Embryo/fetotoxicity	125 mg/kg bw per day[c]	—
Dog	Six-month and 1-year studies of toxicity[d]	Emesis, soft faeces, increased alkaline phosphatase activity	100 mg/kg bw per day	500 mg/kg bw per day

[a] Dietary administration.
[b] Gavage administration.
[c] Highest dose tested.
[d] Capsule administration.
[e] More than one study combined.
[f] Lowest dose tested.

Estimate of acceptable daily intake for humans

0–0.1 mg/kg bw

Estimate of acute reference dose

0.1 mg/kg bw

Information that would be useful for the continued evaluation of the compound

Results from epidemiological, occupational health and other such observational studies of human exposure

Critical end-points for setting guidance values for exposure to procymidone

Absorption, distribution, excretion and metabolism in mammals	
Rate and extent of oral absorption	Rapid, C_{max} 2–8 h; extensive, > 80% excreted in the urine
Dermal absorption	4% concentrate, 13% for dilution in rats in vivo
Distribution	Extensive, highest concentrations in fat
Potential for accumulation	Low
Rate and extent of excretion	Rapid, > 80% in 24 h; enterohepatic recirculation in rats
Metabolism in animals	Hydroxylation, oxidation and conjugation. Some species differences.

Toxicologically significant compounds in animals, plants and the environment	Procymidone, hydroxyprocymidone
Acute toxicity	
Rat, LD_{50}, oral	> 5000 mg/kg bw
Rat, LD_{50}, dermal	> 2500 mg/kg bw
Rat, LC_{50}, inhalation	> 1.5 mg/l
Rabbit, skin irritation	Slight transient irritant
Rabbit, eye irritation	Not irritating
Guinea-pig, skin sensitization (test method used)	Negative (Magnusson & Kligman maximization test)
Short-term studies of toxicity	
Target/critical effect	Decreased body-weight gain, liver effects, testes
Lowest relevant oral NOAEL	25 mg/kg bw per day
Lowest relevant dermal NOAEL	1000 mg/kg bw per day
Lowest relevant inhalation NOAEC	No data
Genotoxicity	
	Not genotoxic
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Body weight, liver effects
Lowest relevant NOAEL	14 mg/kg bw per day (rat)
Carcinogenicity	Liver tumours in mice; testes tumours in rats.
Reproductive toxicity	
Reproduction target/critical effect	Hypospadias; altered testes and epididymis weight
Lowest relevant reproductive NOAEL	12.5 mg/kg bw per day
Developmental target/critical effect	Hypospadias (rat)
Lowest relevant developmental NOAEL	12.5 mg/kg bw per day
Neurotoxicity/delayed neurotoxicity	
	No evidence in conventional studies
Special studies	
	Kinetic and metabolism studies in rats, monkeys, rabbits, mice and with human tissues showed differences between species. Rats having a relatively high area-under-the curve and a low capability to metabolize procymidone and hydroxyprocymidone.
	Receptor-binding assays showed that procymidone and hydroxyprocymidone could bind to rat and human androgen receptors. Both procymidone and hydroxyprocymidone were extensively bound (≥ 77%) to plasma proteins.
	Procymidone produced increases in luteinizing hormone and human chorionic gonadotrophin-stimulated production of testosterone in rats at 6000 ppm and ≥ 700 ppm respectively.
	Ejaculate mass was reduced in monkeys exposed to procymidone for 13 weeks at ≥ 100 mg/kg bw per day. NOAEL was 30 mg/kg bw per day
	Hydroxyprocymidone induced hypospadias in rats at ≥ 62.5 mg/kg bw per day; $BMDL_{10}$ was 43.8 mg/kg bw per day.
Medical data	
	No adverse findings reported

Summary

	Value	Study	Safety factor
ADI	0–0.1 mg/kg bw	Rat, studies of developmental and reproductive toxicity	100
ARfD	0.1 mg/kg bw	Rat, study of developmental toxicity	100

References

Arai, M., Hasegawa, R. & Ito, N. (1980a) Three-month subacute toxicity study of S-7131 (Sumilex, Sumisclex) in mice. Unpublished report No. BT-00-0058 from Nagoya City University Medical School, Nagoya, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd, Osaka, Japan

Arai, M., Hasegawa, R. & Ito, N. (1980b) Six-month subacute toxicity study of S-7131 (Sumilex, Sumisclex) in mice. Unpublished report No. BT-00-0057 from Nagoya City University Medical School, Nagoya, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd, Osaka, Japan.

Bee, W. (1992) S-7131 90-day oral (gavage) subchronic study in the cynomolgus monkey. Unpublished report No BT-21-0147 from Hazleton Deutschland GmbH, Kesselfeld, Germany. Submitted to WHO by Sumitomo Chemical Co., Ltd, Osaka, Japan.

Dalgard, D. & Machotka, S. (1992) Chronic toxicity study in dogs with procymidone. Unpublished report No. BT-21-0162 from Hazleton Washington, Vienna, Virginia, USA. Submitted to WHO by Sumitomo Chemical Co., Ltd, Osaka, Japan.

Filler, R. & Parker, G. A. (1988) Oral chronic toxicity study and oncogenicity study in mice. Unpublished report No. BT-81-0126 from Hazleton Laboratories America Inc., Rockville, Maryland, USA. Submitted to WHO by Sumitomo Chemical Co., Ltd, Osaka, Japan.

Fujita, T., Fukuda, T., Kato, T. & Miyamoto, J. (1976) Toxicity of S-7131 technical material to rats in prolonged dietary administration over 9 months. Unpublished report No. BT-60-0003 from Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd, Osaka, Japan

Fukunishi, K. (2003a) A preliminary study of the effects of procymidone on external genitalia development in male fetuses by oral administration to cynomolgus monkeys. Unpublished report No. BT-0207 from Shin Nippon Biomedical Laboratories Ltd., Kagoshima, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd, Osaka, Japan.

Fukunishi, K. (2003b) A study of the effects of procymidone on external genitalia development in male fetuses by oral administration to cynomolgus monkeys. Unpublished report No. BT-0208 from Shin Nippon Biomedical Laboratories Ltd., Kagoshima, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd, Osaka, Japan.

Hara, M. (1991a) In vitro unscheduled DNA synthesis (UDS) assay of Sumilex in rat hepatocytes. Unpublished report No. BT-10-0142 from Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Hara, M. (1991b) In vitro chromosomal aberration test of Sumilex in Chinese hamster ovary cells (CHO-K1). Unpublished report No.BT-10-0141 from Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Hara, M. (1992) Comments on EPA's 'data evaluation report' of the report entitled in vitro unscheduled DNA synthesis (UDS) assay of Sumilex in rat hepatocytes. Unpublished report No. BT-20-0149 from Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Hara, M. & Suzuki, H. (1980) Cytogenetic test of procymidone in mouse bone marrow. Unpublished report No. BT-00-0043 from Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Harada, T. (1983) A review on medical examinations of factory workers possibly exposed to procymidone technical materials. Unpublished report No BG-31-0019 from Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Hoberman, A. (1992a) Dose-range developmental toxicity (embryo-fetal toxicity and teratogenic potential) study of procymidone administered orally via gavage to Crl:CD®BR VAF/Plus® presumed pregnant rats. Unpublished report No BT-21-0160 from Argus Research Laboratories, Inc., Horsham, Pennsylvania, USA. Submitted to WHO by Sumitomo Chemical Co., Ltd, Osaka, Japan.

Hoberman, A. (1992b) Developmental toxicity (embryo-fetal toxicity and teratogenic potential including a postnatal evaluation) study of procymidone administered orally via gavage to Crl:CD®BR VAF/Plus® presumed pregnant rats. Unpublished report No BT-21-0161 from Argus Research Laboratories, Inc., Horsham, Pennsylvania, USA. Submitted to WHO by Sumitomo Chemical C., Ltd, Osaka, Japan.

Hoberman, A. (1992c) Dose-range developmental toxicity (embryo-fetal toxicity and teratogenic potential including a postnatal evaluation) study of procymidone administered orally via gavage to Crl:CD®BR VAF/Plus® presumed pregnant rats (amendment). Unpublished report No BT-21-0164 from Argus Research Laboratories, Inc., Horsham, Pennsylvania, USA. Submitted to WHO by Sumitomo Chemical Co., Ltd, Osaka, Japan.

Hodge, M., Williams, M., Hollis, K., Moreland, S., Greenwood, M., MacLean Head, L. & Foster, P. (1991) Procymidone: one-generation study in the rat. Unpublished report No. BT-11-0144 from Imperial Chemical Industries, Macclesfield, Cheshire, England. Submitted to WHO by Sumitomo Chemical Co., Ltd, Osaka, Japan.

Hosokawa, S. (1984) Toxicity of S-7131 technical material to rats in prolonged dietary administration over 9 months (BT-60-0003). addendum to final report. Unpublished report No. BT-40-0095 from Takarazuka Research Centre, Takarazuka, Japan. Submitted to WHO by Sumitomo Chemical Co. Ltd., Osaka, Japan.

Inawaka, K. (2003) Study for effects of procymidone on development of fetal external genitalia in rabbits. Unpublished report No. BT-0211 from Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd

Inawaka, K. (2005) Study for determination of minimum toxic dose of the effects on male external genitalia in rats with procymidone. Unpublished report No. BT-0217 from Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Izumi, H. (2005) Teratogenicity study of hydroxylated procymidone in rats by oral administration (observations in male offspring). Unpublished report No. BT-0222 from Panapharm Laboratories Co., Ltd, Kumamoto, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd, Osaka, Japan.

Kadota K, Kohda H &Miyamoto J (1976a) Acute toxicity studies with S-7131 technical material in mice and rats; oral, subcutaneous, intraperitoneal, dermal. Unpublished report No. BT-60-0002 from Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Kadota, K. & Miyamoto, J. (1976) Eye and skin irritation study with S-7131 technical material in albino rats. Unpublished report No. BT-60-0004 from the Institute for Biological Sciences, Hyogo, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd, Osaka, Japan.

Keller, J. & Cardy, R. H. (1986) Oral chronic toxicity and oncogenicity study in rats TB0100 Sumisclex. Unpublished report No. BT-61-0112 from Litton Bionetics Inc., Rockville, Maryland, USA. Submitted to WHO by Sumitomo Chemical Co., Ltd, Osaka, Japan.

Kimura, K., Shiba, K. & Iba, K. (1988) Comparative metabolism of procymidone in rats and mice. Unpublished report No. BM-80-0019 from Takarazuka Research Center, Sumitomo Chemical Co. Ltd., Takarazuka, Japan. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Kinsey, D, Banham, P., Godley, M., Ishmael, J. & Blackburn, D. (1985) Procymidone: six-month feeding study in mice. Unpublished report No BT-51-0102 from Imperial Chemical Industries plc., Macclesfield, England. Submitted to WHO by Sumitomo Chemical Co., Ltd, Osaka, Japan.

Kogiso, S. (1991) Reverse mutation test of Sumilex in bacterial system. Unpublished report No. BT-10-0138 from Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Kogiso, S., Kasaoka, Y., Kato, H. & Nakatsuka, I. (1992) Reverse mutation test of CCA in bacterial systems. Unpublished report No. BT-20-0148 from Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Kohda, H., Nakamura, M. & Kadota, T. (1980) Acute oral and subcutaneous toxicity of three metabolites of procymidone. Unpublished report No. BT-00-0037 from Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Kumagai, H. (2007). Statement on medical examinations at Sumitomo Osaka Works, dated 29 August, 2007. Submitted to WHO by Sumitomo Chemical Co., Ltd, Osaka, Japan.

Kurihara, T. (2001) A review on medical examination of factory workers possibly exposed to procymidone technical materials. Unpublished report No. BT-0197 from Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Matsui, M, (2005a) In vitro plasma protein binding of procymidone and PCM-CH_2OH in human, rat, rabbit and monkey. Unpublished report No. BM-0109 from Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Matsui, M. (2005b) In vitro metabolism of procymidone and PCM-CH_2OH in S9 fraction of human, rat, rabbit and monkey female livers. Unpublished report No. BM-0118 from Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Mikami, N. & Yamamoto, H. (1976) Metabolism of *N*-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide in rats. Unpublished report No BM-60-0002 from Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Milburn, G. & Moreland, S. (1991) Procymidone: multigeneration reproduction study in the rat (first supplement – additional histopathology). Unpublished report No. BT-11-0140 from Imperial Chemical Industries, Macclesfield, England. Submitted to WHO by Sumitomo Chemical Co., Ltd., Osaka, Japan.

Mogi, M. (2005a) Pharmacokinetics and excretion of [phenyl-^{14}C]procymidone after single oral administration to cynomolgus monkeys. Unpublished report No. BM-0103 from Shin Nippon Biomedical Laboratories, Wakayama, Japan. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Mogi, M. (2005b) Pharmacokinetics and excretion of [phenyl-^{14}C]procymidone during and after repeated oral administration to cynomolgus monkeys. Unpublished report No. BM-0104 from Shin Nippon Biomedical Laboratories, Wakayama, Japan. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Mogi, M. (2005c) Biliary excretion after single oral administration of [carbonyl-^{14}C]procymidone to monkey. Unpublished report No. BM-0106 from Shin Nippon Biomedical Laboratories, Wakayama, Japan. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Mogi, M. (2005d) Biliary excretion after single oral administration of [carbonyl-^{14}C] procymidone to rabbit. Unpublished report No. BM-0107 from Shin Nippon Biomedical Laboratories, Wakayama, Japan. Submitted to WHO by Sumitomo Chemical Co, Ltd.

Mogi, M. (2005e) Tissue distribution of pregnant monkey and fetus after single oral administration of [phenyl-^{14}C]procymidone to pregnant monkey. Unpublished report No. BM-0125 from Shin Nippon Biomedical Laboratories, Wakayama, Japan. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Moore, M.R. (1993) Two-year oral toxicity and carcinogenicity study in mice: supplement A to the final report. Unpublished report No. BT-31-0172 from Hazleton Laboratories America Inc., Rockville, Maryland, USA. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Moriya, M, & Katom K, (1977) Mutagenicity of S-7131 in bacterial test systems. Unpublished report No. BT-71-0021 from Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Murakami, M., Yoshitake, A. & Hosokawa, S. (1986) Study on serum hormone levels in rats treated with S-7131. Unpublished report No. BT-60-0113 from Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Murakami, M., Ineyama, M., Yamada, T., Koyama, Y., Ito, M., Kimura, J., Hosokawa, S., Yoshitake, A. & Yamada, H. (1988a) Effect of testicular function of male rats and mice by subacute administration of procymidone. Unpublished report No. BT-80-0130 from Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Murakami, M., Ineyama, M., Hosokawa, S., Yoshitake, A. & Yamada, H. (1988b) The affinity of procymidone to androgen receptor in rats and mice. Unpublished report No. BT-80-0131 from Sumitomo Chemical Co. Ltd., Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Murli, H. (1992a) Dose range finding study for in vivo murine micronucleus assay on CCA. Unpublished report No. BT-21-0158 from Hazelton Washington Inc., Rockville, Maryland, USA. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Murli, H. (1992b) Mutagenicity test on CCA: in vivo mammalian micronucleus test. Unpublished report No. BT-21-0159 from Hazelton Washington Inc., Rockville, Maryland, USA. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Nakanishi, T., Nakatsuka, I. & Kogiso, S. (1991) Skin sensitization test of procymidone in guinea-pigs. Unpublished report No. BT-10-0146 from Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted by Sumitomo Chemical Co., Ltd.

Nakashima, N., Ebino, K., Tsuda, S., Kitazawa, T. & Harada, T. (1984) A 6-month subchronic study of Sumilex in beagles. Unpublished report No. BT-41-0091 from Institute of Environmental Toxicology, Tokyo, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Nunziata, A. (1982) Supplementary information to the technical report (BT-00-0050). Unpublished report No. BT-21-0075 from Centro Ricerca Farmaceutica S.p.A., Rome, Italy. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Ogata, H. (2002) 28-Day repeated-dose dermal toxicity study of procymidone TG in rats. Unpublished report No. BT-0200 from Panapharm Laboratories, Kumamoto, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Ohzone, Y. (2005a) Excretions of procymidone in chimera mice. Unpublished report No. BM-0108 from Daiichi Pure Chemicals Co., Ltd, Ibaraki, Japan. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Okuno, Y. (1975) Skin sensitization with S-7131 in guinea-pigs. Unpublished report No. BT-60-0005 from Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO from Sumitomo Chemical Co., Ltd.

Owen, H.M. (2002) Procymidone: in vitro absorption through human and rat epidermis. Unpublished report No. BM-0076 from Central Toxicology Laboratory, Macclesfield, Cheshire, England. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Pence, D.H., Hoberman, A.M. & Spicer K.M. (1980a) Pilot teratology study in rats – S-7131 (technical). Unpublished report No. BT-01-0041 from Hazleton Laboratories America Inc., Rockville, Maryland, USA. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Pence, D.H., Hoberman, A.M., Durloo, R.S.; Andrews, J.P. & Mossburg, P.A. (1980b) Teratology study in rats – S-7131 (technical). Unpublished report No. BT-01-0048 from Hazleton Laboratories America Inc., Rockville, Maryland, USA. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Principe, P., Monaco, M. & Nunziata, A. (1980) Report on mutagenicity experiment on the substance Sumisclex (procymidone). Unpublished report No BT-01-0050 from Centro Ricerca Farmaceutica S.p.A., Rome, Italy. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Saito, K. (1992) Metabolism of CCA in rats. Unpublished report No. BM-20-0032 from Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Savides, M.C. (2002) The in vivo dermal absorption of [^{14}C]procymidone SC in the rat. Unpublished report No. BM-0075 from Ricerca, LLC, Concord, Ohio, USA. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Segawa, T. (1977) Acute oral, subcutaneous, intraperitoneal and dermal toxicities of S-7131 in rats and mice. Unpublished report No. BT-71-0053 from Hiroshima University School of Medicine, Hiroshima, Japan. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Struble, C. (1992a) Metabolism of [^{14}C]procymidone in rats (preliminary and definitive phases): volume 1 of 2: absorption distribution elimination and tissue residues. Unpublished report No BM21-0033 from Hazleton Wisconsin, Madison, Wisconsin, USA. Submitted by Sumitomo Chemical Co. Ltd.

Struble, C. (1992b) Metabolism of [^{14}C]procymidone in rats (preliminary and definitive phases): volume 2 of 2: metabolite identification. Unpublished report No BM21-0034 from Hazleton Wisconsin, Madison, Wisconsin, USA. Submitted by Sumitomo Chemical Co. Ltd.

Sugimoto, K. (2005a) Pharmacokinetics and excretions of [phenyl-^{14}C]procymidone in female rats (single oral administration). Unpublished report No. BM-0096 from Panapharm Laboratories Co., Ltd, Kumamoto, Japan. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Sugimoto, K. (2005b) Pharmacokinetics and excretions of [phenyl-^{14}C]procymidone in female rabbits (single oral administration). Unpublished report No. BM-0102 from Panapharm Laboratories Co., Ltd, Kumamoto, Japan. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Sugimoto, K. (2005c) Pharmacokinetics and excretions of [phenyl-^{14}C]procymidone in female rats (repeated oral administration for 14 days). Unpublished report No. BM-0098 from Panapharm Laboratories Co., Ltd, Kumamoto, Japan. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Sugimoto, K. (2005d) Excretion of [carbonyl-^{14}C]procymidone in bile of female rats (single oral administration). Unpublished report No. BM-0099 from Panapharm Laboratories Co., Ltd, Kumamoto, Japan. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Sugimoto, K. (2005e) Fetoplacental transfer of [phenyl-^{14}C]procymidone in rats (single oral administration). Unpublished report No. BM-0100 from Panapharm Laboratories Co., Ltd, Kumamoto, Japan. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Sugimoto, K. (2005f) Fetoplacental transfer of [phenyl-^{14}C]procymidone in rats (single oral administration). Unpublished report No. BM-0122 from Panapharm Laboratories Co., Ltd, Kumamoto, Japan. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Sugimoto, K. (2005g) Fetoplacental transfer of [phenyl-^{14}C]procymidone in rabbits (single oral administration). Unpublished report No. BM-0101 from Panapharm Laboratories Co., Ltd., Kumamoto, Japan. Submitted to WHO by Sumitomo Chemical Co. Ltd., Osaka, Japan.

Suzuki, H. (1980) Effect of procymidone on sister chromatid exchanges (SCE) in cultured mouse embryo cells. Unpublished report No. BT-00-0042 from Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co, Ltd.

Suzuki, T. & Kato, T. (1988) Acute inhalation toxicity of Sumilex in rats. Unpublished report No. BT-60-0116 from Takarazuka Research Centre, Takarazuka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Suzuki, H. & Miyamoto, J. (1976) Studies on mutagenicity of S-7131 with bacterial systems. Unpublished report No.BT-60-0011 from Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Suzuki, N. (2005) In vitro assays for procymidone and its metabolites with rat and human androgen receptors. Unpublished report No. BM-0120 from Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Tarui, H. (2005a) Transformation of procymidone and its metabolites in various pH conditions. Unpublished report No. BM-0116 from Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Tarui, H. (2005b) In vitro metabolism of procymidone in human hepatocyte. Unpublished report No. BM-0113 from Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Thakur, A. K. (1988) Oral chronic toxicity and oncogenicity study in mice. Unpublished report amendment No. BT-81-0127 from Hazleton Laboratories America, Inc., Rockville, Maryland, USA. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Tomigahara, Y. (2005a) Metabolism of [phenyl-^{14}C]PA-CH_2OH in female rats. Unpublished report No. BM-0115 from Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Tomigahara, Y. (2005b) Metabolism of [phenyl-^{14}C]PCM-CH_2OH in female rats. Unpublished report No. BM-0119 from Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Tomigahara, Y. (2005c) Pharmacokinetics and excretions of [phenyl-^{14}C]PCM-CH_2OH in female rats (single oral and subcutaneous administration. Unpublished report No. BM-0114 from Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Tomigahara, Y. (2005d) Placental transfer of [phenyl-^{14}C]procymidone in rats (repeated oral administration). Unpublished report No. BM-0117 from Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Tsuji, R. (1994) Acute inhalation toxicity of Sumilex in rats (addendum to Suzuki & Kato, 1988). Unpublished report No. BT-40-0178 from Sumitomo Chemical Co., Ltd, Osaka, Japan. Submitted to WHO by Sumitomo Chemical Co., Ltd.

Weir, R., Keller, J., Moe, J., Cardy, R., Kim, G., Paulin, H. & Fitzgerald, J. (1984) 13-week pilot study in mice: Sumilex. Unpublished report No. BT-41-0092 from Litton Bionetics, Kensington, Maryland, USA. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Wickramaratne, G. (1988) Procymidone: teratogenicity study in rabbits – individual animal data supplement. Unpublished report No. BT-71-0177 from Imperial Chemical Industries Ltd., Macclesfield, Cheshire, England. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Wickramaratne, G., Milburn, G., Banham, P., Greenwood, M. & Moreland, S. (1988a) Procymidone: multigeneration reproduction study in the rat. Unpublished report No. BT-81-0132 from Imperial Chemical Industries, Macclesfield, England. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Wickramaratne, G., Kinsey, D., Banham, P. & Greenwood, M. (1988b) Procymidone: teratogenicity study in rabbits. Unpublished report No. BT-81-0128 from Imperial Chemical Industries Ltd., Macclesfield, Cheshire, England. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Wickramaratne, G. & Milburn, G. (1990) Procymidone: multigeneration reproduction study in the rat (supplementary submission – substance intake data) Unpublished report No. BT-81-0169 from Imperial Chemical Industries, Macclesfield, England. Submitted to WHO by Sumitomo Chemical Co. Ltd.

Zühlke U. (1991) S-7131 Seven-day oral (gavage) range-finding study in the cynomolgus monkey. Unpublished report No BT-11-0143 from Hazleton Deutschland GmbH, Kesselfeld, Germany. Submitted to WHO by Sumitomo Chemical Co. Ltd.